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Development and Evaluation of a Gastroretentive Polyherbal Capsule for the Management of Aspirin-Induced Gastric Ulcers in Experimental Rats

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Abstract:

Peptic ulcer disease is one of the most common gastrointestinal disorders caused by an imbalance between aggressive factors and gastric mucosal defense mechanisms. The present study aimed to develop a gastroretentive polyherbal capsule (GPHC20) containing extracts of Glycyrrhiza glabra, Zingiber zerumbet, Andrographis echinoides, Holarrhena mitis, and Aegle marmelos and to evaluate its gastroprotective efficacy in aspirin-induced gastric ulcer rats. Carbopol 971P was employed as the gastroretentive polymer to prolong gastric residence time and improve therapeutic efficacy. Acute oral toxicity was performed according to OECD guideline 423. Gastroprotective activity was evaluated using aspirin-induced gastric ulceration by estimating gastric pH, gastric volume, total acidity, free acidity, ulcer index, histopathology, and inflammatory cytokines (TNF-α, IL-1β, and IL-10). The optimized formulation (GPHC20) demonstrated excellent safety up to 2000 mg/kg and produced significant ($p < 0.05$) gastroprotection by increasing gastric pH while reducing gastric volume, acidity, ulcer index, and mucosal damage. Histopathological examination confirmed marked protection of gastric mucosa. ELISA analysis further demonstrated significant suppression of TNF-α and IL-1β with elevation of IL-10, indicating potent anti-inflammatory activity. The gastroretentive formulation exhibited superior antiulcer efficacy compared with the marketed polyherbal formulation and produced gastroprotective effects comparable to omeprazole. The findings suggest that gastroretentive delivery substantially improves the therapeutic performance of polyherbal preparations in gastric ulcer management.

Keywords: Gastroretentive capsule, Polyherbal formulation, Aspirin-induced ulcer, Carbopol 971P, ELISA, Gastroprotection.

Introduction

Peptic ulcer disease (PUD) remains one of the most prevalent gastrointestinal disorders worldwide and is characterized by erosion of the gastric or duodenal mucosa resulting from an imbalance between aggressive factors such as gastric acid, pepsin, reactive oxygen species, Helicobacter pylori infection, and non-steroidal anti-inflammatory drugs (NSAIDs), and the protective mechanisms of the gastric mucosa (Malfertheiner et al., 2022). Among these factors, prolonged

NSAID therapy is recognized as one of the leading causes of gastric ulceration because of its inhibitory effect on cyclooxygenase-mediated prostaglandin synthesis, leading to impaired mucosal defense.

Although proton pump inhibitors and H2-receptor antagonists effectively suppress gastric acid secretion, their long-term administration is frequently associated with adverse effects and recurrence after discontinuation. Consequently, medicinal plants have gained increasing attention owing to their antioxidant, anti-inflammatory, cytoprotective, and mucosal healing properties (Ekor, 2014).

The selected medicinal plants, namely Glycyrrhiza glabra, Zingiber zerumbet, Andrographis echinoides, Holarrhena mitis, and Aegle marmelos, are rich sources of flavonoids, tannins, terpenoids, alkaloids, and phenolic compounds with documented antioxidant and antiulcer activities. However, conventional herbal formulations often exhibit limited gastric residence time, reducing their therapeutic effectiveness.

Gastroretentive drug delivery systems prolong gastric residence, allowing sustained release of active constituents directly at the site of action. Carbopol 971P possesses excellent swelling and floating characteristics that facilitate prolonged gastric retention while maintaining structural integrity.

Therefore, the present investigation aimed to develop a gastroretentive polyherbal capsule using Carbopol 971P and evaluate its antiulcer activity in aspirin-induced gastric ulcers. The study also investigated inflammatory cytokines TNF-α, IL-1β, and IL-10 to understand the anti-inflammatory mechanism responsible for gastroprotection.

Materials and Methods

Plant Material

Roots of Glycyrrhiza glabra, rhizomes of Zingiber zerumbet, whole plant of Andrographis echinoides, bark of Holarrhena mitis, and unripe fruits of Aegle marmelos were collected from the Konkan region of Maharashtra, India. Plant materials were authenticated before use.

Preparation of Extracts

The dried plant materials were powdered separately and extracted using ethanol by Soxhlet extraction. The concentrated extracts were dried under reduced pressure and preserved in airtight containers until formulation.

