RESEARCH ARTICLE

Comparative Antioxidant and Antiulcer Potential of In-house and Marketed Polyherbal Formulation

Medha A Khade,[1] Manish K Gupta,[2*] Birendra Srivastava,[3] Supriya R Hyam,[4] Amol B Khade[5]

[1]*Research Scholar, Department of Pharmacology, School of Pharmaceutical Sciences, Jaipur National University, Jagatpura, Jaipur, Rajasthan, India*
[2]*Department of Pharmaceutics, School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India*
[3]*Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India*
[4]*Department of Pharmacognosy, Vijayrao Naik College of Pharmacy, Kankavali, Maharashtra, India*
[5]*Department of Pharmaceutical Chemistry, Indira Institute of Pharmacy, Sadavali, Maharashtra, India*

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ABSTRACT

The prime objective of the present research was to assess the antioxidant and comparative anti-ulcer potential of a gastroretentive polyherbal formulation with a conventional oral marketed formulation. The antioxidant capacity was estimated by 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenylpicrylhydrazyl (DPPH) methods. For the assessment of comparative anti-ulcer potential, the aspirin-induced rat ulcer model was utilized. Enzyme-linked immunosorbent assay (ELISA) studies were performed to study the effect on pro- and anti-inflammatory markers. ABTS and DPPH free radical scavenging capacity signified the antioxidant activity of the formulation. Evaluation parameters such as pH of the gastric content, total and free acidity, ulcer index, and histopathological changes were studied in the aspirin-induced anti-ulcer activity. The study's outcomes reveal the significant anti-ulcer potential of the in-house gastro-retentive polyherbal formulation and marketed formulation as indicated by the reduction in ulcer index, free and total acidity. The results of the histopathological investigation further strengthened the anti-ulcer potential. Moreover, pre-treatment with gastro-retentive formulation decreased the ulcer index to a greater degree than the conventional marketed formulation. The results conclude that the antioxidant, antisecretory, cytoprotective, and anti-inflammatory actions might be responsible for the anti-ulcer activity. Further, the gastro-retentive formulation has an improved potential in treating ulcers over conventional oral formulations.

Keywords: Antioxidant, Antiulcer, Enzyme-linked immunosorbent assay, Gastroretentive, Peptic ulcer, Polyherbal formulations

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INTRODUCTION

One of the major health issues of concern is the prevalence of peptic ulcer disease among a wide range of populations.[1] A peptic ulcer is an ailment of the gastrointestinal tract resulting from the perturbed relative balance between the contributing endogenous and exogenous factors. Normal gastrointestinal physiology entails the undisturbed equilibrium between the defending and belligerent factors. The defending features comprise bicarbonate secretion, mucus production, and prostaglandin generation. Acid, pepsin, stress, *Helicobacter pylori,* non-steroidal anti-inflammatory drugs (NSAIDs), alcohol intake, and abridged blood flow to the mucosa are among the belligerent aspects.[2,3] Current approaches to treat ulcerogenic lesions involve prescribing proton pump inhibitors, H_2 blockers, prostaglandin analogues, anticholinergics, ulcer protective, antacids, and antibiotics. However, none of these drugs assure a complete cure or freedom from side effects and recurrence.[4] Thus, began the quest for substitute remedies with safe and effective therapeutic potential.[5] It is well known that crude herbs have been used as folklore medication for centuries.[6] A tremendous amount of society still depends on traditional medications for their regular healthcare requirements.[7] Among the alternate medicines, plant-origin drugs are the most preferred sources due to their relatively low cost, adverse effects, and toxicity with high efficacy in treating ulcers.[4,8]

The oral route is the highly convenient and hence favored route of administration for systemic effects. However,

*Author for Correspondence: guptarx@gmail.com

conventional oral formulations release the drug quickly, which is absorbed systemically and eliminated from the body at a faster rate with short half-lives. Thus, frequent dosing is needed to attain the desired therapeutic effect.[9] Hence, ample research had been carried out on the design of dosage forms to retain them in the upper gastrointestinal tract.[10] Over the past three decades, several attempts have been made to develop gastro-retentive drug delivery systems to enhance the gastric retention period of the drugs.[11] The benefits offered by these drug delivery systems over conventional dosage forms include improvement in the therapeutic efficacy, reduction in the dose, local action, enhanced bioavailability, decrease in the frequency of dosing with improved patient compliance, and minimization of fluctuations in the plasma levels.[9-13] Recent studies have also revealed the rationale of using gastro-retentive formulations in treating ulcers to be beneficial over conventional formulations.[10,14,15]

The core objective to take up the present research work is to evaluate the comparative anti-ulcer potential of the in-house gastro-retentive polyherbal formulation with the marketed formulation AV gastro (by Amsarveda Pvt. Ltd., Goa). The composition of the formulation includes the hydroalcoholic extracts of *Zingiber officinale* Rosc. rhizomes, *Glycyrrhiza glabra* Linn. root*, Andrographis paniculata* Nees. whole herb*, Aegle marmelos* Correa. unripe fruits*,* and *Holarrhena antidysenterica* wall stem. *Z. officinale* Rosc. had been reported by researchers to possess antioxidant, anti-ulcer, and anti *H. pylori* activity.[16-18] *G. glabra* Linn. had been documented to be evident of anti-ulcer potential.[19] Antiulcer and antioxidant properties of *A. paniculata* Nees. had been reported in previous studies.[20-22] *A. marmelos* Correa. had been testified for antioxidant and anti ulcer properties.[23,24] Antioxidant property of *H. antidysenterica* Wall. had been proven.[25]

MATERIALS AND METHODOLOGY

Collection and Authentication of Plant Material

The rhizomes of *Z. officinale* Rosc., the root of *G. glabra* Linn.*,* whole herb of *A. paniculata* Nees.*,* unripe fruits of *A. marmelos* Correa.*,* and stem of *H. antidysenterica* Wall. were collected from the Konkan region of Maharashtra and shade dried. The plant material was authenticated by Dr. Sangram Keshari Das Professor and HOD Dravyaguna (Pharmacology) at Gomantak Ayurved Mahavidyalaya and Research, Centre, Shiroda, Goa.

