Original Article

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Floating Microballoons of *Rhynchosia densiflora* Leaf Extract for the Treatment of Peptic Ulcer

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Abstract

Peptic ulcer is one of the most common gastrointestinal problems diagnosed all over the world. The aim of the present study is to develop floating microballoons of *Rhynchosia densiflora* (Roth) DC leaf extract and to evaluate its peptic ulcer activity. An ethanolic extract of *R. densiflora* (Roth) DC leaves was prepared by Soxhlet extraction and subjected to phytochemical analysis. Microballoons loaded with ethanolic extract of *R. densiflora* leaf were developed by solvent evaporation method and characterized by optical microscopy, floating time, and release characteristics. Acute oral toxicity study of microballons was carried out following OECD guidelines 423 and antiulcer activity was performed by pylorus ligation, indomethacin and cystamine induced duodenal ulcer methods. *R. densiflora* extract was found to contain glycosides, proteins, flavonoids, phenolic compounds, tannins and saponins. The particle size of microballoons of *R. densiflora* extract was found to be in the range of 300 µm. Acute toxicity studies of microballoons did not produce any toxic symptoms and mortality in animals, hence 100 and 200 mg/kg concentrations were selected to screen antiulcer activity. Both the doses showed significant gastric ulcer healing effect and gastric antisecretory effect in pylorus ligated rats, gastric cytoprotective effect in indomethacin induced gastric ulcer and also produced a significant reduction in duodenal ulcers. Dose of 200 mg/kg showed highly significant antiulcer activity than 100 mg/kg. Thus, it is concluded that floating microballoons loaded with *R. densiflora* extract significantly reduced gastric acid secretion, increased healing of gastric ulcers and also prevent the duodenal ulcers in rats.

Keywords: Peptic Ulcer, Microspheres, *Rhynchosia densiflora* (Roth) DC, Pylorus Ligation, Gastric acid, Antiulcer agents

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1. Introduction

Peptic ulcer is a serious gastrointestinal disorder which affects more than 10% of total world population. Various reports illustrate that annual incidence of peptic ulcer is about 0.03 to 0.19% both in the case of hospitalized as well as diagnosed by physician. The main pathology of peptic ulcer is over secretion of aggressive factors such as pepsin, hydrochloric acid and decreased

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defensive factors like bicarbonates, mucus, and prostaglandins. Other factors such as over consumption of alcohol, smoking, H-Pylori bacteria, Non-Steroidal Anti-Inflammatory Drugs (NSAID) and stress may lead to peptic ulcer (1).

The maximum therapeutic activity cannot be reached through the oral route because of certain factors such as first pass metabolism, gastric emptying time, gastric retention, the half-life of the drug etc. In case of drugs with shorter half-life, controlled release formulations will help to increase the therapeutic activity by extending the release profile of the drug. By this way dose dumping can be eliminated, frequencies and fluctuations in dosing can be minimized (1). Gastroretentive drug delivery system (GRDDS) is a type of formulation in which retention time of the drug in the stomach can be increased. Floating drug delivery system is a type of GRDDS, in which the drug will float on the gastric juice for a longer period and while floating the drug will be released from the system into the stomach. After complete release, the rest of the system will be emptied through the stomach. Floating systems can be made into tablets, capsules, powders, hollow microspheres, and beads. There are two types of floating system, effervescent and non-effervescent floating systems. Effervescent system is also called as gas generated system in which the film is made of volatile liquid where as Non-effervescent system consists of microballoons, alginate beads, micro porous compartment system, hydro colloidal gel barrier system, etc (2).

Microballoon is a type of GRDDS in which the drug is delivered with the help of hollow microspheres. The core of microballoons is hollow which will make them suitable for buoyancy properties and the thin film is made with polymers or proteins. Microballoons are prepared by various techniques such as simple evaporation, hot melt encapsulation, emulsion solvent evaporation method, polymerization technique etc. The active ingredient will be incorporated in between the layers of the formulation and when it comes in contact with the gastric fluid, because of its hollow core float over gastric fluid. The polymers will become hydrated and control the drug release through the system. When the outer layer is dissolved the next layer will become hydrated and this will continue until the drug is completely used. For complete utilization of the formulation a minimum amount of gastric juice must be present in thestomach (3). Floating dosage forms are also regarded as a low-density system and absorption of the drug from the gastrointestinal tract is a complex procedure subjected to many variables. Hence, designing the controlled release systems to constrain the dosage form in the target area of the gastrointestinal tract becomes difficult (4).

