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EVALUATION ANTI-ULCER ACTIVITY OF PISTIA STRATIOTES IN RODENTS MODEL

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Abstract-

After evolution human changes its life style and arrested from several diseases one of them is ulceration. It is well known fact that ulceration is a common disorder of life and significantly involved in the maintenance of the health or the development of other diseases. Ulceration needs good research opinion and medical assessment for improved therapeutics. Peptic ulcer disease (PUD) has been a major health problem throughout the world. This study for Screening and isolation of extract from natural products to protect the gastric mucosa and provide a new rapid acid suppression therapy. Effect of natural products in NSAIDs induced ulcer models in order to identify its anti-ulcer efficacy.

Keywords- Peptic Ulcer, Anti-cancer activity, Pistia Stratiotes, rodent model, NSAIDs

INTRODUCTION

Peptic ulcer is the most prevalent gastro-intestinal disorder and has been a major health problem with a high rate of global incidence. The basic cause of peptic ulcer has not been understood until the recent times. In the early 20th century, peptic ulcer disease was believed to be related to excessive acid secretion resulting from stress full lifestyle. Important advances have occurred in the last two decades that improved our understanding for the disease and as well improved our therapeutic strategies. The major forms of peptic ulcer include gastric and duodenal ulcer both of which are chronic in nature (1-3). Gastric ulcer is a very common global problem today. The pathophysiology of ulcer involves an imbalance between offensive (acid, pepsin,and *Helicobacter pylori*) and defensive (mucin, prostaglandin, bicarbonate, growth factors and nitric oxide) factors. Consequently reduction of gastric acid production as well as reinforcement of gastric mucosal protection has been the major therapeutic approaches of peptic ulcer disease (4-6). Pharmacological management of gastric ulceration thus is mainly directed at the reduction or neutralization of gastric acid secretion. Hence inhibition of gastric acid secretion acts as an important target for the treatment of ulcer. The modern approach to control gastric ulceration via inhibition of gastric acid secretion (7). Despite the rapid and clear efficacy of these antisecretory drugs, there are a number of shortcomings that need to be addressed. For example, H2RAs and PPIs induce rapid tolerance during therapy and rebound hyper secretion following drug withdrawal which leads to high ulcer relapse rate. Furthermore, *H. pylori* negative suffering patients are being encountered gastric ulcers and it recur even after the successful eradication of the bacteria. In 1981, Warren and Marshall observed that a bacterium, *Helicobacter pylori* was associated with peptic ulcer disease and that its elimination with antimicrobial drugs could effectively cure the disease. The identification that *H. pylori* play a key role in the pathogenesis of acid peptic diseases stimulated new approaches to prevention and therapy. (8)

As a result, more and more drugs are coming up offering newer and better options for the treatment of peptic ulcer, but their critical clinical evaluation has recorded several discrepancies including incidence of relapses, adverse side effects, and drug interactions (9). Drugs from native sources of natural products are thus now a target for development, refinement and pharmacological modification for anti-ulcer treatment. The need of new chemical entities (NCEs) for health care is investigated and served through the plant sources. 80% of the populations abiding in the developing countriesrely on traditional medicine for their primary health care needs clarified by the World Health Organization (WHO). Roughly estimated that 50% of the NCEs comes during the last two decades are from plant products. In almost all the traditional systems of medicine, the medicinal plants play a major role and constitute their backbone. Indian medicinal plants possess enormous healing power and only a part of this potential is known to mankind. Evolution of Ayurveda and plant-based remedies for health care through day-to-day life experiences is a part of the cultural heritage of India. Various Indian medicinal plants have been reported to possess anti-ulcer activity. In this study we will evaluate the role of natural products in gastric ulceration (10)