Extraction and Formulation Development

The dried plant parts were ground coarsely and subjected to soxhlet extraction using 70% ethanol as solvent. The extracts were mixed in the proportion: *A. paniculata* 15 mg, *A. marmelos* 150 mg, *H. antidysenterica* 150 mg, *Z. officinale* 15 mg, and *G. glabra* 120 mg. The mixture was granulated using the optimized concentration of carbopol 971 G and filled into cellulose capsules to obtain a gastro-retentive polyherbal formulation (GRPF).

Phytochemical Investigation

The developed polyherbal formulation was subjected to various phytochemical tests to confirm the extraction of prime phytobiological classes viz., glycosides, flavonoids, saponins, alkaloids, terpenoids, tannins, and steroids.[26]

In-vitro Antioxidant Study

2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) Method

To determine the ABTS radical scavenging estimation, a 7 mM solution was prepared in methanol. ABTS radical cation generation was achieved by reacting ABTS solution with 2.4 mM potassium persulfate. This blend was allowed to incubate at room temperature in the darkness for 12 hours. One mL of the mixture was then diluted with potassium phosphate buffer (0.1 M, pH 7.4) to attain the absorbance of 0.706 ± 0.001 units spectrophotometrically at 734 nm. Diverse concentrations of the test sample were added to ABTS solution, and after 7 minutes the absorbance of this mixture was read spectrophotometrically at 734 nm. The phosphate buffer solution was used as a blank with standard ascorbic acid. ABTS radical scavenging ability of the test and standard was calculated using the formula.

1,1-diphenyl-2-picrylhydrazyl (DPPH) Method

The free radical scavenging potential of the formulation was estimated by the DPPH method. A 0.135 mM solution of DPPH was prepared using methanol. To 1-mL of the test sample at varying concentrations, 1-mL of DPPH solution was added. The absorbance of this mixture was read spectrophotometrically at 517 nm after incubation in darkness at room temperature for 30 minutes. The test result was compared against ascorbic acid as standard. The radical scavenging capacity was computed by using the formula.

$$\%\text{radical scavenging capacity} = [(A_C - A_S) \times 100] \div (A_C)$$

where A_C was the absorbance of radical

A_S was the absorbance of radical and either the formulation or standard[24]

Ethical Permission

The study was conducted per the CPCSEA guidelines after obtaining ethical permission from the Institutional Animal Ethical Committee, Indira Institute of Pharmacy under protocol number IIP/IAEC/11/2019-20.

Experimental Animals

Animals of either gender were procured from Global Bioresearch Solution Pvt. Ltd, Shirwal, Maharashtra. Swiss albino mice weighing between 20 to 25 g were utilized for the acute toxicity study. The wistar rats weighing in the range of 180 to 200 g were used for the anti-ulcer study. The housing and handling of all the animals were conducted following the CPCSEA guidelines. All the animals were freely accessible to a pellet diet and water *ad libitum.* A 12 hours light-dark cycle was followed while housing the animals in an environment with a relative humidity of 55 ± 1% and temperature of 23 ± 1°C.

Acute Toxicity Study

The safety dosage evaluation of the formulation was carried out according to guideline 423 of OECD. It was seen that up to 2000 mg/kg, there were no signs of toxic effects or mortality. Hence, the high dose ($^{1}/_{10}^{th}$) selected was 400 mg/kg and the low dose ($^{1}/_{5}^{th}$) was 200 mg/kg for in-house formulation.[27] The animal dose of AV gastro was calculated from the human dose using the table of Paget and Barnes utilizing the body surface area. As the human dose is 1800 mg/day the experimental dose was estimated to be 250 mg/kg.[28]

Investigational Animal Groups

The wistar rats were clustered arbitrarily into six groups of 6 rats each as follows:

- Group I : Negative control (0.9% Saline)
- Group II : Positive control (Aspirin 200 mg/kg)
- Group III : Marketed formulation (AV gastro 250 mg/kg)
- Group IV : GRPF 200 mg/kg
- Group V : GRPF 400 mg/kg
- Group VI : Omeprazole 20 mg/kg

Anti-ulcer Activity

Normal saline (0.9% 10 mL/kg) was administered to groups I and II, which served as negative control and positive control, respectively. Group III received the marketed formulation, AV gastro (250 mg/kg). Groups IV and V were administered with the in-house formulation in dosages of 200 and 400 mg/kg. Groups III, IV, and V served as study treatment groups.[29] Group VI served as the standard group and was administered with omeprazole (20 mg/kg). All the groups received respective treatments for consecutive 14 days. Following 14 day treatment, all the animals were deprived of water and food for 24 hours.[30] Post starvation, on day 15 all the groups excluding Group I were administered a single dose of aspirin (200 mg/kg).[29] After 4 hours of administration of aspirin, all the animals were sacrificed following euthanasia with excess anesthetic ether.[30,31] With a mid-line incision the abdomens were opened. The stomachs were excised carefully, and cut along the greater curvature and the gastric contents were collected in measuring cylinders to note the gastric volume. For about 10 minutes the contents were centrifuged at 2000 rpm. For analysis of parameters namely pH, free acidity, and total acidity of the gastric contents, 1-mL of the supernatant was utilized. The glandular region of the stomach was observed for the severity and scoring of lesions.[32]

