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Research Article

Development and Evaluation of Herbal Drug Formulation for Anti Ulcer Treatment

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Abstract

Aim of the study: In the present study, ulcer healing activity of ethanolic extracts of Symplocos Racemosa was evaluated in rats using indomethacin induced ulcer and ethanol induced ulcer model along with estimation of biochemical parameters.

Materials and Methods: The leaves of Symplocos Racemosa are reported to have anti-inflammatory, antioxidant, and antimicrobial properties. In the present study, ulcer healing activity of ethanolic extracts of Symplocos Racemosa was evaluated in rats using indomethacin induced ulcer and ethanol induced ulcer model along with estimation of biochemical parameters. In the indomethacin induced model, the score of ulcer was evaluated respectively. While in the methanol induced ulcer model, the efficacy of drugs against the Mean ulcerative index was evaluated. The treatment of ulcer with oral containing ethanolic extracts of Symplocos Racemosa and Trigonella foenum graecum exhibited significant ulcer healing.

Results: The results were comparable to standard drug omeprazole, in terms of ulcer index and ulcer scoreing. It has been observed that flavonoids like Quercetin seem to play a very important role in the prevention and treatment of peptic ulcer. It acts by promoting mucus secretion, thereby serves as gastroprotective agent.

Keywords: Ulcer healing, Symplocos Racemosa (SR), antioxidant, Indomethacin Ulcer

Introduction

Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, the last few years have seen a major increase in their use in the developed world. The human being exploited to alleviate his suffering from injuries of deceases utilizing plant growing around him

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The plant kingdom still hold many species of plant containing substance of medicinal value which have yet to be discovered and the large no. of plant are constantly being screened for their possible pharmacological value in addition to already exploited plants. As the results of modern isolation technique and pharmacological screening procedure, new plant drugs usually find their way into modern medicines. Now a days maximum world's population depends on herbal medicines.

Medicinal plants often contain additional active principles other than the major active principles and physiologically inert substances like cellulose and starch. Unlike the chemical entities, which contains one active ingredient pulps a number of inert substances, which makeup the dosage form (like tablet, capsules and syrups).

Material and Methods

Collection and authentification of plants:

Bark of **Symplocos racemosa** was purchased from Bhopal and authenticated from Botany Department Safia College, Bhopal. Barks were coarsely pulverized separately after sufficient shade drying. Materials were passed through 120 meshes to remove fine powders and coarse powder was used for extraction.

Leaves of **Psidium guajava** were also collected from Bhopal and authenticated from Botany Department Safia College, Bhopal. Leaves were dried in shade and pulverized. Materials were passed through 120 meshes to remove fine powders and coarse powder was used for extraction

Phytochemical Screening

The ethanolic extracts of both the plants were tested qualitatively for different phytoconstituents analysis using various chemical tests. Total phenolic content (TPC) was determined using the various chemical method and total flavonoid content , tannins, steroids, terpenoids, amino acids, proteins and trace elements was determined. The result indicated the presence of flavonoids (kaempeferol, quercetin, myricetin) and phenolic compound (chlorogenic acid rutin and quercetin, phenolic compound (chlorogenic acid rutin and quercetin). **Symplocos racemes.**

Induction of unhealed ulcers:

Chronic ulcers were induced in the rats. The rats were fasted for 24 hours before the induction of ulcers. The rats were anesthetized with ketamine (60 mg/kg i.p). An epigastric incision was made through midline and stomach was exposed. 0.3 ml of a 20% solution of acetic acid was injected into the sub serosal layer of the glandular portion of the stomach with the aid of a tuberculine syringe. Subsequently stomach was re-internalized; the abdomen was closed and sutured. The animals were maintained in individual cages with meshed bottom to prevent coprophagy. The size of the mesh (4 x 4mm) allowed feces to fall to the floor of the cage below the mesh. After the induction of ulcers, five days were required for the ulcers to develop fully. The fifth day after ulcer induction was considered day 0. These ulcerated animals were administered indomethacin1mg/kg/day p.o. for 4 weeks to produce unhealed ulcers. High mortality of rat after subcutaneous indomethacin necessiated change of route from subcutaneous to per oral. Thus a modification was made in the original protocol. Unhealed ulcers were produced after oral administration of indomethacin. To infect the pyloric antrum tissue of the animals with H. pylori, a broth of H. pylori (1 ml p.o.) was administered three times a week for four weeks. During this period indomethacin administration was uninterrupted.