Rhynchosia densiflora (Roth) DC is a small climber belongs to Fabaceae family, which is widely distributed in Africa and India and reported for antioxidant and anti-inflammatory properties (5). Considering the merits of pharmacokinetic and pharmacodynamic parameters, drugs can be incorporated into this controlled release system, to develop a floating dosage form. Furthermore, a prolonged drug release from this floating system can be accomplished by using enteric polymers. Hence, in this study, floating microballoons loaded with ethanolic extract of Rhynchosia densiflora leaves were prepared, characterized and evaluated for anti-ulcer activity.

2. Materials and methods

2.1. Collection and authentication of Rhynchosia densiflora leaves

The plant was collected from Tirumala hills, AndhraPradesh, identified and authenticated by Dr. K. Madhava Chetty, Botanist (Add accession Number)

2.2. Preparation of extract

The leaves of *Rhynchosia densiflora* were collected, washed and dried at room temperature. It was thenpowdered and passed through a 60# mesh sieve and stored in an airtight container. 100 g of the powder drug was defatted with petroleum ether for 72 hrs and then extracted with 70% ethanol in Soxhlet apparatus and filtered. The filtrate was concentrated using rotary flash evaporator at a temperature not exceeding 60 ^{o}C to dryness. The obtained extract was subjected to preliminary phytochemical analysis and physicochemical constants such as moisture content, total ash, acid insoluble ash, water soluble and alcohol soluble

extractive values were determined following standard procedure (6).

2.3. Ethanol induced-erosions

Animals were deprived of food for 24 h before the experiments but had free access to water. Then, Group 1 received 2. 5 ml/kg saline, as vehicle control, followed by 5 ml/kg ethanol after 1 h, and Group 2 received 20 mg/kg pantoprazole, as a standard control, followed by 5 ml/kg ethanol (% 50) after 1 h. Groups 3, 4, 5 respectively received 5, 10 and 20 mg/kg TFP followed by 5 ml/kg ethanol (% 50) after 1 h. All treatment were carried out by intragastric gavage. One hour after the experimental period, all animals were sacrificed by ether anesthesia.

2.4. Preparation of microballoons (4, 7)

Rhynchosia densiflora extract was loaded with the polymer HPMC and Eudragit S-100 in different ratios and dissolved in the mixture of dichloromethane and ethanol, added to poly vinyl alcohol. An approximately 400 ºC temperature was set to create a stable formulation. The organic phase was removed by evaporation technique to develop stable micro balloons of *R. densiflora* extract. Resulted microballoons were filtered, washed with distilled water and dried at 40 °C in hot air oven and characterized by particle size analysis, floating time, buoyancy percentage and In- vitro drug release analysis (8).

2.5. Animals

Albino Wistar rats weighing 150-200 g were procured from the departmental animal house. Ethics Committee approval was obtained for this study (XXII/MSRFPH/COL-08). The animals were kept on a controlled light-dark cycle under constant temperature (26 °C) and humidity (50%) with free access to food and water.

2.6. Acute oral toxicity test

The acute oral toxicity studies for floating microballoons loaded *R. densiflora* extract was performed as per OECD guideline 423 and a total of 6 Albino Wistar rats were used. The animals were administered with 2000 mg/Kg of formulation and observed for various clinical signs and mortality (9).

2.7. Antiulcer Activity

The efficacy of microballoons loaded *R. densiflora* extract in Pylorus ligation induced ulcer, Indomethacin induced ulcer; and Cystamine induced duodenal ulcer were studied following Azamthulla et al., with slight modifications (10).

2.8. Pyloric ligation method

Pylorus ligation model is used to study anti-secretary activity. Rats were randomly divided into 4 groups (n=6). Group 1 received distilled water, while group 2 was intra-duodenally administered with pantoprazole 40 mg/Kg (standard). Group 3 and 4 received 100 and 200 mg/Kg, p.o., of microballoons, respectively. Subsequently, the stomach was opened by midline incision using xiphoid process under anaesthetic condition. The pyloric portion was ligated without damaging the surrounding blood vessels. After 6 h of ligation, all animals were sacrificed and the abdomen was cut open to isolate ligated stomach. Soon after, the total volume of gastric fluid and various biochemical parameters viz., total acidity, free acidity, total proteins, and pepsin content in the stomach contents were analysed. Later, the upper curvature of the stomach was cut open to establish ulcer score and ulcer index. The ulcer index was calculated using the formula, Ulcer Index (UI) =10/X, where X=total mucosal area/total ulcerated area. A part of the stomach was stored in 10% formalin for histopathological studies.