2. MATERIAL AND METHOD

2.1 Materials:

2.1.1 Chemicals: Chemicals used in the present study have been listed below along with the name of the manufacturers:

Chemical	**Manufacturer**
Boric acid	SD Fine Chemicals Ltd., India
Bovine serum albumin	Sigma Chemicals, USA
Bradford's reagent	Sigma Chemicals, USA
Bromophenol blue	Sigma Chemicals, USA
Chloroform	Merck, Germany
Copper sulphate	Sisco Research Laboratories, India
Dimethyl sulphoxide (DMSO)	Qualigens, India
Ethanol	Merck, Germany
Folin's Reagent	Sisco Research Laboratories, India
Formaldehyde	Sisco Research Laboratories, India
Formamide	Sisco Research Laboratories, India
Glucose	Sigma Chemicals, USA
Hydrogen peroxide	M.P. Biomedicals, India
Indomethacin	Fluka, USA
Magnesium chloride ($MgCl_2$)	Sisco Research Laboratories, India
Methanol	Merck, Germany
Omeprazole	Sigma Chemicals, USA
Perchloric acid	Sigma Chemicals, USA
Phenophthalein	Sisco Research Laboratories, India
Potassium dihydrogen phosphate	Sisco Research Laboratories, India
Sodium acetate	Sisco Research Laboratories, India
Sodium Chloride (NaCl)	Sisco Research Laboratories, India
Sodium hydroxide (NaOH)	Sisco Research Laboratories, India
Topfer's reagent	Sisco Research Laboratories, India
Triton X-100	Sisco Research Laboratories, India

2.1.2 Instruments used in the study:

- Autoclave, Vertical SMI-102 (Jindal, SM Scientific Pvt. Ltd, Delhi, India).
- Digital pH meter (Model- CL-54, Toshniwal Instruments Manufacturing Pvt. Ltd. Chennai, India).
- Electrophoresis chambers (Miniphor UVT System, Maxiphor UVT System Banglore Genei, Banglore, India.
- Freezers (-20ºC Refrigerated, RQFV-265(D), Remi Instruments (-85ºC Ultrafreezer, Model-U41085, New Brunswick, Canada), 4ºC Refrigerators, Samsung India Electronics Pvt. Ltd., New Delhi, India).
- Fluorescent Spectrophotometer (Cary Eclipse Fluorescent Spectrophotometer, PCB 150 Water Peltier System, Varian Inc., the Netherlands).
- Homogenizer with 1/8 Hp motor and auto-transference.
- Ice machine (Ice Boy, Model-ZX-120 ELW, New Delhi, India).
- Incubator (Jindal, SM Scientific Pvt Ltd, Delhi, India).
- Magnetic Stirrer (Remi laboratory Instruments, Mumbai, India).
- Microplate reader (Powerwave ^{TM}-XSL, Biotek India, Mumbai, India).
- Microscopes (Trinocular Stereozoom Microscope SZ-CTV Olympus Optical Co., Tokoyo, Japan).
- Microwave oven (LG Electronics India Pvt. Ltd. Greater Noida, India).
- Spectrophotometer (Shimadzu UV1201, Shimadzu Scientific Instruments, Columbia, USA).
- Shaking Water Bath (LabTech, Diahan Labtech Co. India Pvt. Ltd. New Delhi, India).
- Vortex Mixer (Lead Instrument Pvt. Ltd., Banglore, India).
- Weighing Balances (Micro Electronic Balance, JS 110, Chyo, Japan).

Plants material:

All the plants were collected and the voucher specimen numbers were assigned and preserved in the Herbarium of the Institute. All the extraction procedures of plant extracts were performed. In the present study, the active constituents of plant were used to evaluate their anti-ulcer potential.

2.1.3 Pistia stratiotes:

The dried extract of Pistia stratiotes were purchased from the local herbal market and the authentification was done by the Institute. The dried Pistia stratiotes (1 kg) after removing the rotten Pistia stratiotes were cut into small pieces and were placed in glass percolator with 5 lit of ethanol: distilled water (1:1) and allowed to stand at room temperature for 24h. The percolate was collected and this process was repeated for four times. The combined percolate was concentrated under vacuum using rotary evaporator at 40^0C. The weight of extract obtained was found to be 310 gm.

2.2 Methods:

2.2.1Experimental Animals:

Experimental protocols were approved by the Institutional Ethical and Usage Committee following the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals Reg. No.- 2005/PO/RcBT/S/18/CPCSEA) which complies with International norms of INSA (Indian National Science Academy). Adult female Sprague Dawley rats, weighing 140–220 g were used in this study. Animals were housed in raised bottom mesh cages to prevent coprophagy and were kept in environmentally controlled rooms with temperature regulated at 22 ± 2◦C, 12/12 h light and dark cycle.