Formulation of Gastroretentive Polyherbal Capsules

Four formulations containing Carbopol 971P (45–75 mg/capsule) were prepared by wet granulation. Polyherbal extracts were blended uniformly with Carbopol 971P, lactose, PVP K30, magnesium stearate, and talc. The wet mass was passed through sieve #18, dried at 60°C, resieved through #24, lubricated, and filled into size "00" cellulose capsules. Among the prepared formulations, GPHC20 containing 75 mg Carbopol 971P exhibited optimum pharmaceutical characteristics and was selected for biological evaluation.

Experimental Animals

Female Swiss albino mice were used for acute toxicity studies, whereas Wistar rats (180–200 g) of either sex were employed for gastroprotective evaluation. Animals were maintained under standard laboratory conditions with free access to pellet diet and water. Experimental protocols were approved by the Institutional Animal Ethics Committee (Approval No. IIP/IAEC/11/2019-20).

Acute Oral Toxicity

Acute toxicity was performed according to OECD Guideline 423. Animals received escalating oral doses of the optimized formulation up to 2000 mg/kg and were observed continuously during the first 24 h

and daily for 14 days for mortality and behavioral abnormalities.

Gastroprotective Activity

Gastroprotective activity was evaluated using the aspirin-induced gastric ulcer model. Rats were divided into six groups:

- Group I: Normal control
- Group II: Aspirin control (200 mg/kg)
- Group III: GPHC20 (100 mg/kg)
- Group IV: GPHC20 (200 mg/kg)
- Group V: AV Gastro (250 mg/kg)
- Group VI: Omeprazole (20 mg/kg)

Animals received respective treatments orally for 14 consecutive days followed by ulcer induction using aspirin. Four hours after aspirin administration, animals were sacrificed and gastric juice was collected.

Evaluation Parameters

The following parameters were evaluated:

Estimation of pH

The gastric juice pH was determined by making use of a calibrated pH meter (EQUIP- TRONICS) after diluting 1 mL of supernatant gastric content with 1 mL of distilled water.

Assessment of free acidity

One mL of distilled water was used to dilute the supernatant liquid (1 mL) and titrated with 0.01 N NaOH utilizing Topfer's reagent as an indicator. The achievement of a stable canary yellow tint indicated the attainment of the endpoint of the titration. The volume of NaOH used up in the titration was recorded.

Estimation of total acidity

The valuation of total acidity was performed utilizing the procedure for free acidity utilizing phenolphthalein as an indicator. The amount of NaOH consumed was recorded after the solution turned pink.

Calculation of acidity

The following formula was used to compute the total and free acidity of the stomach content in mEq/L:

Gross mucosal assessment

Along the greater curve, the individual stomachs of the animals were opened and cleaned with saline. These were then pinned to waxed Petri plates to reveal the glandular portion.[98] With the assistance of a magnifying lens (10X), the entire gastric mucosal layer was meticulously examined to count the number of ulcers and rate their severity.

Scoring of severity

The severity of lesions was examined censoriously and scored using the scale:

0 : Normal-colored mucosa,

0.5 : Red colored mucosa,

1: Spot ulcers 1.5 : 2: Hemorrhagic streaks: Deep ulcers, 3: Perforations

Computation of Ulcer Index (UI) and % Protection

Calculation of ulcer index was accomplished utilizing the formula:

$$\text{Ulcer Index} = \frac{\text{UAS} + \text{UAN} + \text{UP}}{10}$$

Where UAS = Average severity score UAN = Average number of ulcers

UP = Percent of rats with ulcers [100]

The degree of protection against ulceration by the various treatments was computed in comparison with the ulcer index of the Aspirin-treated group as follows:

$$\% \text{ Protection} = \frac{Control_{UI} - Treatment_{UI}}{Control_{UI}} \times 1$$

Where ControlUI = Ulcer index of the positive control group TreatmentUI = Ulcer index of treatment group [94]

Histopathology

Stomach tissues were fixed in 10% buffered formalin, processed routinely, sectioned, stained with hematoxylin and eosin, and examined microscopically for epithelial erosion, congestion, edema, and inflammatory cell infiltration.

ELISA

Serum TNF-α, IL-1β, and IL-10 concentrations were determined using rat-specific ELISA kits according to the manufacturer's instructions.

Statistical Analysis

Data were expressed as Mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. A value of $p < 0.05$ was considered statistically significant.

Results and Discussion

Acute Oral Toxicity

The acute oral toxicity study demonstrated that the optimized gastroretentive polyherbal capsule (GPHC20) was safe up to an oral dose of 2000 mg/kg. No mortality or treatment-related signs of toxicity, including behavioral abnormalities, tremors, salivation, diarrhea, convulsions, or changes in locomotor activity, were observed during the 14-day observation period. Based on OECD Guideline 423, doses of 100 and 200 mg/kg were selected for subsequent pharmacological studies. These findings indicate a wide safety margin and support the suitability of the formulation for long-term therapeutic application.