2.9. Indomethacin induced peptic ulcer

The cytoprotective activity of microballoons loaded *R. densiflora* extract was evaluated using indomethacin induced erosion of epithelial cells, eventually leading to ulcers. The rats were randomly divided into 4 groups (n=6).Group 1 received distilled water, while group 2 received misoprostol 100 µg/Kg, p.o., (standard). Group 3 and 4 were administered with 100 and 200 mg/Kg b.w., of microballoons loaded Rhynchosia densiflora extract, p.o., Initially, indomethacin (5 mg/Kg., p.o) was administered for 5 days to develop ulcers in all the groups, and from 6th day, the animals were treated with standard and various doses

Table 1. Physicochemical Constants of Rhynchosia densiflora extract.

Sl No	Parameters	Value (%w/w)
1	Loss of drying	10.23
2	Total ash	6.31
3	Acid insoluble ash	1.05
4	Water soluble extractive	5.13
5	Alcohol soluble extractive	13.68

of microballoons loaded *R. densiflora* extract. After 21 days of administration, the animals were sacrificed and various parameters such as total acidity, free acidity, total proteins, pepsin content, ulcer index, and ulcer score were analyzed. Mucin content in the stomach was also analyzed by dipping an isolated stomach tissue in 10% Alacian blue solution.

2.10. Cysteamine induced duodenal ulcer

The study animals were randomly divided into four groups (n=6). Group 1 was administered with distilled water, while group 2 with standard drug, ranitidine 50 mg/Kg, p.o. Group 3 and 4 were administered with 100 and 200 mg/Kg b.w., of microballoons loaded *R. densiflora* extract p.o. Post 30 min administration of microballoons loaded *R. densiflora* extract and ranitidine, cysteamine HCl (400 mg/Kg, p.o.,) was administered in two divided doses at an interval of 4 h to develop duodenal ulcers. The animals were sacrificed after 24 h from the last dose of cysteamine HCl, abdomen was cut open to isolate stomach and duodenum. Gastric content was taken out to measure gastric volume while, duodenum was opened to analyze ulcer score and ulcer index.

2.11. Statistical Analysis

All results are represented as Mean±Standard error of the mean (SEM). The obtained values were compared using the One-way Analysis of Variance (ANOVA) followed by the Tukey Grammar test for multiple comparisons. The *P* values <0.05 were considered as statistically significant.

3. Results

3.1. Phytochemical analysis of Rhynchosia densiflora extract

Preliminary phytochemical analysis confirmed the presence of glycosides, phenolic compounds and tannins, proteins, and flavonoids.

3.2. Physicochemical analysis of Rhynchosia densiflora extract

The results of physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive and loss on drying are shown in table 1.

3.3. Preparation of floating microballoons loaded Rhynchosia densiflora (FMRD) extract

Floating microballoons loaded *R. densiflora* (FMRD) extract was successfully developed by solvent evaporation method using HPMC and Eutragit S-100 as polymers in a ratio of (1:1).

3.4. Characterization of floating microballoonsloaded Rhynchosia densiflora

3.4.1. Floating time of microballoons

The floating time of microballoons was performed and the formulation showed a floating time of 5 hours.

3.5. In-vitro buoyancy percentage

The buoyancy percentage of microballoons showed in between the range of 70-85%.The rise in buoyancy percentage can be attributed to the gel-forming polymer that induced swelling.

3.6. In-vitro drug release studies

USP rotating paddle apparatus (37±0.5 °C and 100 rpm) is used for the determination of drug release from the microballoons and the percentage drug release was shown in figure 1.

The drug release profile of microballoons loaded with *R. densiflora* showed cumulative drug release on the 300 minute is 106% and also observed that drug is releasing from the surface of microballoons in a controlled manner.

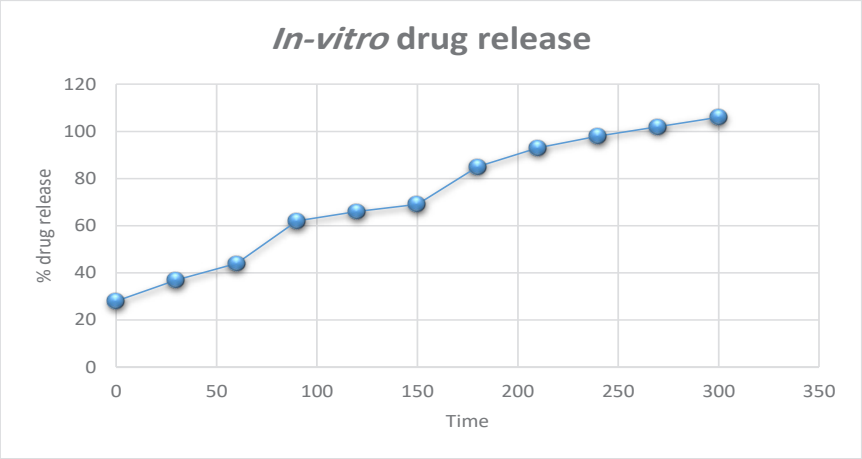

Figure 1. *In-vitro* drug release of microballoons.

