

Antiulcer Activity of Developed Herbal Floating Microspheres of *Mangifera indica* using Human Gastric Adenocarcinoma (AGS) Cell Lines

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ABSTRACT

Background: *Helicobacter pylori* infection is a major etiological factor in the development of gastric ulcers and other gastric disorders. The need for safe and effective gastroretentive delivery systems has led to increasing interest in herbal formulations; however, their gastric safety must be thoroughly evaluated before further development. **Objective:** The present study aimed to evaluate the in vitro cytotoxicity and gastric epithelial safety of anti-*Helicobacter pylori* herbal microspheres using the human gastric adenocarcinoma (AGS) cell line. **Methods:** AGS cells were cultured under standard conditions and treated with different concentrations of the herbal microsphere formulation. Cell viability was assessed using the MTT assay, and the percentage of viable cells was calculated relative to untreated controls. Morphological evaluation of treated and control cells was performed using an inverted light microscope to observe changes in cellular integrity, attachment, and overall morphology. **Results:** The MTT assay demonstrated high cell viability across all tested concentrations, indicating minimal cytotoxic effects of the formulation. No significant reduction in metabolic activity was observed when compared with control cells. Morphological examination further confirmed the preservation of normal epithelial characteristics, with treated cells exhibiting intact cell structure, proper attachment, and uniform distribution. The IC₅₀ value was found to be significantly higher than the effective concentration range, supporting the biocompatible nature of the microspheres. **Conclusion:** The anti-*Helicobacter pylori* herbal microspheres exhibited excellent compatibility with AGS gastric epithelial cells, confirming their in vitro gastric safety. These findings suggest that the formulation holds strong potential for further development as a gastroretentive therapeutic system for gastric ulcer management.

Keywords: *Helicobacter pylori*; Herbal microspheres; AGS cell line; In vitro cytotoxicity; Gastric safety; Gastroretentive drug delivery; Gastric ulcer

How to cite this article: Yadav R, Shukla Y, Chaturvedi S, Arun, Singh A, Ananya. Antiulcer Activity of Developed Herbal Floating Microspheres of *Mangifera indica* using Human Gastric Adenocarcinoma (AGS) Cell Lines. Int J Drug Deliv Technol. 2026;16(6s): 676-681; DOI: 10.25258/ijddt.16.6s.93

1. INTRODUCTION

A Peptic ulcer is a disorder in which the mucosal lining of the stomach or the duodenum (first part of the small intestine) breaks down, forming a painful inflammatory sore or cavity. The term “peptic” refers to Pepsin, a digestive enzyme in the stomach that breaks down proteins. When the protective lining of the stomach or duodenum is damaged, acid and pepsin can injure the tissue, leading to an ulcer (1). There is substantial epidemiological and experimental evidence about Peptic Ulcer

Disease (PUD) from studies conducted around the world. These studies help researchers understand the causes, risk factors, prevalence, and treatment of the disease in different populations (2). Worldwide, It is the most frequent clinical condition among gastro-intestinal illnesses, affecting 10 % of the world’s population and having an annual prevalence of 0.1–0.3 % according to study from 1990 to 2019. According to study from 1997 to 2002 in Wuhan area of China, the percentage of PUD is 22–47 % among the patient with

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gastrointestinal system. According to study from 2019 to 2022, in general Chinese population also, the prevalence of PUD was 22.6 % among the patients with gastrointestinal ailments [3]. The disease causes persistent pain, loss of weight, appetite, and working hours, as well as the occasional mortality. The condition can cause upper gastrointestinal bleeding and perforation, which are both fatal and morbid [4]. Research on creating efficient and safer anti-ulcer drugs from both synthetic and natural sources has increased significantly during the past many years. Although these therapies can well manage the condition of ulcer but relapses occur, and there is a risk of drug interactions during ulcer therapy, according to clinical evaluation reports on synthetic drugs. Proton pump inhibitors (PPIs) and H₂ blocker reduces the absorption of drug for which acidic medium is required such as ketoconazole, itraconazole, digoxin, and atazanavir, they also reduce the polymerization of sucralfate. All PPIs are metabolized by hepatic P450 cytochromes, including CYP2C19 and CYP3A4. FDA has issued the notice regarding significant drug interaction between clopidogrel and PPIs. PPIs reduce the activation of clopidogrel and its antiplatelet action [5–7]. Further long-term use of omeprazole can lead to osteoporosis due to decrease absorption of calcium. Antacids reduces the absorption of tetracyclines, iron salts, fluoroquinolones, ketoconazole, isoniazid, ethambutol and H₂ blocker [6].