Gastroprotective Activity

Administration of aspirin (200 mg/kg) produced severe gastric ulceration characterized by a significant reduction in gastric pH together with marked increases in gastric juice volume, total acidity, free acidity, and ulcer index. Pretreatment with GPHC20 significantly ($p < 0.05$) protected the gastric mucosa against aspirin-induced injury.

The optimized formulation produced a dose-dependent increase in gastric pH. At 200 mg/kg, GPHC20 increased gastric pH to 6.49 ± 0.07, which was higher than the marketed formulation (4.52 ± 0.06) and comparable to omeprazole (5.90 ± 0.03). Simultaneously, gastric juice volume was markedly reduced from 5.63 ± 0.07 mL in the aspirin-treated group to 1.83 ± 0.04 mL, indicating significant inhibition of gastric secretion.

Similarly, free acidity and total acidity were reduced to 15.67 ± 0.33 mEq/L and 24.00 ± 0.37 mEq/L, respectively, demonstrating potent antisecretory activity. The superior reduction in acidity observed with GPHC20 compared with the marketed formulation indicates improved therapeutic performance, probably resulting from prolonged gastric residence produced by Carbopol 971P.

Table 3.1 Relative representation of evaluation parameters in Aspirin-induced ulcer model in rats

Groupings	pH	Volume of gastric content (mL)	Free acidity (mEq/L)	Total acidity (mEq/L)
Negative 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
GPHC20 100 mg/Kg	5.17±0.023*	2.75±0.035*	25±0.365*	42.17±0.307*

GPHC20 200 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*

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

Ulcer Index and Percentage Protection

Aspirin administration produced severe gastric lesions with an ulcer index of 10.58. Treatment with GPHC20 significantly decreased ulcer formation in a dose-dependent manner. At 100 mg/kg, the ulcer index was reduced to 5.11, while administration of 200 mg/kg further reduced the ulcer index to 1.70, corresponding to 83.94% gastroprotection. The marketed formulation exhibited only 52.05% protection, whereas omeprazole produced 84.02% protection. Thus, the gastroprotective efficacy of GPHC20 at 200 mg/kg was almost identical to that of the standard antiulcer drug.

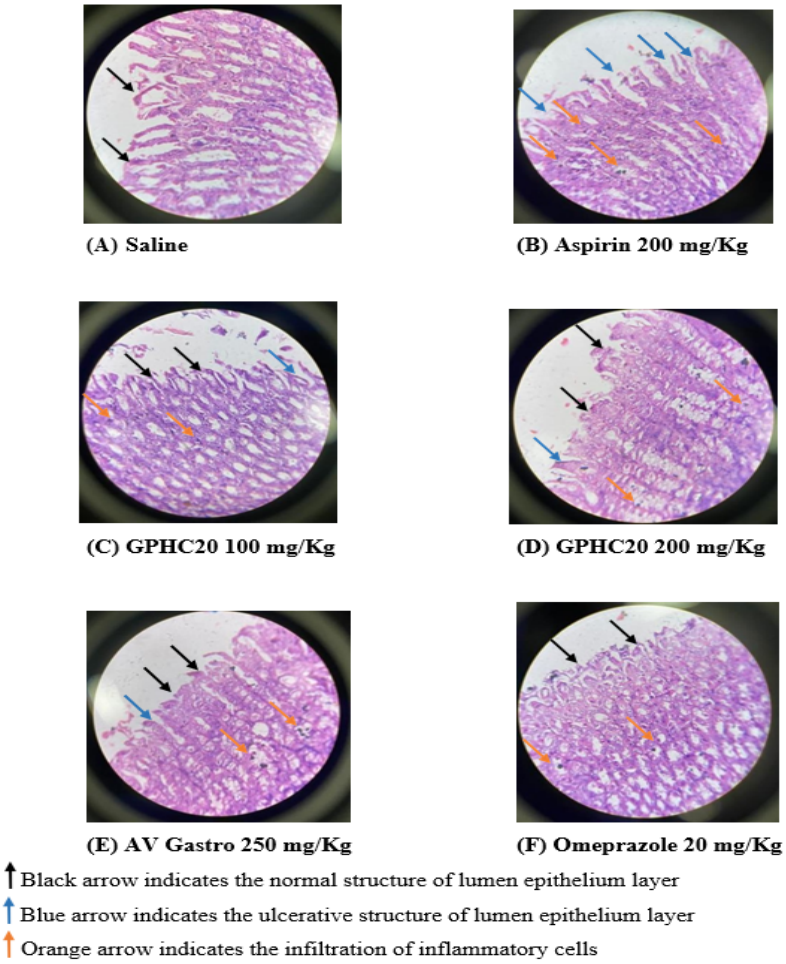
The improved gastroprotective effect may be attributed to prolonged gastric retention, sustained release of phytoconstituents, antioxidant activity, increased mucus production, and inhibition of gastric acid secretion.