3.7. Particle size determination of microballoons loaded Rhynchosia densiflora

Scanning electron microscopy studies showed the particle size are in a range of 200-300 microns and exhibited characteristic spherical shape revealing balloon formation, and displayed the presence of pores after exposure to simulated gastric fluid revealing their floating behavior (Figure 2).

3.8. Oral acute toxicity studies

The safety profile of *R. densiflora* extract was evaluated by administering 2000 mg/Kg orally to Wistar rats. The administered dose was well tolerated by the animals with no significant alteration on any of parameters observed. Considering safe, 1/20th (100 mg; low dose) and 1/10th (200 mg; high dose) of tolerated dose was employed for further studies.

3.9. Antiulcer activity models

3.9.1. Anti-ulcer activity: Pylorus ligation induced ulcers

In pylorus ligation model, gastric secretion was evoked in animals treated with FMRD extract (100 and 200 mg/Kg) resulting in significant reduction in total and free acidity. In addition, *R.*

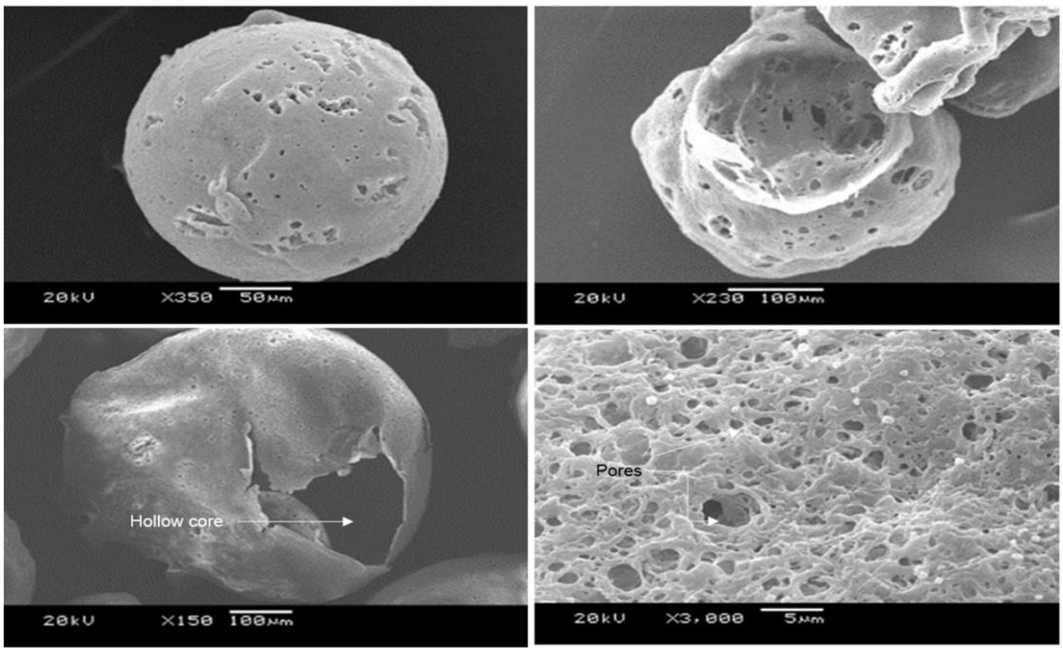

Figure 2. Scanning electron microscopy photomicrograph of microballoons loaded *R. densiflora* extract.