Helicobacter pylori is a Gram-negative, microaerophilic bacterium that colonizes the gastric mucosa and is recognized as a major etiological factor in the development of chronic gastritis, peptic ulcer disease, and gastric carcinoma. Persistent infection with *H. pylori* induces epithelial damage, inflammation, and disruption of gastric mucosal integrity, thereby contributing significantly to ulcer formation and delayed healing. Despite the availability of conventional antibiotic-based therapies, increasing drug resistance, adverse effects, and poor patient compliance have highlighted the need for alternative and safer therapeutic strategies [8-9].

Herbal-based systems have gained considerable attention in the management of gastric disorders due to their multi-targeted mechanisms, favorable safety profile, and long history of traditional use. Several plant-derived bioactive compounds exhibit anti-*H. pylori* activity along with gastroprotective properties. However, while antimicrobial efficacy is essential, the safety and compatibility of such formulations with gastric epithelial cells remain equally critical. Any therapeutic system intended for gastric application

must demonstrate minimal cytotoxicity toward host cells to ensure safe and effective treatment [10-11].

In vitro cell line-based evaluation serves as a reliable and ethically acceptable approach for preliminary safety assessment of pharmaceutical and herbal formulations. The human gastric adenocarcinoma (AGS) cell line is widely used as a representative gastric epithelial model to study host-pathogen interactions, cytotoxicity, and cellular responses to therapeutic agents. Cytotoxicity assays, particularly the MTT assay, provide quantitative information on cell viability, while microscopic examination offers qualitative insights into morphological changes, cell attachment, and overall cellular integrity [12-13].

Therefore, the present study was designed to evaluate the antiulcer effects of herbal floating microsphere using the AGS gastric cell line. The findings of this study aim to establish the in vitro gastric cell compatibility of the formulation, thereby supporting its potential suitability for further development in the management of *H. pylori*-associated gastric disorders.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

The MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin solution, trypsin-EDTA, dimethyl sulfoxide (DMSO), and phosphate-buffered saline (PBS) were procured from standard commercial suppliers. All chemicals and reagents used were of analytical grade. The anti-*Helicobacter pylori* herbal microsphere formulation used in the present study was previously developed and characterized [14-15].

2.2 AGS Cell Line Culture

The human gastric adenocarcinoma (AGS) cell line was used as an in vitro gastric epithelial model. Cells were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum and 1% penicillin-streptomycin solution. The cells were maintained under standard culture conditions at 37 °C in a humidified atmosphere containing 5% CO₂. The culture medium was replaced every 2–3 days, and cells were sub-cultured upon reaching approximately 80–90% confluency using trypsin-EDTA [16].

2.3 Sample Preparation and Treatment

The herbal floating microsphere formulation was dispersed in culture medium to prepare test samples of different concentrations. AGS cells were seeded in 96-well culture plates at an appropriate density and allowed to attach overnight. After cell attachment, the culture medium was replaced with fresh

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medium containing various concentrations of the test formulation. Untreated cells served as the control group. The cells were incubated with the formulation for a predetermined exposure period under standard culture conditions.