Polymer Characterization: Sodium alginate was characterized for following properties.

1) Swelling Index:

Swelling index was calculated by introducing the specified quantity concerned, previously reduced to the required fineness and accurately weighed, into a 25-ml glass-stoppered measuring cylinder. 25 ml of water was added and the mixture was thoroughly shaked for every 10 minutes for 1 hour. Then the mixture was allowed to stand for 3 hours at room temperature, or as specified. The volume in ml occupied by the polymer was measured, including any sticky mucilage. The mean value of the individual was calculated, related to 1 gm material.

2) Determination of viscosity:

Polymer solutions of 1% w/v were prepared in water. The viscosities of prepared polymeric solution were determined using Brookfield viscometer CAP 2000.

Spindle used: Flat type spindle

Method: The spindle was attached to the spindle coupling nut. The spindle was immersed in the test material and while immersing it was tilted to avoid the air trapping. Parameters were selected and the viscosity was recorded. The spindle was cleaned before the samples were changed. The motor was turned off and the spindle was removed and cleaned.

3) Ash value:

2 g powdered was weighed and taken into porcelain dish. The dish was supported on a pipe-clay triangle placed on a ring of retort stand. It was heated with a burner, using a flame about 2 cm. high and supported the dish about 7 cm. above the flame, it was heated till vapors almost cease to be evolved, then lower the dish and heat more strongly until all the carbon was burnt off. It was cooled in desiccators. Obtained ash was weighed and calculates the percentage of total ash with reference to the air dried sample of the powder.

4) DSC Studies: The DSC analysis of extracts, Sodium alginate, sodium citrate & calcium chloride were carried using a Shimadzu DSC 60 to evaluate any possible drug – polymer interaction. Accurately weighed 1mg samples were hermetically sealed in aluminium crucible & heated at constant rate at 10 C /min. over a temperature range of 40-300 C. Inert atmosphere was maintained by nitrogen gas at a flow rate of 50ml/min.

Formulation of in-situ gel of SRME & PGME:

Preparation insitu gelling system of SRME & PGME:

The different concentration of sodium alginate solutions was prepared in ultra pure water containing sodium citrate at 60oC. Calcium chloride was added to the solution after cooling at below 40oC with stirring. Extracts were dissolved separately in 0.1N HCL solution and then added slowly to the above sodium alginate solution while stirring on a magnetic stirrer to get the homogeneous dispersion of the drug. 0.1 N NaOH was added to the above solution to neutralize the hydrochloric acid while stirring.The above formulations were sonicated in a bath sonicator for 15 minutes & then checked the viscosity of the solutions and then add the prepared solutions in pH 1.2 buffer, to see the gel formation and checked its physical appearance and took the dissolutions of the prepared gels.

Experimental design:

A factorial design experiment was conducted to study the effect two factors. The levels of the two factors were selected on the basis of the preliminary studies carried out before implementing the experimental design. All other

formulation and processing variable were kept constant throughout the study of 32 factorial experimental design layouts.

Optimized method for Sodium Alginate in situ oral gel preparation: (Floating system):

Sodium alginate solutions of different concentration were prepared by adding the alginate to ultrapure water containing sodium citrate and different concentration of calcium chloride and heating to 600C while stirring on a magnetic stirrer. SRME, PGME and preservatives was then dissolved in the resulting solution after cooling to below 40°C. Prepared sols finally stored in amber color bottles until further use.

Evaluation of anti-ulcer activity of formulations (SRME & PGME)

I) Animals: Studies were carried out using Wistar albino rats (120–150 gm) of either sex. They were obtained from the animal house, National Institute of Biological Sciences, Pune India. All the animals were housed in polypropylene cages maintained in controlled temperature (27 ± 2°C) and light cycle (12 h light and 12 h dark). They were provided with standard rat pellet diet and water adlibitum. All the animals were given a week time to get acclimatized with the laboratory conditions. The experiments were carried out according to guidelines of Committee for Prevention and Control of Scientific Experimentation on Animals (CPCSEA).