Measurement of pH

The pH of 1-mL of supernatant gastric juice was determined using a pH meter after diluting it by means of 1 mL of distilled water.[30]

Estimation of Free Acidity

Distilled water (1-mL) was used to dilute 1-mL of the supernatant liquid. The resultant mixture was titrated against 0.01 N NaOH using topfer's reagent as an indicator. The titration continued until the solution attained stable canary yellow color indicating the endpoint. The volume of NaOH consumed was documented.[32]

Estimation of Total Acidity

One-mL of the supernatant gastric liquid was mixed with 1-mL of distilled water and the mixture was titrated against 0.01 N NaOH utilizing a phenolphthalein indicator. The endpoint was considered once the solution turned pink. The volume of NaOH consumed was documented.[32]

Calculation of Free and Total Acidity

Making use of the volume of NaOH consumed and normality, the free and total acidity of the gastric content in mEq/L was computed using the formula:[32]

Acidity = (Volume of NaOH × N × 100) ÷ 0.1 mEq/L

Where N is the normality of NaOH

Gross Mucosal Assessment

The stomach cut along the greater curvature were washed with saline and pinned to waxed petri plates to expose the glandular portion.[33] Careful inspection of the entire gastric mucosal layer was carried out through the magnifying lens (10X) to examine the number and severity of ulceration.[29,32] The number of ulcerogenic lesions per stomach was logged.[34]

Scoring of Severity

The severity of lesions in each rat was examined critically and scored as follows:[31,35]

Normal-colored stomach: 0

Red colouration: 0.5

Spot ulcer: 1

Hemorrhagic streaks: 1.5

Deep ulcers: 2

Perforations: 3

Computation of Ulcer Index (UI) and %Protection

Calculation of ulcer index was executed utilizing the formula:

$\text{Ulcer Index} = (U_{AS} + U_{AN} + U_P) \div 10$

Where U_{AS} = Average ulcer severity score in the group

U_{AN} = Average number of ulcers in the group

U_P = Percent of animals in the group showing ulcers[36]

The extent of protection against ulceration by the various treatments was computed against the positive control group as follows:

$\%\text{Protection} = [(UI_{\text{Positive control}} - UI_{\text{Test/Standard}}) \times 100] \div UI_{\text{Positive control}}$

Where $UI_{\text{Positive control}}$ = Ulcer index of the positive control group

$UI_{\text{Test/Standard}}$ = Ulcer index of the test or standard group[29]

Histopathological Investigation

After recording the ulcer numbers and severity the stomachs were preserved in sample bottles containing 10% formalin for histopathological examinations within 2 days. The central portions of the damaged or ulcerated parts of the glandular tissue were cut into halves and 2 to 5 samples were isolated. Several samples from the stomachs with no lesions were isolated from various parts from the antrum to the corpus.[37] The tissue samples were processed by making use of a tissue processor and embedded in paraffin blocks. By means of a rotary microtome, about 5 µm thin sections were obtained.[27]

Table 1: Phytochemical evaluation of the in-house formulation

Phytobiological class	*Test*
Alkaloids	+
Flavonoids	+
Carbohydrates	-
Glycosides	+
Tannins	-
Saponins	+
Terpenoids	-
Steroids	+

+ Present; - Absent

Table 2: ABTS and DPPH antioxidant activity

Sample	*DPPH IC50 value*	*ABTS IC50 value*
GRPF	651.84	634.33
Ascorbic acid	26.46	35.43

The values are expressed as mcg/mL

These sections were placed on the glass slides and stained by hematoxylin and eosin (H & E) stains. The stained tissue was examined under a photographically equipped light microscope.[38]

ELISA

The formulation was tested for quantitation of pro- and anti-inflammatory cytokines namely TNF-α, IL-1β, and IL-10 using a rat-specific enzyme immune-assay kit (RayBiotech Inc, USA) as per the manufacturer's directions. The ELISA results were recorded at 570 nm with an ELISA reader (Bio-Rad Laboratories, CA, USA). The concentrations were determined for three wells for each cytokine and values were derived from the standard plot and stated as pg/mL.[39,40]

Statistical Analysis

All the results are stated as mean ± SEM (n=6). Statistical study to assess the significance of the difference among mean values of the groups was accomplished using One-way ANOVA followed by Dunnett's multiple comparisons test. The statistical evaluation and graphical representation were carried out using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA.

RESULTS

The tests for detecting the presence of various phytobiological constituents in the developed formulation were performed, confirming the occurrence of glycosides, flavonoids, saponins, alkaloids, and steroids (Table 1).

The developed formulation was subjected to antioxidant activity by DPPH and ABTS scavenging methods. The results show that the formulation possesses significant free radical scavenging capacity (Table 2, Figures 1 and 2).

The developed formulation was subjected to an acute toxicity study by 423 OECD guidelines. The formulation was found to show no signs of toxicity at the dosage of 2000 mg/kg. Hence, the study was carried out at two dosage levels of 200 and 400 mg/kg.