2.2.1.2Treatment schedule:

Pilot investigation with the graded doses of test compounds were tested against Cold restraint ulcer (CRU) model to identify the effective dose and selected for further studies in other ulcer models. Test extract Pistia stratiotes; reference drug omeprazole (Omz) (10 mg/kg) and sucralfate (SUC) (500 mg/kg) were freshly prepared in 1% carboxymethyl cellulose (CMC) as suspension and administered orally to the animals. All animals were deprived of food for 16 h before ulcerogens exposure. The rats were randomly divided into various groups, each consisting of 6 animals.

Treatment groups for acute gastric ulcer model:

Rats were divided into three groups.

Group I (Control group): Control group of animals were treated with 1% CMC, 45 min prior to the induction of gastric ulcer in all ulceration models.

Group II (Treatment groups): Rats were treated with test extract Pistia stratiotes, 45 min prior to the induction of gastric ulcer in all ulceration models.

Group III (Reference drug treated): Third group was sub categorizes into further sub-groups based on selection of models.

2.2.2Acute gastric ulcer models:

Cold Restraint ulcer**,** Alcohol induced ulcer **,**Pyloric ligation induced ulcer **,**Aspirin induced ulcer

2.2.3 Cold restraint ulcer model:

The method described by (11) was used in this assay. The rats were subjected to cold and restraint stress after 45 mins of treatment with test drugs and reference drug omeprazole. All the animals were immobilized in restraint cage and kept at 4°C in an environmental chamber. Two hours later the animals were sacrificed and stomachs were observed and scored under Magnascope (5X magnification) for ulcers.

2.2.4 Alcohol induced gastric ulcer model:

Absolute alcohol (1 ml/200 g, body weight of animals) was induced gastric ulcer in rats by administering orally (12). The test compounds and reference drug sucralfate were administered 45 minutes before alcohol treatment. After 1 hour of alcohol administration, the animals were sacrificed and stomach was cut open along the greater curvature to observe the gastric lesions which appear as hemorrhagic bands along the mucosal ridges of the stomach. The lengths of the lesions were measured using Biovis image analyzer software and summated to give a total lesion score.

2.2.5 Pyloric ligation induced ulcer model:

After 45 mins of administration of test drug and reference drug Omeprazole (Omz), ulcer was induced in rats by pyloric ligation. Under Chloral hydrate anesthesia (300mg/kg, i.p.), the abdomen was opened and the pyloric end of the stomach was ligated avoiding any damage to the adjacent blood vessels (13). Stomach was replaced carefully and the animals were allowed to recover with free access to water. After 4 hours the animals were sacrificed and the stomach was dissected out. Lesions were scored and gastric fluid was collected and centrifuged at 2000 rpm for 10 mins. The collected supernatant was used for the estimation of gastric secretion studies and mucin estimation.

2.2.6 Aspirin induced gastric ulcer model:

The experiment was carried out according to the method of (14). Gastric lesions were induced with Aspirin (150 mg/kg) administered to rats after 45min of treatment of test compounds and reference drug omeprazole (Omz). The animals were sacrificed after 5 hours of aspirin treatment and the stomach was dissected out, incised along the lesser curvature and the lesion was scored.

2.2.6.1 Measurement of ulcer:

Ulcers formed in stomach of rats in Cold restraint, pyloric ligated and aspirin induced gastric ulcer models was observed under Magnascope (5X magnification) and scored according to the arbitrary scoring system and graded as following:

Shedding of epithelium = 10; Petechial and frank hemorrhages = 20; One or two ulcers = 30; More than two ulcers = 40; Perforated ulcers = 50. (15)

In Alcohol induced gastric ulcer model after sacrificed the rats take images of stomach by Olympus trinocular zoom microscope and then length of the lesions were measured using Biovis image analyzer software (Expert Vision Lab Private Ltd., Mumbai, India) and summated to give a total lesion score.