Table 2. Effect of FMRD extract on pylorus ligation induced gastric ulcer

Groups	Positive control	Standard	FMRD extract	
			100 mg	200 mg
Free acidity	21.31±3.15	7.3±617**	9.71±1.25***	6.17±0.764
Total acidity	16.8±2.63	5.27±0.85***	4.1±0.468***	3.25±0.632***
Pepsin content	0.06±0.136	0.03±0.246 ns	0.064±0.087 ns	0.047±0.002 ns
Total protein	0.09±0.004	0.34±0.016ns	0.12±0.0005 ns	0.24±0.1518 ns
Gastric volume	2.8±0.145	3.68±0.1875**	4.15±0.1196***	4.74±0.191***
Ulcer score	2.75±0.25	0.9±0.128***	1.25±0.25**	1±0.20***
Ulcer index	0.97±0.25	0.10±0.036**	0.29±0.13*	0.237±0.005*

All Values are expressed as Mean ± SEM (n=6), ***p<0.001, **p<0.01, *p<0.1, nsp>0.05 Vs control.

densiflora extract exhibited significant reduction in ulcer score and ulcer index (Figure 3), with mild changes in pepsin and total protein levels (Table 2).

3.9.2. Anti-ulcer activity: Indomethacin induced ulcers

Animals administrated only with indomethacin displayed gastric ulcer in glandular region of the stomach. Treatment with FMRD extract (100 and 200 mg/Kg) significantly reduced the ulcer index compared to positive control and also compared to standard. Additionally, FMRD extract at either dose also exhibited a significant effect on total acidity, free acidity and gastric volume compared to positive control. At the same time, an insignificant effect on pepsin, total protein and mucin contents were observed (Table 3 and Figure 4).

3.9.3. Anti-ulcer activity: Cysteamine induced duodenal ulcers

Administration of cysteamine HCl resulted in the development of ulcer on the anti-mesenteric side of the duodenum. *R. densiflora* extract treatment at both the doses (100 and 200 mg/Kg)

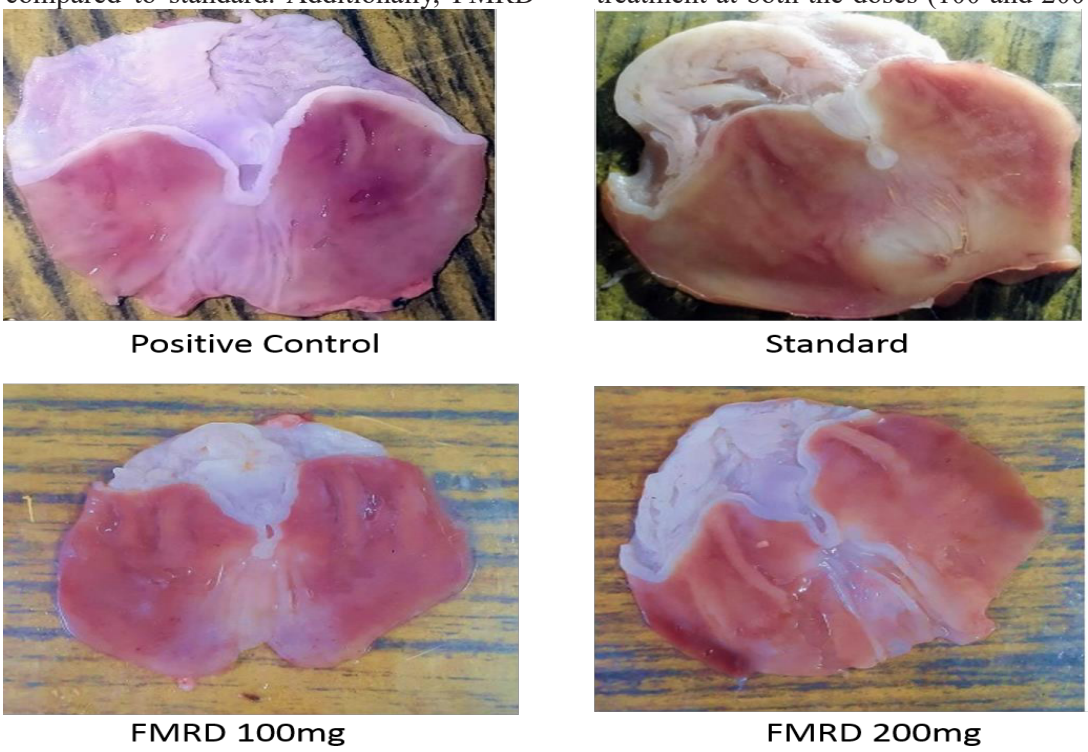

Figure 3. Effect of FMRD extract in pylorus ligation model.

Table 3. Effect of FMRD in Indomethacin induced gastric ulcers.