2.4 Cell Viability Assay (MTT Assay)

The cytotoxicity of the different concentration of herbal floating microspheres of *Mangifera indica* was studied on AGS (Procured from NCCS Pune) cell line using MTT Assay. The cells (10000 cells/well) were cultured in 96 well plate and incubated (Air-Jacketed CO₂ incubator, Heal Force- HF90) for 24 h in Ham's F12K medium supplemented with 10% FBS (Fetal Bovine Serum - HIMEDIA-RM 10432) and 1% antibiotic solution (Penicillin-Streptomycin-Sigma-Aldrich P0781) at 37°C with 5% CO₂. Next day cells were treated from different concentrations of the samples (concentration as per mentioned in excel sheet). Stock solution of sample was prepared in Ethanol and further diluted to get different concentrations in incomplete Cell culture Medium (Without FBS). Cells without treatment were considered as Control and cells without MTT were considered as Blank. After incubation for 24 hours, MTT Solution (5 mg/ml) was added to cell culture and further incubated (Air- jacketed CO₂ incubator-Heal force-HF90) for 2 h. At the end of the experiment, culture supernatant was removed, and cell layer matrix was dissolved in 100 µl Dimethyl Sulfoxide (DMSO-SRL- Cat no.- 67685) and read in an Elisa plate reader (iMark, Biorad, USA) at 540 nm. IC₅₀ was calculated by using software Graph Pad Prism 6. Images were captured under inverted microscope (Olympus ek2) using Camera (AmScope digital camera 10 MP Aptima CMOS). 50% inhibitory concentration (IC₅₀) was presented as Mean ± SEM (Standard Error of Mean).

The percentage cell viability was calculated using the following equation:

Calculation:

$$\% \text{ Viable cells} = (A_{\text{test}} / A_{\text{Control}}) * 100$$

(A_{test} = Absorbance of test sample)
(A_{Control} = Absorbance of Control)

2.5 Morphological Evaluation Using Inverted Light Microscope

Morphological changes in AGS cells following treatment with the herbal microsphere formulation were examined using an inverted light microscope (Olympus ek2) using Camera (AmScope digital camera 10 MP Aptima CMOS). Control and treated cells were observed for alterations in cell shape, attachment, density, and overall cellular integrity. Microscopic images were captured to compare the morphology of treated cells with control cells.

2.6 Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using appropriate software. Differences between control and treated groups were considered statistically significant at $p < 0.05$.

3. RESULTS

3.1 Effect of Herbal Floating Microspheres of *Mangifera indica* on AGS Cell Viability

The cytotoxicity of the herbal floating microspheres of *Mangifera indica* was evaluated on the human gastric adenocarcinoma (AGS) cell line using the MTT assay. AGS cells were treated with different concentrations of the formulation, and cell viability was assessed after the treatment period.

Based on the results obtained from the MTT assay, it was observed that when the AGS cell line was exposed to different concentrations (0, 1, 10, 50, 100, 250, 500 and 1000 µg/ml) of the herbal floating microspheres of *Mangifera indica*, cytotoxic activity was estimated for the sample and 50% inhibitory concentration as mentioned in table 1. The herbal floating microspheres of *Mangifera indica* was found to be less cytotoxic. IC₅₀ is the concentration of herbal floating microspheres of *Mangifera indica* at which the viable cells are reduced by half.

Sample code	IC ₅₀ value (µg/ml) Mean ± SEM
Formulation	799.7±0.06

As shown in figure 1 a concentration-dependent response; however, a high percentage of cell viability was observed across the tested concentration range. At lower and intermediate concentrations, the formulation did not produce any significant reduction in cell viability when compared with the untreated control group. Even at higher concentrations, AGS cells maintained substantial viability, indicating minimal cytotoxic effects of the formulation.

The percentage cell viability remained above the acceptable cytotoxicity threshold, confirming the biocompatibility of the herbal microspheres with gastric epithelial cells. The IC₅₀ value of the formulation was found to be considerably higher than the effective concentration range, further indicating that the formulation is non-toxic to AGS cells under the tested conditions. These findings suggest that the herbal microspheres of *Mangifera indica* exhibit excellent in vitro gastric cell

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safety.

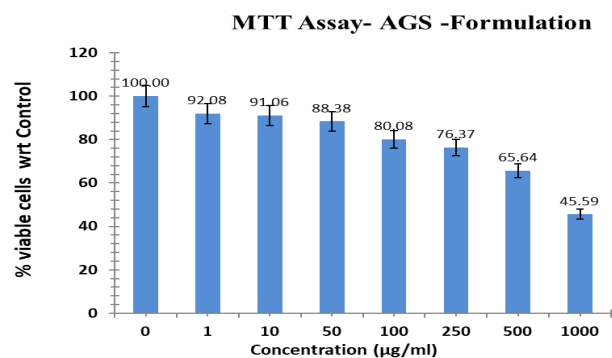


Figure 1: Effect of different concentration of Herbal Floating Microspheres of *Mangifera indica* on AGS cells