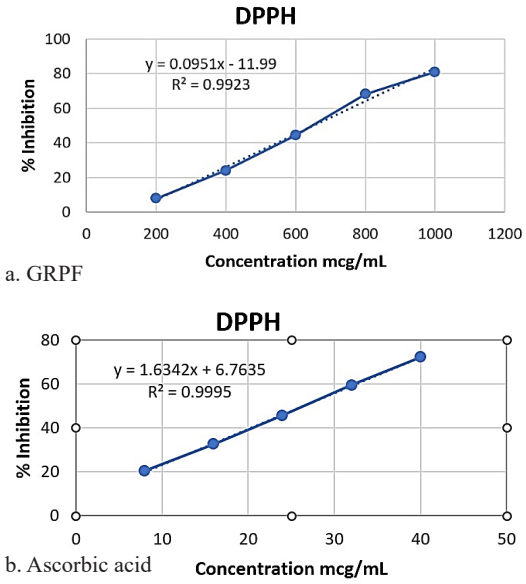


Figure 1: Graphical illustration of DPPH assay

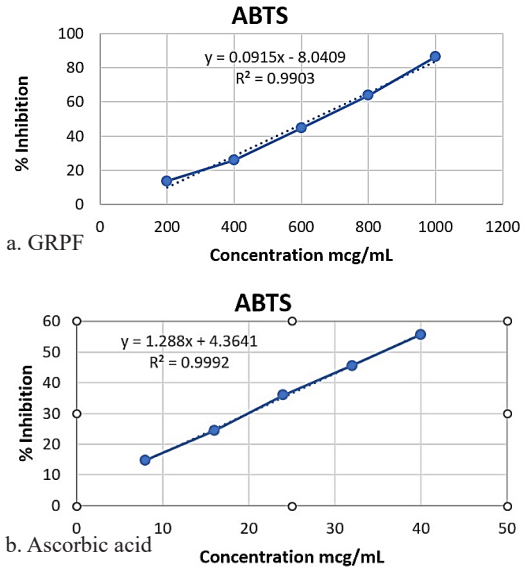


Figure 2: Graphical illustration of ABTS assay

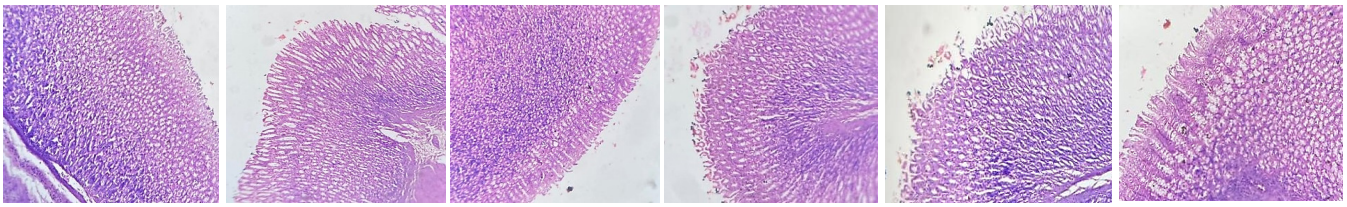
In the present study, a comparative anti-ulcer activity was performed between a gastro-retentive in-house and conventional marketed formulation by aspirin (200 mg/kg) induced ulcer model in wistar rats. The study findings revealed significant alterations in the parameters under study. The test groups of the in-house formulation lowered the gastric secretory volume, ulcer index, total, and free acidity, to a greater extent than the marketed formulation. The pH was significantly raised in the in-house formulation treated groups than in the marketed formulation (Table 3). The in-house formulation (200 and 400 mg/kg) significantly diminished the average number and severity of ulcers in the treated rats

Table 3: Comparative representation of pH, volume of gastric content, free and total acidity in Aspirin-induced ulcer model

Groups	*pH*	*Volume of gastric content (mL)*	*Free acidity (mEq/L)*	*Total acidity (mEq/L)*
Control (Saline)	2.37 ± 0.036	4.40 ± 0.044	55.83 ± 0.307	84.33 ± 0.558
Positive control Aspirin 200 mg/kg	1.83 ± 0.032	5.63 ± 0.073	94.17 ± 0.401	123.3 ± 0.667
GRPF 200 mg/kg	5.17 ± 0.023*	2.75 ± 0.035*	25 ± 0.365*	42.17 ± 0.307*
GRPF 400 mg/kg	6.49 ± 0.074*	1.83 ± 0.043*	15.67 ± 0.333*	24 ± 0.365*
AV Gastro 250 mg/kg	4.52 ± 0.063*	2.85 ± 0.034*	37.17 ± 0.307*	51 ± 0.258*
Standard Omeprazole 20 mg/kg	5.90 ± 0.032*	2.27 ± 0.051*	22.67 ± 0.211*	35 ± 0.365*

Table 4: Comparative representation of Ulcer index and %protection in Aspirin-induced ulcer model

Groups	*Average number of ulcers*	*Average severity score*	*% of animals with ulcers*	*Ulcer index*	*% Protection*
Control (Saline 0.9%)	0	0	0	0	-
Positive control Aspirin 200 mg/kg	3.33 ± 0.211	2.5 ± 0.224	100	10.58	-
GRPF 200 mg/kg	0.67 ± 0.333*	0.42 ± 0.201*	50	5.11	51.73
GRPF 400 mg/kg	0.17 ± 0.167*	0.17 ± 0.167*	16.67	1.70	83.94
AV Gastro 250 mg/kg	0.50 ± 0.224*	0.25 ± 0.112*	50	5.08	52.05
Standard Omeprazole 20 mg/kg	0.17 ± 0.167*	0.08 ± 0.083*	16.67	1.69	84.02



a. Saline **b. Aspirin:** 200 mg/kg **c. GRPF:** 200 mg/kg **d. GRPF:** 400 mg/kg **e. AV Gastro:** 250 mg/kg **f. Omeprazole:** 20 mg/kg