Groups	Positive control	Standard	FMRD extract	
			100 mg	200 mg
Free acidity	11.03±0.32	7.6±0.76**	7.73±1.68**	7.63±0.47**
Total acidity	5.76±0.83**	5.76±0.83**	6.18±1.14**	2.83±0.71***
Pepsin content	0.06±0.01	0.07±0.02 ns	0.62±0.10 ns	0.10±0.04 ns
Total protein	0.08±0.07	0.29±0.06 ns	0.11±0.008 ns	0.26±0.13 ns
Mucin content	0.16±0.005	0.14±0.98 ns	0.14±0.019 ns	0.15±0.002 ns
Gastric volume	2.8±0.14	3.68±0.18**	4.15±0.119***	4.74±0.1912***
Ulcer score	3±0.00	1±0.00**	1.25±0.2**	1.25±0.07**
Ulcer index	1.55±0.02	0.595±0.31 ns	0.44±0.14ns	0.72±0.04ns

All Values are expressed as Mean ± SEM (n=6), ***p<0.001, **p<0.01, *p<0.1, nsp>0.05 Vs control.

increase the gastric volume and was comparable to standard drug. Similarly, the ulcer score and ulcer index was also significantly reduced compared to positive control and comparable to standard (Table 4 and Figure 5).

3.10. Histopathological studies of stomach in pylorus ligation induced ulcers

Histology of positive control rats showed abnormal microscopic architecture and glands. The parietal cells located in the upper half of gastric glands with eccentric nuclei and pale eosinophilic vacuoles were irregularly arranged. Also, severe inflammatory reaction, manifested by submucosal oedema with local mononuclear leucocytic infiltration in the lamina propria, muscularis mucosa and submucosal layers were also noticed. The submucosal blood vessels were congested and the muscular coat was hypertrophied. A marked appearance of haemorrhage, inflammation and mucosal erosions resulting in the formation of gastric lesions were observed in gastric mucosa. Additionally, formation of gastric pits with the detachment of surface epithelium and loss of glandular

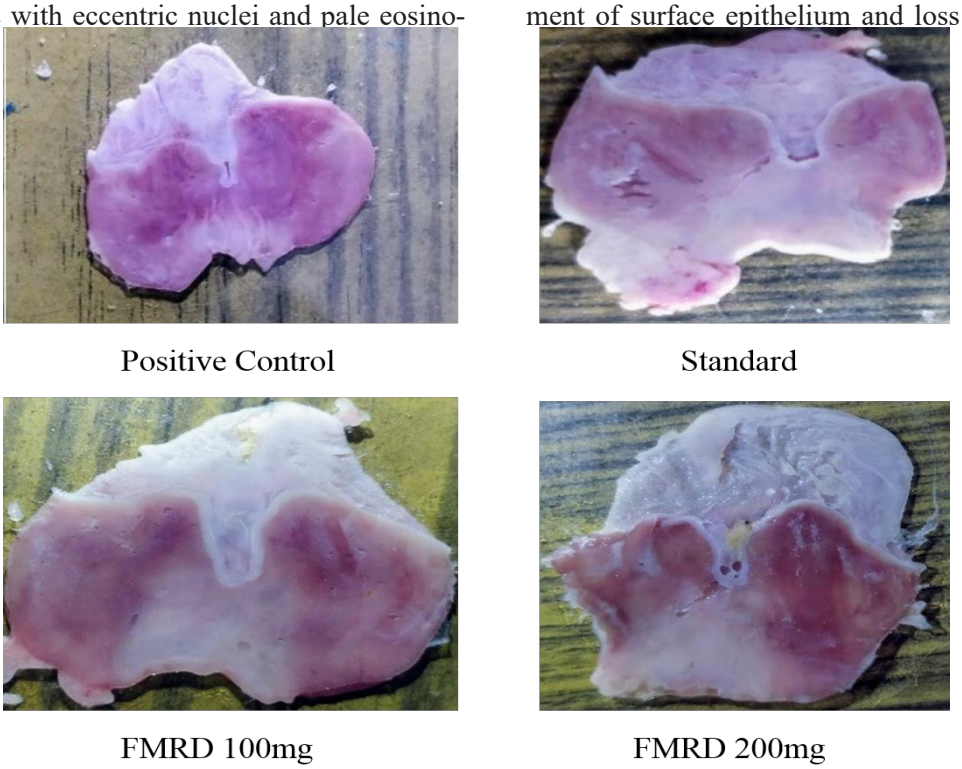

Figure 4. Effect of FMRD in indomethacin induced gastric ulcers.