3.2 Morphological Evaluation of AGS Cells

Morphological examination of AGS cells was carried out using an inverted light microscope to support the cell viability findings. As shown in **figure 2**, the different images are given a, b, c, d, e, f, g and h. The images are shown as different concentration of (a-1 µg/ml, b-10 µg/ml, c-50 µg/ml, d-100 µg/ml, e-250 µg/ml, f-500 µg/ml and g-1000µg/ml and h-control) herbal floating microspheres of *Mangifera indica* was given. Untreated control cells exhibited normal epithelial morphology with well-defined cell boundaries, firm attachment to the culture surface, and uniform cell distribution.

As shown in **figure 2** different images of AGS cells treated with different concentration of the herbal floating microsphere of *Mangifera indica* showed no noticeable morphological alterations when compared with the control group. The different concentration of herbal floating microspheres treated cells retained their normal shape, adhesion, and cellular integrity, with no evidence of cell shrinkage, membrane disruption, or detachment. Cell density in the treated groups was comparable to that of the control cells, further supporting the absence of cytotoxic effects.

The microscopic observations indicate the MTT assay results and confirmed that the formulation did not induce structural damage or morphological abnormalities in AGS gastric cells.

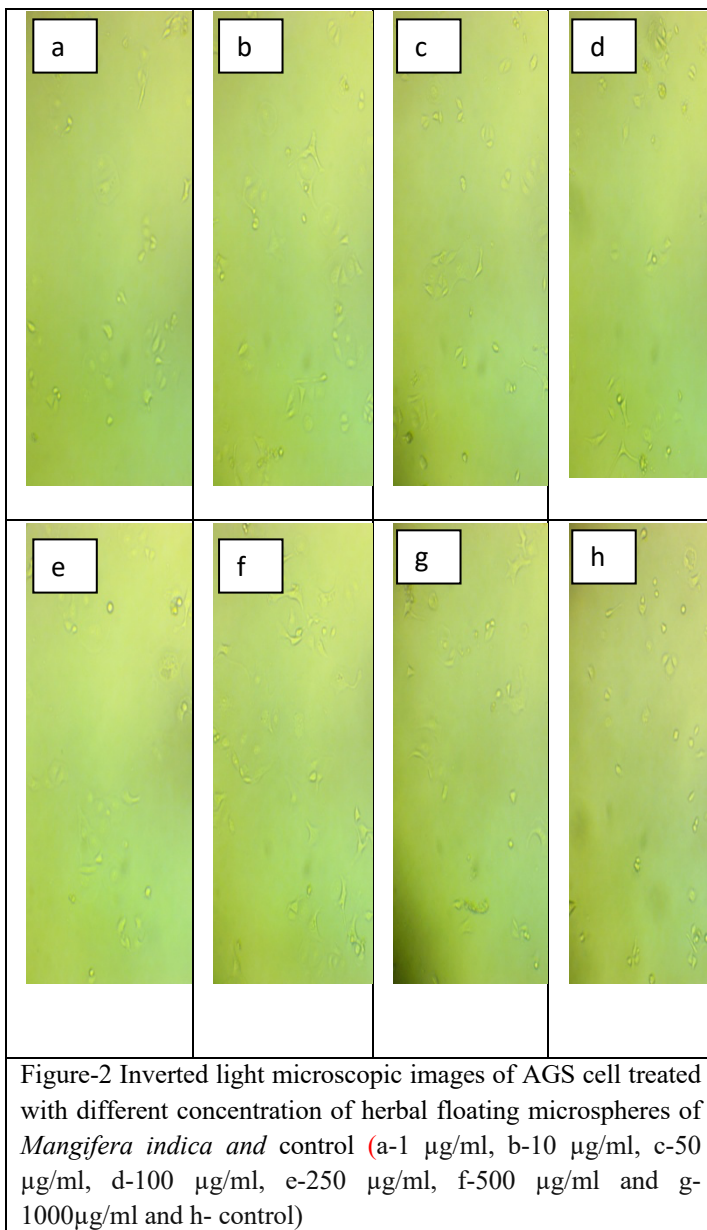


Figure-2 Inverted light microscopic images of AGS cell treated with different concentration of herbal floating microspheres of *Mangifera indica* and control (a-1 µg/ml, b-10 µg/ml, c-50 µg/ml, d-100 µg/ml, e-250 µg/ml, f-500 µg/ml and g-1000µg/ml and h- control)