Figure 3: Histopathological investigation of Aspirin-induced ulcer model

with the resultant reduction in the ulcer index (51.73 and 83.94%). The marketed formulation AV gastro (250 mg/kg) also displayed a significant diminution in ulcer index (52.05%) analogous to the lower dose (200 mg/kg) of the in-house formulation (51.73%). It was observed that the higher dose (400 mg/kg) in-house formulation (83.94%) had gastroprotective and anti-ulcer effects, which were comparable with standard omeprazole (84.02%) (Table 4). Thus, the study concluded a significantly better gastroprotective activity of the gastro-retentive formulation when compared with the conventional oral polyherbal preparation.

The values are expressed as mean ± SEM (n=6) considering the p-values of <0.05 as significant when compared with the untreated control groups denoted as *

Ulcer index is expressed as $(U_{AS} + U_{AN} + U_P) \div 10$ and %protection is calculated in comparison with the disease control group. The values are stated as mean ± SEM (n=6) considering the p-values of <0.05 as significant when compared with the untreated control groups denoted as *

Moreover, the histopathological investigations supported the above findings with the less distorted architecture of gastric mucosa at a 400 mg/kg dose of the in-house formulation which was analogous to the standard omeprazole (20 mg/kg). The negative control group (Figure 3a) receiving saline revealed normal and intact gastric mucosa with a continuous epithelial layer. There was no infiltration of inflammatory cells and congestion in the submucosa. The positive control group (Figure 3b) receiving aspirin (200 mg/kg) showed severe distortion in the mucosal layer with the erosion of the superficial epithelial cells. The submucosa was evident of marked congestion of blood vessels, inflammatory cell infiltration, and moderate to severe edema. The mucosa of the group administered with the GRPF at 200 mg/kg (Figure 3c) dose showed distortion to a lesser extent as compared to the positive control group. The congestion in the submucosal layer was also not seen. The group administered with GRPF at 400 mg/kg (Figure 3d) dose exhibited intact mucosa with negligible congestion and infiltration in the submucosal layer. The group pre-treated with AV gastro at 250 mg/kg (Figure 3e) dose showed limited damage to the gastric mucosa with few epithelial detachments as compared to positive control. The submucosa showed mild infiltration and congestion.

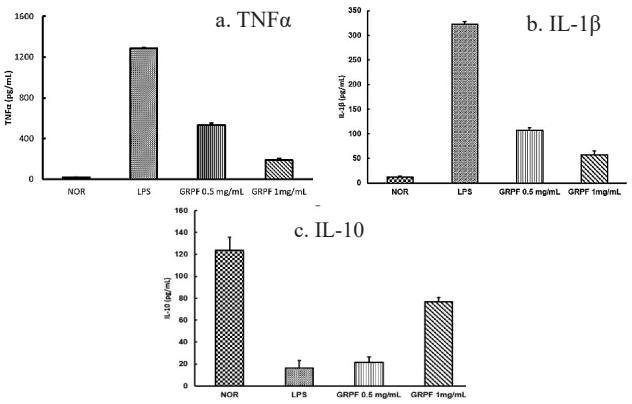


Figure 4: ELISA study

Pre-treatment with omeprazole 20 mg/kg (Figure 3f) showed complete protection with normal mucosa with the absence of infiltration and congestion in the submucosal layer.

Investigation of inflammatory markers was carried out using rat-specific ELISA kits. The study revealed a significant reduction in the inflammatory markers TNF-α and IL-1β. There was also a rise in the levels of marker IL-10. (Figure 4).

The above findings prove the gastro-retentive polyherbal formulation to be a more potent anti-ulcer remedy than the conventional polyherbal preparation. Further, it can be related that the existence of phytoconstituents for instance, flavonoids, saponins, alkaloids, and steroids could be responsible for the antioxidant, gastro-protective, and antisecretory actions of the PHFs. However, exploring the probable mechanism of action and exact constituents accountable for the pharmacological actions is necessary.

DISCUSSION

As mentioned earlier, the interest in the therapeutic use of herbs and polyherbal preparations in treating several diseases has increased in recent years due to obvious reasons such as being economical, easily available, and having lower side effects tendency when compared to drugs of synthetic origin.[33] In the present study, *Z. officinale* Rosc. rhizomes, *G. glabra* Linn. root*, A. paniculata* Nees. whole herb*, A. marmelos* Correa. unripe fruits*,* and *H. antidysenterica* Wall. stem. were selected for the formulation of a gastro-retentive capsule. *Z. officinale* Rosc.,[16,17] *G. glabra* Linn.,[19] *A. paniculata* Nees.,[20] and *A. marmelos* Correa.[23] had been reported to possess anti-ulcer activity. Antioxidant properties of *Z. officinale* Rosc.,[18] *A. paniculata* Nees.,[22] *A. marmelos* Correa.,[24] and *H. antidysenterica* Wall.[25] had been reported in previous studies. This was the rationale behind selecting these herbs for the formulation of the PHF.