Table 4. Effect FMRD extract on cysteamine induced duodenal ulcers

Groups	Positive group	Standard	FMRD extract	
			100 mg	200 mg
Gastric volume	2.8±0.145	3.68±0.187**	4.15±0.119***	4.74±0.191***
Ulcer score	2.5±0.287	0.75±0.250**	1.25±0.250*	0.25±0.250***
Ulcer index	0.916±0.08	0.431±0.21 ns	0.353±0.15 ns	0.042±0.013**

All Values are expressed as Mean±SEM (n=6), ***p<0.001, **p<0.01, *p<0.1, nsp>0.05Vs control.

Figure 5. Effect of FMRD in Cysteamine induced duodenal ulcers.

cells were also evident from stomach histopathology.

R. densiflora extract treated group displayed mild inflammatory reaction. The reaction severity was less compared with positive control animals. The standard group exhibited good tissue architecture with uniform epithelial, glandular tissue; but with mild leucocytic inflammatory cells. Pre-treatment with Rhynchosia densiflora extract considerably reduced these changes in the gastric mucosa (Figure 6).

4. Discussion

Ulcer develops due to imbalance between the defensive and aggressive factors (11) and also due to different factors such as chronic use of alcohol, smoking, long term use of NSAIDs, excessive stress and *H. pylori* bacterial infection. In general, 20-50% population is infected with *H. pylori* infection and becomes more severe with an increase in age while the duodenal ulcer is more frequent in 30-55-year-old individuals (12).

The ethanolic extract of *R. densiflora* leaves was prepared using Soxhlet apparatus and

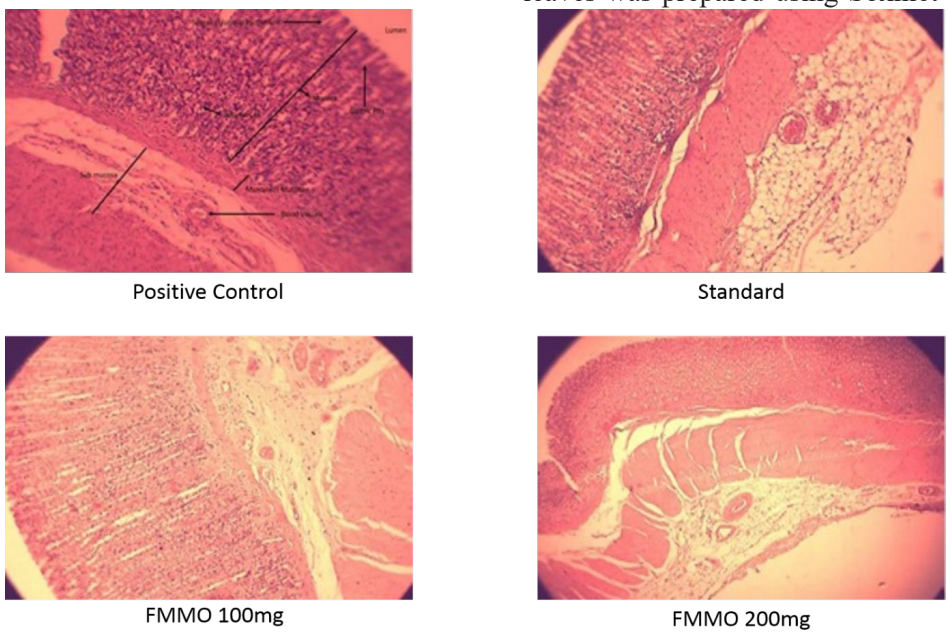

Figure 6. Histopathological studies of stomach in pylorus ligation method.

the percentage yield of the extract was found to be 5% w/w. Preliminary phytochemical analysis was performed and revealed the presence of glycosides, phenolic compounds and tannins, proteins, and flavonoids, physicochemical properties such as total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive and loss on drying was optimized with respect to standard values which was in correlation with Aluru *et al* (13).

Floating delivery system has been developed which extends the gastric residence time. In floating delivery system, the drug remains buoyant and floats on the gastric fluid with sustained release of drug (14). The advantages of floating delivery system over immediate release dosage forms are reduction in alteration of plasma-level concentration, prolonged duration of residence at the site of action, advanced therapeutic potency and minimized side effects, reduction in administration of total dose and administration frequency, improved bioavailability and solubility of poorly soluble drugs higher pH (15). In the present study, floating time of microballoons was 5h with a buoyancy percentage in range of 70-85%. Floating behavior of microballoons was revealed by the presence of pores that would have been formed due to the matrix destruction caused by solubilization of HPMC and Eutragit S-100 upon exposure to simulated gastric fluid.