2.2.7 Biochemical estimation in acute gastric ulcer models:

2.2.7.1Gastric secretion study:

Free and total acidity was measured from the collected gastric juice by titrating against 0.01N NaOH, using phenolphthalein as an indicator and expressed in terms of µequiv./ml (7). Mucin level in gastric juice was quantified as described by Crowther et al. 1987 (16)

2.2.7.2 Direct fluorometric assay:

In gastric juice, mucin was quantified with a fluorometric assay as described by Crowther et al. 1987(16). Before the fluorometric assay, gastric juice was delipidate using the method of Wessel et al., 1984(17). Briefly describe, 50 µl of gastric juice was diluted 1: l (v/v) in PBS buffer and 400 µl methanol was added. After a short centrifugation (9000 g for 1 min), 200 µl of chloroform and 300 µl of distilled water were added, thoroughly mixed, and centrifuged once more. The upper phase was discarded and 300 µl of methanol was added. After a further centrifugation (9000 g for 2 min), lipid-free proteins were recovered in the pellet. For the fluorometric mucin determination, the pellet was resuspended in 200 µl of PBS, 250 µl of alkaline reagent (1 ml 0.15 N NaOH and 200 µl of 0.6 M 2-cyano-acetamide) was added and the mixture was incubated at 100°C for 30 min. Subsequently, 2 ml of 0.6 M borate buffer (pH 8) was added and the fluorescence was measured by varion fluorimeter at 383 nm (excitation 336 nm).

2.2.7.3 Protein assay:

The Protein content of the samples was estimated by the method of Lowry et al. 1951 (18) using Folin Phenol reagent. Bovine serum albumin (1 mg/ml) was used as standard.

2.2.8 Protein estimation of lowery method:

This method is reasonably sensitive, detecting 10µg/cm^{-3} of protein. This method is based on the reaction when the folin's reagent together with copper sulphate solution is mixed with protein solution, blue purple colour is formed which can be quantified at 660nm range.

Requirements BSA Solution (Standard Protein solution), 1mg/ml. Solution A: Mix solution (i) and (ii) 2% Sodium carbonate**;**0.1N NaOH**;**Solution B: Mix solution (i) and (ii)**;**1.56% $CuSO_4$**;**2.37% Sodium Potassium Tartarate**;** Solution C: Mix 2 ml solution B in 100 ml solution A. **;** Solution D: Dilute Folin Ciocalteau reagent (1:1), at the time of use.

Procedure

Prepare standard solution of stach in increasing concentration and add distilled water to make up volume 1ml in each tube, as shown in table Took 2 ml of solution C in each test tube and added 200 µl of protein solution of each concentration. Incubated for 10 minutes at RT. After incubation added 200 µl of 1X Folin reagent, further incubated for 30 minutes dark. Took the O.D at 660 nm.

2.2.9 Statistical analysis: Data were expressed as mean ± S.E.M. Analysis will be performed with Prism version 5.0 software using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. $P<0.05$ was considered to be statistically significant.

3. RESULT AND DISUSSION: -

3.1 Pistia stratiotes:

Pistia stratiotes, also known as Jalkumbhi, is an aquatic plant, stoloniferous, floating on lakes, streams, and stagnant water ponds and in lime-rich water, throughout India. It is distributed in the tropical and subtropical region of Asia, Africa, and America. Four varieties are distinguished. The Indian variety is known as var. Cuneta. It is propagated by seeds or more rapidly by stolons. It forms a dense mat on the water surface and causes serious clogging on water ways. It is also responsible for harboring mosquito larvae, which carry the filarial parasites. It flowers in hot season and fruits appear after the rain.

P. stratiotes is a floating, stoloniferous herb found in ponds and streams almost throughout India up to a height of 1000 m. Leaves are green in colour, odourless, and bitter in taste. The leaves are approximately 13cm long and 17cm wide and of fan-shaped having parallel venation, blunt apex, and entire margin

Figure 1: Image of plant Pistia stratiotes

3.2 Evaluation of anti-ulcer effect of Cactus against cold restraint induced gastric ulcer model in rats:

Extract of Pistia stratiotes at graded doses (100, 200 and 400 mg/kg, p.o.) showed percentage protection of 37.5, 50.0 ($P<0.05$) and 52.5 ($P<0.05$) respectively. Whereas the effect of omeprazole, a substituted benzimidazole (reference drug) showed a percentage protection of 77.4 ($P<0.01$) at 10 mg/kg, p.o. was also investigated for comparison. From this observation 200mg/kg dose of extract was identified as the effective dose and selected for further studies. The results are graphically represented in Table 1.

Table 1: Effect of graded dose of Pistia stratiotes extract and reference drug omeprazole (Omz) on percentage protection of ulcer against cold restraint induced gastric ulcer models in rats. Data expressed as mean of % protection of Pistia stratiotes and reference drugs after ulcer induction ± S.E.M.