4. DISCUSSION

The present study was designed to evaluate the cytotoxicity and gastric safety of anti-*Helicobacter pylori* herbal floating microspheres of *Mangifera indica* using the human gastric adenocarcinoma (AGS) cell line. Assessment of cell viability and morphological characteristics is a critical prerequisite for the development of gastroretentive systems intended for prolonged contact with the gastric mucosa. The findings of this study provide important insights into the biocompatibility and selective activity of the developed formulation.

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4.1 Significance of High Cell Viability

High cell viability observed in the MTT assay across all tested concentrations indicates that the herbal microsphere formulation is well tolerated by gastric epithelial cells. Maintenance of cellular metabolic activity suggests that the formulation does not interfere with mitochondrial function or essential cellular processes. This is a desirable characteristic for formulations intended for long-term gastric residence, as preservation of gastric epithelial integrity is essential to avoid irritation or tissue damage.

4.2 Low Cytotoxicity and Gastric Safety

As shown in **table 1**, the herbal floating microspheres of *Mangifera indica* was found to be less cytotoxic. IC_{50} is the concentration of herbal floating microspheres of *Mangifera indica* at which the viable cells are reduced by half. The IC_{50} value of herbal floating microsphere was found 799.7 ± 0.06 $\mu\text{g/ml}$, as evidenced by sustained AGS cell viability and normal cellular morphology, confirms its gastric safety. Since *H. pylori* infection requires prolonged therapeutic exposure within the stomach, any formulation designed for gastric retention must demonstrate minimal toxic effects on host cells. The absence of morphological abnormalities such as cell shrinkage, detachment, or membrane disruption further supports the non-toxic nature of the herbal microspheres and their suitability for gastric application.

4.3 Selective Activity Against *H. pylori*

An important observation of the present study is the selective biological behavior of the formulation. While the herbal microspheres exhibit antibacterial activity against *H. pylori* (as demonstrated in previous antibacterial evaluations), they show minimal cytotoxicity toward gastric epithelial cells. This selective action is highly advantageous, as it ensures targeted eradication of the pathogen without compromising host tissue viability.

The results demonstrate selective antibacterial activity with minimal cytotoxicity toward gastric epithelial cells.

4.4 Advantage of Microsphere System for Gastric Application

The microsphere-based delivery system offers several advantages for gastric applications, including enhanced gastric retention, controlled drug release, and prolonged local therapeutic action. Encapsulation of herbal extracts within microspheres may reduce direct exposure of gastric cells to high concentrations of active constituents, thereby minimizing cytotoxic effects while maintaining antibacterial efficacy. Additionally, the microsphere system supports sustained

release at the site of infection, improving therapeutic efficiency against *H. pylori*.

4.5 Comparison with Published AGS Cell Line Studies

Previous studies using the AGS cell line to evaluate gastroprotective and antimicrobial formulations have reported varying degrees of cytotoxicity depending on the nature of the active compounds and delivery systems. In contrast, the present formulation exhibited significantly higher cell viability, indicating superior biocompatibility. Similar studies have emphasized that formulations showing more than 80% cell viability are considered safe for gastric epithelial application, aligning well with the findings of the current study. These results place the developed herbal microspheres favorably in comparison with other reported gastric delivery systems.

5. CONCLUSION

The anti-*Helicobacter pylori* herbal microspheres exhibited excellent compatibility with AGS gastric epithelial cells, as evidenced by high cell viability and preserved cellular morphology. These findings confirm the in vitro gastric safety of the formulation and support its suitability for further development as a gastroretentive therapeutic system for the management of gastric ulcer and *H. pylori*-associated disorders.

6. ACKNOWLEDGEMENT

The authors gratefully acknowledge Aakaar Biotechnologies Private Limited for providing the cell line facility and technical support required to carry out the in vitro AGS cell line studies. The authors also express sincere gratitude to the Supervisor and the Faculty of Pharmaceutical Sciences, Baba Mastnath University, Rohtak, for their continuous guidance, encouragement, and institutional support throughout the course of this research work.

7. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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