II) Drugs and Chemicals Omeprazole (Dr.Reddy's Lab, India) and Topfers reagent (Nice Chemicals, India) were used in this study. All other chemicals used in present study were of analytical grade.

Methods:

Pylorus ligation ulcer model:

The animals were divided into following groups of six animals each.

Group 1: Served as control and was Animals were treated vehicle only

Group 2: Animals were treated with Fomulation 1 p.o (SRME)

Group 3: Animals were treated with Formulation 2 p.o (PGME)

Group 4: Animals were treated with Formulation 3 p.o (NO extract)

Group 5: Animals were treated with standard drug Omeprazole (20mg/kg, p.o) Overnight fasted rats were anaesthetized with anaesthetic ether. Then an incision of 1cm long was given in the abdomen just below the sternum. The stomach was exposed and a thread was passed around the pyloric sphincter and a tight knot was applied. Abdomen wall was closed by putting the sutures. After 45 minutes of extracts (SRME & PGME) treatments pyloric ligation was performed. After 4 hr of pyloric ligation animals were sacrificed by decapitation. Abdomen was opened and the oesophagus was tied at the end of the stomach. A small cut to the pyloric region just above the knot was given and contents of the stomach were collected in a centrifuge tube. The following parameters were analyzed:

1. Volume of gastric juice (in ml): Gastric content was centrifuged at 1000 rpm for 10min and measured the volume.

2.Determination of free and total acidity: Pipetted out 1ml of supernatant liquid and diluted it to 10ml with distilled water. The PH of this solution was noted with the help of pHmeter.

Acidity was calculated by using the formula: Acidity = meter. The solution was titrated against 0.01N NaOH using topfers reagent (Dimethyl-amino-azo-benzene with phenolptheline) as indicator. The end point was noted when the solution turns to orange color; this corresponds to the free acidity. Titration was continued further till the solution regained pink color. This volume corresponds total acidity.

volume of NaOH x Normality x 100 mEq/lt/100g 0.1

3. Ulcer Scoring & Ulcer Index Determination

0 - Normal Mucosa
0.5 - Red coloration
1.0 - Spot ulcers
1.5 - Hemorrhagic streaks
2.0 - Ulcers >3 but <5
2.5 - Ulcer >5.

Mean ulcer score of each group were calculated, which was designated as the ulcer index and percentage of protection was calculated as C – T / C x 100

(C = ulcer index in control group; T = ulcer index in test group)

4. Ethanol induced Ulcer model: Administration of Formulations and control drugs was done for 10 days after which 90% ethanol was administered to the overnight fasted rats of all groups the next day; at a dose of 1 mL per animal, irrespective of the weight of the animal through oral route. One hour after ethanol administration, all rats were sacrificed by an overdose of chloroform and the stomachs were rapidly removed,

opened along their greater curvature and gently rinsed under running tap water. They were then spread on a paraffin plate and the inner surface was examined with a 6x hand held magnifier. The scores for each single lesion were then totalled .Mean ulcerative index was calculated as follows:

I) Presence of oedema, hyperaemia and single submucosal punctiform haemorrhages.

II) Presence of submucosal haemorrhagic lesions with small erosions.

III) Presence of deep ulcer with erosions and invasive lesions:

Ulcer index = (number of lesion. I) + (number of lesion. II) × 2 + (number of lesion. III) ×3

The percentage inhibition was determined as follows:

= (Control mean lesion index-Test mean lesion index) /X 100Control mean lesion index

Statistical analysis: The data were represented as Mean± SEM. The data on antiulcer activity of formulations were analyzed by one way Analysis of Variance (ANOVA), 'P' value less than 0.05 was considered as statistically significant.

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Result and Discussion

Development of formulation: buccal patch

Preformulation studies:

A) Polymer characterization:

Name of Polymers: HPMC K15 & Carbapol

I) HPMC K15:

Description: Hypromellose is an odorless and tasteless, white or creamy-white fibrous or granular powder

Physical Properties:

Ph - 5.2.8.0 for a 1% w/w aqueous solution.

Ash value -1.4.3.0%, depending upon the grade and viscosity.

Density (bulk) - 0.361 g/cm3

Density (tapped) - 0.567 g/cm3

Density (true) - 1.333 g/cm3

Melting point - Browns at 190.200°C; chars at 225.230°C.

Glass transition temperature is 170.180°C.