Toxicology is any harmful effect of a chemical or drug on a target organism. Acute toxicity involves harmful effects in an organism through a single or short-term exposure. The purpose of toxicity testing is to provide adequate database to make decision concerning the toxicology properties of chemicals and commercial products and to decide whether a drug or chemical is safe or not (16). In acute toxicity study, Floating microballoons loaded *R. densiflora* extract did not show any mortality or toxic effect up to the dose of 2000 mg/Kg during the observational period of 24 h. There were no significant changes in behaviour, breathing, cutaneous effects, sensory nervous system responses and gastrointestinal effects. These results showed that in single dose, there are no adverse effects of Floating microballoons loaded *R. densiflora* extract indicating that the medium lethal dose (LD_{50}) is higher than 2000 mg/Kg in rats. Accordingly, 100 mg/Kg (low dose) and 200 mg/Kg (high dose) was selected for the present study.

The pylorus ligation induced ulcer was used to study the effect on gastric secretion. The ligation of pyloric end of the stomach causes accumulation of gastric acid in the stomach that produces ulcers. Agents that reduce secretion of gastric aggressive factors such as acid and pepsin and/or increase secretion mucin are effective in reducing development of gastric ulcers in this model (17). *R. densiflora* extract at 200 mg/Kg decreased gastric acid, pepsin secretion and increased gastric mucus secretion indicating its antisecretory and cytoprotective effects. *R. densiflora* extract at 100 mg/Kg was effective only in reducing gastric acid and pepsin secretion. Based on these results it can be concluded that Rhynchosia densiflora extract offered anti-secretory activity in pylorus ligation induced ulcers.

Indomethacin is known to produce erosions and ulcers in the gastrointestinal tract of experimental animals such as rats and guinea pigs. A layer of mucus that apparently forms a barrier covers the gastric mucosa. The gastric mucus production is stimulated by prostaglandins. Prostaglandin deficiency has been regarded to be primarily responsible for ulceration. The administration of indomethacin results in the production of gastric mucosal damage mainly in the glandular portion of the stomach. Indomethacin is a known prominent inhibitor of prostaglandin synthesis that in turn damages the mucosal barrier; the damage in the mucosal barrier causes the permeation of sodium ions from the mucosa in to the lumen (18). The agents having cytoprotective effect are efficient in preventing ulcers induced by indomethacin. *R. densiflora* extract, at both doses was effective in reducing ulcer index, in decreasing free acidity, total acidity and increasing in mucus content. A decrease in acid secretion, free acidity, total acidity, ulcer score, ulcer index and an increase in mucus content can be correlated to cytoprotective activity (11). *R. densiflora* extract exhibited similar results and hence can be attributed to its cytoprotective property.

Cysteamine induced ulcer in rats is a widely used method to evaluate effect on

duodenal ulcer. Cysteamine inhibits the alkaline mucus secretion from the Bruner glands in the proximal duodenum and stimulates gastric acid secretion. Gastric emptying is also delayed, and serum gastrin concentration is increased (19). *R. densiflora* extract 200 mg/Kg was more effective in preventing ulcer development than the low dose, 100 mg/kg revealing anti-duodenal ulcer activity.

Histology of positive control rats showed abnormal microscopic architecture and glands. Also, severe inflammatory reaction, manifested by submucosal oedema with local mononuclear leucocytic infiltration in the lamina propria, muscularis mucosa and submucosal layers were also noticed whereas *R. densiflora* extract treated group displayed mild inflammatory reaction. The reaction severity was less compared with positive control animals. The standard group exhibited good tissue architecture with uniform epithelial, glandular tissue; but with mild leucocytic inflammatory cells. Pre-treatment with *R. densiflora* extract considerably reduced these changes in the gastric mucosa (Figure 5).

5. Conclusion

R. densiflora extract exhibited a promising antiulcer effect on the various screened models. Anti-secretory effect was observed in pylorus ligation model thus decreasing HCl secretion, while cytoprotecitve nature was observed in indomethacin induced ulcer model. *R. densiflora* extract also displayed a promising effect on cysteamine induced duodenal ulcers. Anti-ulcer effect was prominent at 200 mg compared to the low dose, 100 mg. Further studies are to be undertaken to identify the bioactive responsible and propose the underlying anti-ulcer mechanism of Rhynchosia densiflora extract.

Ethics code (number) of project

Institutional Animal Ethical Committee (IAEC) Proposal Reference Number: XXII/MSRFPH/COL/M-19 and CPCSEA Registration Number: 220/PO/ReBi/S/2000.

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Conflict of Interest

The authors have no conflict of interest.

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