Extract and doses	**Mean Severity of ulcer score**	**% Protecction**
Control	**20.0±1.44**	**0.0**
Extract (100)	12.5±1.5	37.5±5.8
Extract (200)	**10.0**±2.7	**50.0**±4.5
Extract (400)	9.6 ±2.8	52.5±2.1
Omeprazole (10)	**4.5**±1.3	**77.5**±4.7

3.3 Anti-ulcer effect of plant extract against Alcohol, Pyloric ligation and Aspirin induced acute gastric ulcer model in rats:

Graded doses of plant extract Pistia stratiotes at (200 mg/kg, p.o.) were screened. Extract was showing 58.79% protection in alcohol model (Table 2. and Figure 1.) and 50% protection in aspirin model both of models is following the cytoprotective mechanism. Extract also exhibiting percentage protection in pyloric ligation model which is following the antisecretory mode of action. The results are represented in Table 2.

Table 2: Effect of plant extract Pistia stratiotes, reference drug Omeprazole and Sucralfate (Omz and SUC) on percentage protection of ulcer against alcohol, pyloric ligation and aspirin induced gastric ulcer models in rats. Data expressed as mean of % protection of Pistia stratiotes and reference drugs after ulcer induction ± S.E.M. Statistical analysis was done by one way ANOVA followed by Dunnett's Multiple Comparison Test. *Statistically significant at P<0.05 and **P< 0.01, in comparison to control. n = 6 in each group.

S.N.	Extract and Reference	% protection in alcohol model	% protection in aspirin model	% protection in pyloric model
1	Control	0.0	0.0	0.0
2	Extract	57.5±4.32	42.5±3.5	50.3±2.4
3	Omeprazole	--	50.0±2.3	62.5±5.5
4	Sucralfate	64.4±5.5	--	--

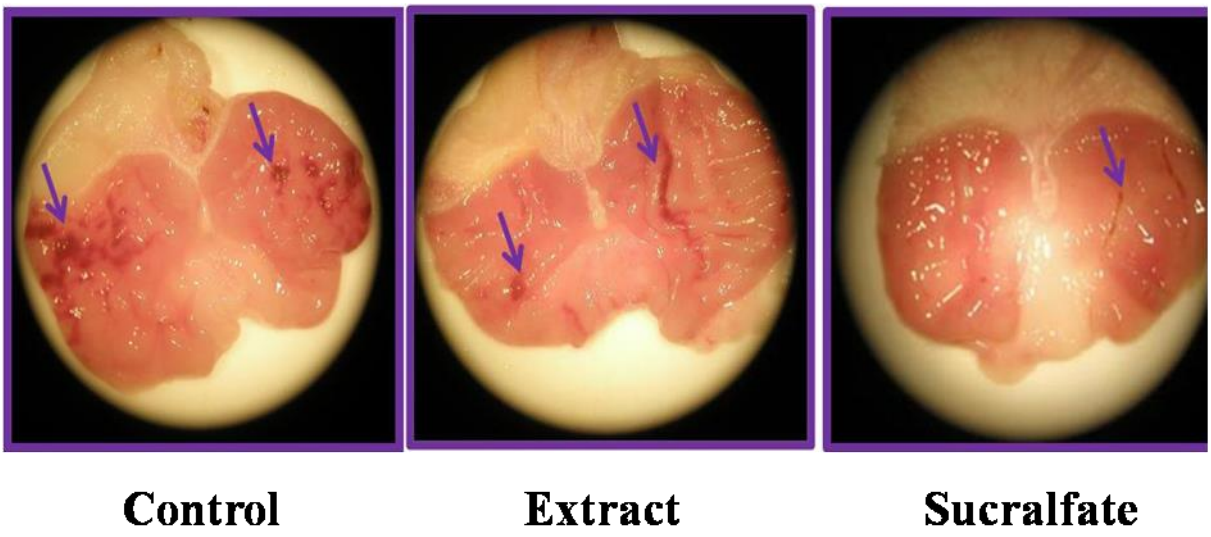

Figure 2: Representative photographs of ethanol induced gastric lesions and protection with Pistia stratiotes and reference drug sucralfate.