Solubility: Soluble in cold water, forming a viscous colloidal solution, practically insoluble in chloroform, ethanol (95%), and ether, but soluble in mixtures of ethanol / methanol and dichloromethane, and mixtures of water and alcohol.

Specific gravity: 1.26

II) Carbopol :

Description: Carbomers are white-colored, .fluffy. Acidic, hygroscopic powders with a slight characteristic odor.

Physical Properties:

Acidity/alkalinity - pH = 2.5.3. for a 0.5% w/v aqueous dispersion; pH - 2.5.3.0 for a 1% w/v aqueous Dispersion.

Density (bulk) - 1.87.2.08 g/cm3

Density (tapped) - 1.5 g/cm3

De Glass transition temperature- 100.105°C

Melting point - Decomposition occurs within 30 minutes at 260°C.

Specific gravity - 1.31

Moisture content: Normal water content is up to 2% w/w. However, carbomers are hygroscopic and typical equilibrium moisture content at 25°C and 50% relative humidity is 9.10% w/w.

Solubility: Soluble in water and, after neutralization, in ethanol (95%) and glycerin. Although they are described as soluble.Carbomers do not dissolve but merely swell to a remarkable extent, since they are three-dimensionally crosslinked microgels.

Thickness uniformity & diameter: As the total amount of polymerncreases the thickness of the film were found to be increased. The thickness for formulation B-1 to B-varied from to 0.28±0.02 to 0.4±mm

Swelling Index: The swelling of the patches were observed in phosphate buffer solution (pH 6.8) and shown in table. Swelling was more pronounced in patches B4 and B5 which contain HPMC and Carbopol in a ratio of (1.5:1) and (2:0.5) respectively. Patches B2, and B6 showed less swelling (weight basis), may be due to the presence of Eudragit RL 100 and ethyl cellulose, respectively. These results were in agreement with the increase in area due to swelling. The results revealed that all the formulations provide an acceptable swelling index in the range of formulation B-1 to B-6.

Mucoadhesion strength: As the amount of mucoadhesive polymer increases the mucoadhesion was found to be increase. In formulation B-1 to B-6 four different polymer was used in which Carbopol 934P have better mucoadhesion property than other so B-4 shows greater mucoadhesion strength (10.37 gm). Mucoadhesion strength of formulation B-1 to B-6 varied from 9.35 gm to 23.4 gm

Mucoadhesive Time: In formulation B-1 to B-6 four different polymer was used in which Carbopol 934P have better mucoadhesion property than other so B5 shows greater mucoadhesion time 310 min than the formulation containing Eudragit RL-100 and ethyl cellulose. Mucoadhesion time of formulation BP-1 to BP-6 varied from 178 minute to 310 minute.

Evaluation of mucoadesive herbal buccal patchs:

Physical properties: Physical appearance and surface texture of PGME & SRME Buccal Patch:

Table 1: Physical appearance and surface texture

SI NO.	Formulation	Colour PGME & SRME	Flexibility	Surface Texture
1	F1	Light green/Brown	+	smooth
2	F2	Light green/Brown	+	Smooth
3	F3	Light green/Brown	+	Smooth
4	F4	Light green/ Brown	+	Smooth
5	F5	Light green/Brown	+	Smooth
6	F6	Light green/Brown	+	Smooth
7	F7	Light green/Brown	+	Smooth
8	F8	Light green/Brown	+	Smooth
9	F9	Light green/Brown	+	Smooth

Table: 2 Uniformity weight of Patch of PGME & SRME Buccal Patch: Table: Uniformity weight of Buccal patches

Formulation Batch	Uniformity weight(gm) PGME	Uniformity weight(gm) SRME
F1	0.250±0.013	0.250±.21
F2	0.251±0.02	0.250±.44
F3	0.252±0.002	0.250±0.007
F4	0.251±0.004	0.251±0.21
F5	0.251±0.002	0.250±0.014
F6	0.252±0.03	0.251±0.11
F7	0.252±0.008	0.252±0.13
F8	0.250±0.005	0.250±0.20
F9	0.250±0.045	0.251.4±0.07

Weight uniformity in buccal patch (PGME) F1 to F9 varied from 0.250±0.013 gm to 0.252±0.03gm. Similarly weight uniformity in buccal patch (SRME) F1 to F9 varied from 0.250±0.014gm to 0.252±0.13gm.The patches were found uniform

Evaluation of formulations(In-situ gel) :

1) Physical appearance:

All the prepared sodium alginate based in situ gel of SRME & PGME were checked for their clarity were found to be satisfactory.