Table 3.2 Relative representation of % protection and Ulcer index in Aspirin- induced ulcer model in rats

Groupings	Mean of number of ulcers	Mean of severity score	Percent of animals with ulcers	Ulcer index	% Protection
Positive control Aspirin 200 mg/Kg	3.33±0.211	2.5±0.224	100	10.58	-
GPHC20 100 mg/Kg	0.67±0.333*	0.42±0.201*	50	5.11	51.73
GPHC20 200 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

Ulcer index is expressed as (UAS + UAN + UP) ÷10 and percent 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 related with the untreated control group denoted as *

Histopathological Evaluation



Histopathological observations strongly supported the biochemical findings. Normal control animals exhibited intact gastric mucosa with well-preserved epithelial architecture and no evidence of inflammatory cell infiltration.

The aspirin-treated control group demonstrated severe erosion of the mucosal epithelium, extensive edema, vascular congestion, and marked inflammatory infiltration. Animals treated with GPHC20 at 100 mg/kg showed moderate improvement with partial restoration of gastric architecture.

In contrast, rats treated with GPHC20 at 200 mg/kg exhibited nearly normal gastric mucosa with minimal epithelial disruption, negligible edema, and very limited inflammatory infiltration. These histological findings were comparable with those observed in the omeprazole-treated group and confirmed the excellent gastroprotective potential of the developed formulation.

Effect on Inflammatory Cytokines

Inflammation plays an essential role in gastric ulcer pathogenesis. Therefore, inflammatory cytokines were quantified using ELISA.

Treatment with GPHC20 significantly reduced the levels of the pro-inflammatory cytokines TNF-α and IL-1β, while simultaneously increasing the anti-inflammatory cytokine IL-10. Suppression of TNF-α and IL-1β may reduce neutrophil infiltration, oxidative damage, and apoptosis within gastric tissue. Elevated IL-10 promotes mucosal healing by suppressing inflammatory signaling and enhancing tissue repair.

These findings indicate that the gastroprotective mechanism of GPHC20 involves both antioxidant and anti-

inflammatory pathways in addition to gastric acid suppression.

Discussion

The present investigation demonstrated that the gastroretentive polyherbal capsule significantly enhanced protection against aspirin-induced gastric ulcers. Carbopol 971P provided prolonged gastric retention, allowing sustained release of herbal bioactive constituents directly at the site of ulceration. The selected medicinal plants possess flavonoids, tannins, phenolics, triterpenoids, and glycyrrhizin, which are well known for their antioxidant, anti-inflammatory, cytoprotective, and mucus-enhancing properties.

Reduction in gastric acidity together with increased gastric pH indicates inhibition of aggressive factors responsible for mucosal injury. Simultaneously, marked reduction in ulcer index and restoration of normal gastric histology demonstrate effective protection of gastric tissue.

The superior therapeutic efficacy of GPHC20 compared with the marketed formulation confirms the importance of gastroretentive delivery in improving herbal drug performance. The ELISA findings further suggest that suppression of inflammatory cytokines contributes substantially to mucosal protection.

Overall, the developed gastroretentive formulation exhibited efficacy comparable to omeprazole while utilizing naturally derived herbal ingredients, indicating its potential as a safe and effective alternative for long-term management of gastric ulcers.

Conclusion

The present study successfully developed a gastroretentive polyherbal capsule (GPHC20) using Carbopol 971P for prolonged gastric residence and sustained drug release. The optimized formulation was found to be safe up to 2000 mg/kg and demonstrated significant gastroprotective activity in aspirin-induced gastric ulcer rats. GPHC20 effectively increased gastric pH while reducing gastric secretion, acidity, ulcer index, and gastric mucosal damage. Histopathological examination confirmed preservation of gastric architecture, whereas ELISA studies demonstrated suppression of TNF-α and IL-1β with elevation of IL-10, indicating a strong anti-inflammatory mechanism. The gastroretentive formulation exhibited superior efficacy compared with the marketed polyherbal formulation and produced antiulcer activity comparable to omeprazole. These findings suggest that gastroretentive delivery significantly enhances the therapeutic effectiveness of polyherbal formulations and represents a promising strategy for the management of gastric ulcer disease.

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