Etiopathogenesis of ulcers is evident of multifactorial involvement in causing damage to the mucosal lining of the gastrointestinal tract.[27] These are the results of free radical generation and inflammation.[31] It is now well known that inequity between protective and hostile factors is causative in the progression of gastric damage. Stress-induced increases in gastric acid secretion, alcohol, smoking, infection by *H. pylori*, nutritional deficiencies, NSAIDs, and hereditary predisposition are the contributing factors to develop ulcers.[32,41] Under normal circumstances, suppression of the hostile factors and augmentation of the defending factors are attributed to the prostaglandins generated through the cyclooxygenase pathway.[41] NSAIDs such as aspirin are well known to damage the gastric mucosa by increasing gastric acid secretion, back diffusion of H^+ ions, inhibition of prostaglandin synthesis, decrease in bicarbonate and mucus secretion, augmented expression of interleukin-1, induction of apoptosis, and reactive oxygen species generation.

Moreover, the inhibition of thromboxane synthesis leads to interference with platelet functions consequently increasing bleeding complications. This makes it an effective laboratory tool for assessing the anti ulcer potentials of the therapeutic moiety.[27,29,42-44] Thus, the aspirin-induced ulcer model was utilized to explore the comparative anti ulcer potential of the in-house and marketed PHF.

The presence of pharmacologically active metabolites can be identified by various phytochemical tests for the different classes of phytoconstituents.[45] Previous studies have reported secondary metabolites namely alkaloids, terpenoids, flavonoids, and tannins to possess the anti-ulcer potential.[1,46,47] From these implications, it can be resolved that the presence of these phytoconstituents could be responsible for the significant gastroprotective effects of the formulation under study.

Gastroretentive drug delivery system (GDDS) has been an emerging trend in therapeutics over the last three decades.[10] This approach retains the dosage form in the gastric region for an extended period and enhances gastric residence time, thereby releasing the drug into the upper gastrointestinal tract for systemic or local effects.[48] These systems utilize several approaches including bioadhesive, high-density, swelling and expanding, floating, magnetic, and delayed gastric emptying systems.[10,49] Several advantages this drug delivery system offers make them preferred over conventional oral formulations. Local action, increased bioavailability, decreased frequency of dosing, minimization of fluctuation, and biodegradable and biocompatible nature are the prime benefits offered.[9,10,12] Studies have also unveiled the benefits of using GDDS in the therapy of peptic ulcers, especially *H. pylori* infections.[11,14,15] The present study findings have also proven the improved anti ulcer activity of in-house gastro-retentive polyherbal capsules over conventional formulations available in the market.

CONCLUSION

From the present study, we conclude that we have successfully developed a novel gastro-retentive polyherbal capsule with significant anti-ulcer potential in the aspirin-induced ulcer model. Moreover, the histopathological investigations confirmed the dose-dependent improvement in the damaging effects of aspirin on the mucosa. The anti ulcer action may perhaps be attributed to the decline in oxidative stress and the presence of secondary metabolites. Moreover, a synergistic effect could have been attained by the anti ulcer components of the PHF. However, further investigation is required to confirm the molecular mechanisms involved.

ABBREVIATIONS

ABTS: 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; DPPH: 1,1-diphenyl-2-picrylhydrazyl, ELISA: Enzyme-linked immunosorbent assay; NSAIDs: Nonsteroidal anti-inflammatory drugs; GRPF: gastroretentive polyherbal formulation; CPCSEA: Committee for the purpose of control and supervision of experiments on animals; OECD: Organization for economic co-operation and development; TNF-α: Tumor necrosis factor-α; IL-1β: Interleukin-1beta; IL-10: Interleukin-10; ANOVA: Analysis of Variance; PHF: Polyherbal formulation; GDDS: Gastroretentive drug delivery system.

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CONFLICT OF INTERESTS

The authors declare the conflict of interest none.

SOURCE OF SUPPORT

Nil.