The haziness that was observed after autoclaving (due to precipitation of Polymer at elevated temperature) was found to disappear and the original clarity was regained after overnight standing.

2) pH of formulation-

The pH of the formulations was found to be satisfactory and it was in the range of 6.7-7.4.

Table : 3 pH of In-Situ gel

Floating System	pH (SRME)	pH(PGME)
F1	6.7±0.02	6.9±0.04
F2	6.8±0.04	6.9±0.03
F3	7.4±0.03	7.3±0.04
F4	7.1±0.03	7.2±0.02
F5	7.0±0.02	7.1±0.01
F6	7.3±0.03	7.2±0.02

F7	6.8±0.01	6.8±0.02
F8	7.2±0.03	7.2±0.03
F9	7.1±0.05	7.0±0.04

In this range of pH SRME & PGME retains their activity. Therefore, pH adjustment is not required by any other reagent.

Rheological properties: The viscosity two different formulation of in situ oral gel was determined. It is the main parameter that determines the residence time in the GIT and it satisfies in-situ formulation.

Rheology of In- situ oral Gel:

The rheological properties of the sols are of importance in view of their proposed oral administration. Rheology of the two formulations is found to be better as the shear stress decreases with the steady flow rate. F1 shows that fast decrease in shear stress than the shear strain, batch F3 and shows that steady decrease in the shear strain than shear strain.

4) In-Vitro Floating Ability:

Time taken by formulation to emerge on the medium surface (floating lag time) and time for which formulation continuously floated (duration of floating). The released CO2 was entrapped in gel network producing buoyant formulation and then calcium ion reacted with Sodium alginate produced a cross linked 3-D gel network and swelled structure that might further diffusion of CO2 and drug molecule and resulted in extended period of floating and drug release respectively.

Floating Lag time for In-Situ gel varied from 38±0.04 to 89±0.06 seconds for SRME gel 40±0.05 to 90±0.06 seconds for PGME gel. Floating Time varied from 423±1.4 minutes to 573±3.0 minutes for SRME gel & 433.5±1.5 to 572±10.5 minutes for PGME gel.

5) In Vitro Gelling Studies:

Gelling studies were carried out using 0.1N HCl, (pH 1.2). In these studies the gelling capacity (extent and speed of gelation) for all formulations were determined. The in-situ gel so formed should preserve its integrity without dissolving or eroding so as to localize the drug at absorption site for extended duration. Gelation characteristic was assessed on an ordinal scale ranging between - - - to +++. After ingestion, the liquid polymeric solution should undergo a rapid sol-to-gel transition by means of ionic gelation.

6) Drug content: This is one of significant necessity for any type of dosage form that quantity of the drug present in the formulation should not deviate beyond certain specified limits from the labelled amount.

Determination of antiulcer activity

Pyloric Ligation Method:

I) Observations: The number of ulcers was counted by using magnifying glass.Severity score was observed as under:

- Normal ulceration
- Red ulceration
- Spot ulceration
- Hemorrhagic stress
- Deep Ulcer
- Perforation

Ulcer index = (UN+US+UP) * 10^{-1}

UN: Avarage no. of ulcer per animal

US: Average no. of severity score

UP: % of animal with ulcer

Observations from above table revealed that formulations made of SRME & PGME showed good response against all symptoms. Only red colouration & spot ulcers were observed but the intensity was very less. Other symptoms wers not observed. All observations were comparable with standard drug.

(II) Effect of Formulations (SRME & PGME) on various parameters in pyloric ligation induced gastric ulcers: [163]

Values are mean ± SEM for six animals in each group.

***P< 0.05 considered statistically significant as compared with control group.**

****** P< 0.01 considered statistically significant as compared with control group.**

In pyloric ligation model, the Formulation 1 and Formulation 2 showed significant ($p<0.01$) rise in pH as compared to control. The free acidity gastric content is increased in control animals. The Formulation 1 & 2 both showed significant ($p<0.01$) decrease in free acidity as compared to control. Both Formulations significantly reduced the total acidity and ulcer index ($p<0.001$) as compared to control (Table 1).The percentage protection of Formulation 1 & 2 was found to be 65% and 60% respectively whereas the percentage protection of omeprazole was found to be 75%.