3.4 Effect of active extract on gastric secretion:

The antisecretory effect of Pistia stratiotes was evaluated by estimating free and total acid of gastric juice and cytoprotective by estimating the mucin as shown in Table 3. The extract has reduced free acidity 12.34% and total acidity 28.43%, which was comparable with control group and reference drug omeprazole (Omz) exhibiting (45.72%, P<0.01) free acidity and (54.97%, P<0.01) total acidity respectively. It was significantly upregulated mucin secretion by 28.44% (P<0.01) whereas omeprazole (Omz) increased mucin secretion by 31.02% (P<0.05) in comparison to control.

Table 3: *statistically significant at P<0.05 and **P< 0.01, in comparison to control. n = 6 in each group.

Treatment	Free acid µequiv./ml	Total acid µequiv./ml	Mucin µg/ml
Control	73.50±6.832	119.83±5.780	2780.06±281.43
Extract (200mg/kg)	51.75±9.19	87.09±6.830*	4471.63±382.54**
Omz(10mg/kg)	46.14±2.89**	68.70±1.16**	3539.6±264.16*

3.5 Discussion

The present study has been conducted to evaluate the anti-ulcer activity of Pistia stratiotes extract against various models of experimentally induced gastric ulcers and to evaluate its mechanism of action involved in impediment of ulcer development. A preliminary study for the dose fixation of Pistia stratiotes crude extract was conducted. A preliminary study for dose fixation of Pistia stratiotes in CRU model was conducted and 200mg/kg was found to be the optimum dose that can give the highest protection. The screening results were summarized in the table 2. CRU is a well-accepted model for the induction of gastric ulcers, in which peripheral sympathetic activation and increased acid secretion play an important role. (14). Thus, the significant protection imparted by the plant extract in this model reflected the possibility of its involvement in the regulatory mechanism of gastric acid secretion.

Plant extract exerted better protective effect (50.67%) against ethanol-induced gastric lesions (Figure 4 and 5) in comparison to reference drug, sucralfate (64.4%). The ethanol induced acute gastric mucosal injury model is considered to be one of the important and widely used experimental model for ulcer disease (19) Plant extract appears to augment the gastric mucosal defence indicating its latent cytoprotective potential. Ethanol induced a significant macroscopic damage which was evidenced by presence of ulceration hemorrhagic Figure 13. To understand the biological basis of anti-ulcerogenic effect of extract and to determine whether it's anti-secretory effect in vivo, the test compound was given orally to pylorus-ligated (PL) rats (13). The effects of the extract on PL induced gastric ulcer and secretion was examined and compared with the reference drug omeprazole (Table 4). Gastric acid plays a central role in ulcer induction in the pyloric ligation model (20). It is well known that pylorus ligature causes gastric hypersecretion. The cytoprotective nature of extract was evident with the increase in mucin content in pyloric ligation model and protection against ethanol induced ulcer model to a greater extent than the reference drugs. In order to provide more compelling evidence for the gastroprotective effect of Pistia stratiotes, its antiulcer effect against NSAIDs (Non-steroidal anti-inflammatory drugs) induced ulcer model like aspirin was explored. In aspirin induced gastric ulcer model, plant extract showed 50.53% protection, whereas omeprazole (Omz) showed 62.30% protection ($p< 0.05$) in comparison to control as shown in Figure 4. NSAIDs are considered another well established and common cause of peptic ulcers in humans. In conclusion, extract of Pistia stratiotes inhibits the formation of gastric lesions in rats may be by inhibiting acid secretion through the inhibition of H^+, K^+-ATPase proton pump activity and through cytoprotective effects. Plant extract also exhibit antioxidative properties by scavenging the free radicals and reactive oxygen species. Thus plant extract might be a potent therapeutic agent in treating gastric ulcer incidences since it possess anti-secretory, anti-oxidative and cytoprotective potentials.

4. CONCLUSION

Peptic ulcer is one of the major gastro-intestinal disorders and has been a major health problem with a high rate of global incidence. The pathophysiology of these ulcers involves an imbalance between offensive (acid, pepsin, and Helicobacter pylori) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors). As a result more and more drugs are coming up offering newer and better options for the treatment of peptic ulcer. To avoid adverse effects, investigation has been extended to exploit medicinal plant derived novel molecules as new leads which can offer better protection and lower incidence of side effects.

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