REFERENCES

1. Abebaw M, Mishra B, Gelayee DA. Evaluation of anti-ulcer activity of the leaf extract of Osyris quadripartita Decne. (Santalaceae) in rats. Journal of Experimental Pharmacology. 2017;9:1–11.
2. Falcão HDS, Leite JA, Barbosa-Filho JM, de Athayde-Filho PF, Chaves MCDO, Moura MD, et al. Gastric and duodenal antiulcer activity of alkaloids: A review. Molecules. 2008;13(12):3198–3223.
3. Maheshwari R, Balaraman R, Sailor G, Parmar G, Patel A, Seth AK. Antiulcer and antioxidant effects of Normacid syrup (a polyherbal formulation) on experimentally-induced gastric ulcers. Oriental Pharmacy and Experimental Medicine. 2014;14(2):145–155.
4. Vyawahare NS, Deshmukh VV, Gadkari MR, Kagathara VG. Plants with Antiulcer Activity. Pharmacognosy Reviews. 2009;3:118–125.
5. Sanpinit S, Chonsut P, Punsawad C, Wetchakul P. Gastroprotective and Antioxidative Effects of the Traditional Thai Polyherbal Formula Phy-Blica-D against Ethanol-Induced Gastric Ulcers in Rats. Nutrients. 2021;14(1):1–16.
6. Asad I, Anjum I, Alamgeer, Ahsan H, Mushtaq MN. Evaluation of gastroprotective activity of Qarahine: A polyherbal formulation. Indian Journal of Pharmaceutical Education and Research. 2021;55(3):823–828.
7. Hayat Z, Chaudhry MA. Evaluation of a poly-herbal preparation for the treatment of peptic ulcer. Bangladesh Journal of Pharmacology. 2017;12(2):119–124.
8. Lakshmi Srinivas T, Mohana Lakshmi S, Neelufar Shama S, Koteswara Reddy G, Prasanna KR. Medicinal Plants as Anti-Ulcer Agents. Journal of Pharmacognosy and Phytochemistry. 2013;2(4):91–97.
9. Setia M, Kumar K, Teotia D. Gastro-retentive floating beads a new trend of drug delivery system. Journal of Drug Delivery and Therapeutics. 2018;8(3):169–180.
10. Goudanavar PS, Ramesh B, Fattepur SR. Gastro retentive dosage forms: An excellent carrier system for treatment of peptic ulcer. Research Journal of Pharmacy and Technology. 2021;14(4):2316–2320.
11. Bera H, Maiti S, Saha S, Nayak AK. Biopolymers-based gastroretentive buoyant systems for therapeutic management of Helicobacter pylori infection. Polysaccharide Carriers for Drug Delivery, Elsevier; 2019, p. 713–736.
12. Mostafavi A, Emami J, Varshosaz J, Davies NM, Rezazadeh M. Development of a prolonged-release gastroretentive tablet formulation of ciprofloxacin hydrochloride: Pharmacokinetic characterization in healthy human volunteers. International Journal of Pharmaceutics. 2011;409:128–136.
13. Sadhu PK, Baji AA, Shah NV., Seth AK, Dash DK, Aundhia CJ, et al. An approaches and patents on controlled release gastroretentive drug delivery system – a review. International Journal of Pharmaceutical Research. 2020;12(2):2047–2059.
14. Patel AK, Patel VM. A Review: Gastroretentive Drug Delivery Systems and its Rational in Peptic Ulcer Treatment. Journal of Pharmaceutical Science and Bioscientific Research. 2012;2(4):179–188.
15. Garg T, Kumar A, Rath G, Goyal AK. Gastroretentive drug delivery systems for therapeutic management of peptic ulcer. Critical Reviews in Therapeutic Drug Carrier Systems. 2014;31(6):531–557.
16. Anosike CA, Obidoa O, Ezeanyika LUS, Nwuba MM. Anti-inflammatory and anti-ulcerogenic activity of the ethanol extract of ginger (Zingiber officinale). African Journal of Biochemistry Research. 2009;3(12):379–384.
17. Nanjundaiah SM, Annaiah HNM, Dharmesh SM. Gastroprotective effect of ginger rhizome (Zingiber officinale) extract: Role of gallic acid and cinnamic acid in H+, K+-ATPase/ H. pylori inhibition and anti-oxidative mechanism. Evidence-based Complementary and Alternative Medicine. 2011;2011:1–13.
18. Ali AMA, El-Nour MEAM, Yagi SM. Total phenolic and flavonoid contents and antioxidant activity of ginger (Zingiber officinale Rosc.) rhizome, callus and callus treated with some elicitors. Journal of Genetic Engineering and Biotechnology. 2018;16(2):677–682.
19. Jalilzadeh-Amin G, Najarnezhad V, Anassori E, Mostafavi M, Keshipour H. Antiulcer Properties of Glycyrrhiza Glabra L. Extract on Experimental Models of Gastric Ulcer in Mice. Iranian Journal of Pharmaceutical Research. 2015;14(4):1163–1170.
20. Shoaib A, Tarique M, Khushtar M, Siddiqui HH. Antiulcerogenic activity of hydromethanolic extract of Andrographis paniculata in Indomethacin and Indomethacin plus pylorus ligation induced gastric ulcer in rats. Asian Journal of Biomedical and Pharmaceutical Sciences. 2014;4(39):8–15.
21. Kumar PA, Naik R, Karra N, Panara K. Experimental and Clinical Evidence of Andrographis paniculata (Roxb.) Wall. Ex Nees (Bhunimba)-A Review. International Journal of Pharmaceutical & Biological Archives. 2013;4(6):1086–1093.
22. Rajendrakumar T, Rao S, Satyanarayana ML, Narayanaswamy HD, Byregowda SM. Phytochemical and Invitro-Antioxidant Activities of Ethanolic Extract of Andrographis Paniculata. International Journal of Science, Environment and Technology. 2020;9(3):386–394.