Ethanol induced Ulcer model:

The administration of Formulation 1SRME, Formulation 2 PGME (500 mg/kg body weight) causes a decrease in ulcer index of ethanolic induced ulceration in the stomach of Wistar rats. Ethanol induced gastric ulcer was employed to study the ulceroprotective effect of both formulations.

Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intracellular membrane permeability to sodium and water. The massive

Intra cellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium. In ethanol induced gastric ulcer, on induction of gastric ulceration by using ethanol (96%, v/v), the pretreatment with both formulations showed a reduction in the severity of the lesions.

III) Effect of Formulations (SRME & PGME) on ethanol induced gastric ulcers:

0: no changes detected

+: active changes up to less than 25 %

++: active changes up to less than 50 %

+++: active changes up to less 75 %

++++: active changes up to more than 75 %

A) Morphological investigation

Ethanol (50%) induced gastric damage showed marked gross mucosal lesion, including long hemorrhage bands and petechial lesion. On gross examination these hemorrhagic bands were characterized by different sizes along the longitudinal axis of the glandular part of stomach (Fig 1A). Animals pretreated with formulations 1 & showed very mild lesions with interstitial hemorrhage and sometimes no lesion at all.

B) Investigation of gastric lesion

Both formulations significantly increased macroscopic curative ratio compared with control groups (Table 3). Morphometric evaluation was also carried out to evaluate the extent of ulcer. The ulcer index was significantly ($P<0.01$) reduced in animals pretreated with Formulatios compared to distilled water and Omaprazole treated rats.

C) Biochemical investigation

Moreover, SRME & PGME significantly reduced the gastric ulcer in ethanol induced gastric ulcer model by confirmation of significant decreases ($P<0.05$) in acid volume and increases ($P<0.05$) in pH when compared with ($P<0.05$) (Table-2) ethanol treated group.

D) Histopathological investigation

On microscopic examination, ethanol treated rats showed mucosal hemorrhage, segmental mucosal necrosis of gastric epithelium, edema and ample infiltration of leukocytes in submucosa .Only patchy mucosal epithelial loss was seen in pre-treated rats.

Table (B): Evaluation parameters for ethanol induced method

Group	Ulcer index	% Protection
Normal control	79±1.09	0.00
Formulation 1	18.50±.04**	69
Formulation 2	16.75±.03**	61
Formulation 3	65.45±03	10.21
Standard(Omeprazole)	21.70±.09**	70

Values are mean ±SEM for six animals in each group. **

From the selected formulation (F5) of sodium alginate based insitu gel of SRME & PGME “Pyrolus legation method in rats”was used for in vivo study and gel formation was also checked in collected gastric juice from the rats.

In the present study, the control group treated orally with ethanol produced the expected ulceration. Pretreatment at the dose of 500mg/kg, p.o and 500mg/kg, p.o with formulation containing methanolic extracts of significantly ($p<0.01$) decreased the ulcer index when compared with control rats. These results indicate that PGME & SRME extract displays an antiulcerogenic effect related to cytoprotective activity, since it significantly reduced the ethanol induced ulcer. In histopathological examination of stomach mucosa ethanol treated group shows the ulcerated mucosa with haemorrhage and discontinuity of lining of epithelium.Pretreatment with SRME & PGME (500mg/kg, p.o. and 500mg/kg, p.o.) protected the mucosal epithelial from the damage caused by ethanol.The antioxidant activity of flavonoids has been well documented in the literature. Moreover, flavonoids have been reported for their antiulcerogenic activity and gastro protection already.It has been also reported that flavonoids like Quercetin seem to play a very important role in the prevention and treatment of peptic ulcer. It acts by promoting mucus secretion, thereby serves as gastroprotective agent

CONCLUSION

In conclusion, the oral administration of plant extracts displayed a significant antiulcer activity without any apparent toxicological effects, which supports the use of *Psidium guajava & Symplocos racemosa* in herbal medicine of India for ulcer therapy.