23. Rao ChV, Ojha Amresh SK, Mehrota S, Pushpangadan P. Analgesic, Antiinflammatory and Antiulcerogenic Activity of the Unripe Fruits of Aegle marmelos. Acta Pharmaceutica Turcica. 2003;45:85–91.
24. Sivakumar G, Gopalasatheeskumar K, Gowtham K, Sindhu E, Akash RK, Rajaguru B, et al. Phytochemical analysis, Antioxidant and Antiarthritic activities of different solvent extract of Aegle marmelos L. unripe fruit. Research Journal of Pharmacy and Technology. 2020;13(6):2759–2763.
25. Zahin M, Aqil F, Ahmad I. The in vitro antioxidant activity and total phenolic content of four Indian medicinal plants. International Journal of Pharmacy and Pharmaceutical Sciences. 2009;1(Suppl 1, Nov.-Dec.):88–95.
26. Anjali V, Lavanya V, Kumari BR, Girish C. Evaluation of Phytochemical Parameters of Herbal Formulation of Ficus Benghalensis and Panax Ginseng. International Journal of Health Sciences & Research. 2018;8(1):77–84.
27. Bashir A, Hazarika I, Jaikumar S, Akila K, Shefeer K. Anti-Ulcer Activity of Polyherbal Formulation-RO12 on Experimentally Induced Ulcer in Rats. International Journal of Phytopharmacology. 2014;5(6):406–410.
28. Saadh MJ, Haddad M, Dababneh MF, Bayan MF, Al-Jaidi BA. A Guide for Estimating the Maximum Safe Starting Dose and Conversion it between Animals and Humans. Systematic Reviews in Pharmacy. 2020;11(8):98–101.
29. Zaghlool SS, Shehata BA, Abo-Seif AA, El-Latif HAA. Protective effects of ginger and marshmallow extracts on indomethacin-induced peptic ulcer in rats. Journal of Natural Science, Biology and Medicine. 2015;6(2):421–428.
30. Nawale S, Priyanka N, Das S, Ganga Raju M. Data of in vivo screening of antiulcer activity for methanolic extract of Vernonia elaeagnifolia DC. Data Brief. 2019;23:1–13.
31. Alam S, Mohiuddin M, Linga Swamy K, Baig A, Venkata Reddy M, Gupta RK, et al. Evaluation of Antiulcer and Antioxidant Activity of Polyherbal Formulation in Wistar Rats. International Journal of Pharmaceutical and Phytopharmacological Research. 2013;2(6):400–406.
32. Dashputre NL, Naikwade NS. Evaluation of Anti-Ulcer Activity of Methanolic Extract of Abutilon indicum Linn Leaves in Experimental Rats. International Journal of Pharmaceutical Sciences and Drug Research. 2011;3(2):97–100.
33. Ighodaro O, Adejuwon A. Anti-Peptic Ulcer Potential of a Polyherbal Mixture in Indomethacin-Induced Rat Model. Journal of Complementary Medicine & Alternative Healthcare. 2018;5(4):001–005.
34. Ahmed N, Ali Khan MS, Mat Jais AM, Mohtarrudin N, Ranjbar M, Amjad MS, et al. Anti-ulcer Activity of Sandalwood (Santalum album L.) Stem Hydroalcoholic Extract in Three Gastric-Ulceration Models of Wistar Rats. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas. 2013;12(1):81–91.
35. Sayed N, Barve V. Evaluation of Antiulcer Activity of Laghusoothshekhar (an Ayurvedic Formula) in Pyloric Ligature Induced Gastric Ulcers in Albino Rats. American Journal of Undergraduate Research. 2016;13(2):81–86.
36. Sattar A, Abdo A, Mushtaq MN, Anjum I, Anjum A. Evaluation of gastro-protective activity of Myristica fragrans on ethanol-induced ulcer in albino rats. Anais da Academia Brasileira de Ciencias. 2019;91(2):1–8.
37. Shirwaikar A, Ram A, Mohapatra P. Antioxidant and antiulcer activity of aqueous extract of a polyherbal formulation. Indian Journal of Experimental Biology. 2006;44:474–480.
38. Praveen Kumar AVS, Jonnalagadda VG, Joy JM, Lakshmi M, Prathyusha S, Lakshmi M. Pharmacognostical, Phytochemical and Pharmacological Evaluation for the Antiulcer Activity of Polyherbal Suspension. Journal of Pharmacognosy and Phytochemistry. 2013;2(3):124–131.
39. Demircan N, Safran BG, Soylu M, Ozcan AA, Sizmaz S. Determination of vitreous interleukin-1 (IL-1) and tumour necrosis factor (TNF) levels in proliferative diabetic retinopathy. Eye. 2006;20(12):1366–1369.
40. Muniandy K, Gothai S, Badran KMH, Kumar SS, Esa NM, Arulselvan P. Suppression of proinflammatory cytokines and mediators in LPS-Induced RAW 264.7 macrophages by stem extract of Alternanthera sessilis via the inhibition of the NF-κB pathway. Journal of Immunology Research. 2018;2018:1–12.
41. Vemula SK, Chawada MB, Thakur KS, Vahalia MK. Antiulcer activity of Amlapitta Mishran suspension in rats: A pilot study. Ancient Science of Life. 2012;32(2):112–115.
42. Lahiri S, Palit G. An Overview of the Current Methodologies used for Evaluation of Gastric and Duodenal Anti-ulcer Agents. Pharmacologia. 2012;3(8):249–257.
43. Balaji G, Ramesh B, Mahendranath G, Jagadeesh Kumar D, Venu Babu V. Protective Effects of A Poly Herbal Formulation Against Aspirin Induced Ulcers in Wistar Rats. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(5):190–194.
44. Nitin M., Girish M., Chetan M., Javed A, Krunal S., Kumar K. Comparative Influence of Selected Antioxidants with Lansoprazole in Aspirin Induced Ulcer Model in Rats. Research Journal of Pharmacy and Technology. 2011;4(8):1273–1277.
45. Alam F, Us Saqib QN. Pharmacognostic study and development of quality control parameters for fruit, bark and leaf of Zanthoxylum armatum (Rutaceae). Ancient Science of Life. 2015;34(3):147–155.
46. Basak M, Saha S. Phytochemical and Antiulcer potential of Ethanolic leaf extract of Heliotropium indicum Linn. Research Journal of Pharmacy and Technology. 2021;14(4):1938–1944.
47. Jain P. Secondary metabolites for antiulcer activity. Natural Product Research. 2016;30(6):640–656.
48. Ali A, Pillai HH, Mathew P, Beena P, Das C, Abraham E. Formulation and Evaluation of Floating mucoadhesive Microspheres loaded with Antiulcer drug. Research Journal of Pharmacy and Technology. 2020;13(8):3759–3764.
49. Balata G. Design and Evaluation of Gastroretentive Floating Tablet of Nizatidine: A Trial to Improve its Efficacy. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(5):423–429.