The gastric retention approaches as well as herbal drugs described here have application for treatment of *H.pylori* infections although further development is required for each to be fully effective especially, in human studies. Overcoming high mucous turnover rate & resulting limited retention times is challenging for bio adhesive systems & swelling systems must guarantee clearance from the stomach after a certain time to prevent any obstruction.

Floating systems are available commercially, & combination approaches, using floating behavior & mucoadhesion, have also shown promise. Exploiting dual mechanism of retention may provide the strength & reproducibility required to permit successful advancement in this field. So in future, a combination of herbal drugs with a novel drug delivery systems mentioned above, may lead to an important breakthrough in herbal/integrative treatment of *H.pylori* infections & other types of ulcer.

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Table 4. : Floating time for sodium alginate in situ gel

Floating System	Floating Lag Time(Second)SRME	Floating time(hrs)SRME	Floating Lag Time(Second)PGME	Floating time (hrs)PGME
F1	38±0.04	450.6±1.6	40±0.05	447±1
F2	62±0.05	420.5±0.5	65±0.06	433.5±1.5
F3	64±0.03	484±2	64±0.009	463±1
F4	69±0.05	571.5±1.5	70±0.08	572±10.5
F5	42±0.02	573±3	45±0.06	563.5±1.5
F6	48±0.04	512.5±0.5	48±0.08	518.5±1
F7	89±0.06	423.5±1.5	90±0.06	433±1
F8	62±0.02	553±1	65±0.02	535±0.5
F9	60±0.06	542.5±0.5	60±0.008	543.5±1

In Vitro Gelling Studies

Table 5. : In-vitro gelation time

Floating System	Geling Studies (SRME)	Duration of Gelation (SRME)	GelingStudies (PGME)	Duration of Gelation (PGME)
F1	+++	9hr 32min	+++	9hr 38min
F2	+	8hr	+	8hr 10min
F3	++	7hr 30min	++	7hr 10min
F4	+++	8hr 05min	+++	8hr 25min
F5	+++	8hr 18min	+++	8hr 8min
F6	+++	9hr 5min	+++	9hr 15min
F7	+	4hr 38min	+	4hr 26min
F8	+++	8hr 35min	+++	8hr 38min
F9	+	7hr 50 min	++	8hr 40min

Table:6 Drug content in In-Situ gel

Formulation code	% Drug content (SRME)	%Drug content (PGME)
F1	91.20±2.01	92.33±2.45
F2	93.63±2.25	92.76±2.65
F3	94.70±1.97	94.80±1.50
F4	92.85±2.81	92.62±2.61
F5	95.08±2.25	95.50±2.15
F6	96.21±2.55	96.21±2.35
F7	94.25±1.25	94.80±2.25
F8	95.70±2.89	94.93±2.09
F9	95.03±2.50	95.60±2.10

The drug content varied from 91.23% to 96.23% in batches F1 to F9

Table 7 : Observations of pyloric ligation methods

Sl. no.	Treatment	Dose mg/kg	Normal Stomach	Red Coloration	Hemorrhagic Streak	Perforation
1	Normal Control	1ml/animal	-------	+++	++++	++
2	Formulation I(SRME)	300mg	--	+	----	---
3	Formulation II(PGME)	300mg	--	+	------	----
4	Stndard (OMP)	20mg	+++	----	-----	------

Table 8 Evaluation of parameters in pyloric ligation method

Group	Ulcer Index	%Protection	pH of gastric Fluid	Gastric juice in ml
Normal control	4.5±1.5	0.0	2.45±0.02	2.2±0.1
Formulation 1(SRME)	1.9±0.09	62	3.15±0.04**	3.05±0.06**
Formulation 2(PGME)	1.5±0.08	58	2.8±12**	2.82±.08*
Formulation 3(No extract)	4.3±1.05	22	2.45±0.05	2.3±.05
Standard(Omeprazole)	1.2±0.08	70	3.35±0.05**	3.45±.04**

Table 9 : Observationsof different parameters in ethanol induced method

Sr. no	Hyperemia and hemorrhages	Edema	Necrosis	Leucocytic infiltration	Ulceration Protcetion
Normal control	00	00	00	00	00
Formulation 1	+++	++	++	++	+++
Formulation 2	++++	++++	+++	+++	++++
Formulation 3	+	+	+	+	+
Standard	++	+++	++	+++	++