Research Article

Evaluation of Madiphalrasayan having Antiulcer Activity in Wistar Albino Rats

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ABSTRACT

Peptic ulcer is one of the major diseases affecting the human population. It develops due to the imbalance between aggressive factors like acid, pepsin, H. pylori and bile salts and defensive factors like mucous, bicarbonate, blood flow, epithelial cell restoration and prostaglandins.1 The anti-ulcer activity of Madiphal rasayana [MR] was evaluated by using the experimental models of acute gastric lesions induced by ethanol and pylorus ligation in rats. Animals pre- treated with doses of 1.35ml/kg and 2.75ml/kg of MR were statistically analyzed and compared to the standard and control group with the parameters like volume of gastric secretion, PH, free acidity, total acidity and % of ulcer protection, absorbance of gastric mucus & hexosemine. The results suggested that the MR significantly decreased the volume of gastric acid secretion, free acidity, total acidity and % of ulcer protection, absorbance of gastric mucus & hexosemine in comparison with standard drug Omeprozole. MR shown significant reduction in lesion index, total affected area and percentage of lesion in comparison with control group in ethanol induced ulcer in experimental models. The gastric mucosal protective effect of MR is brought by inhibiting the gastric secretion, which shows it may act like a proton pump inhibitor. The anti-ulcer activity of MR which reduced gastric volume and total acidity in pylorus ligation ulcer model reveals that MR may act as a H2 receptor antagonist. Present study indicates that MR has anti-ulcerogenic potency in Etnanol induced and pylorus ligation induced ulcers in rats.

Keywords: Anti ulcer, ethanol, madiphalrasayana, peptic ulcer, pylorus ligation

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INTRODUCTION

Peptic ulcer disease (PUD) is a chronic (long lasting) condition that affects the gastrointestinal (GI) tract or digestive system. PUD causes ulcers (soresorlesions) in the lining (mucosa) of the stomach or first part of the small intestine (duodenum).1 Peptic ulcer disease often results in burning pain in the upper centre of the abdomen [1, 2]. In addition to the foods that we eat, a number of other substances also come in contact with the digestive tract. Some of these substances can be harmful to the gastric (stomach) or intestinal mucosa. Substances that can damage the lining of the stomach and duodenum include oral medications (e.g., nonsteroidal anti-inflammatory drugs [NSAIDs]), microorganisms (e.g. bacteria, parasites), and chemicals produced by the body during digestion (e.g., stomach [gastric] acid, pancreatic enzymes, bile) of these drugs are cardiac arrhythmias, blood dyscrasias, hypertension, central nervous system and gastro intestinal disturbances, nephritis, impairment of sexual drive, hepatitis, healing agents etc. [2, 3] pancreatitis, increased liver enzyme activity and triglycerides, leucocytopenia and thrombocytopenia, pharyngitis, purities and electrolyte imbalance [4, 5]. So, there is a necessity to discover a potent and safe anti ulcer drug with less or no side effect [5].

Ayurveda [rasayana] system of medicine plays a vital role in the treatment of many diseases. Rasayana system of medicine is an ancient medical system of [post8th-century] Indian origin. It was understood by two famous people Nagarjunacharya and Nityanadhiya [6]. It emphasizes the treatment of both body and soul by balancing the principal humours. It is based upon Nagarjunacharya [Buddhistmonk]

famous book Rasaratanakaram of who ran a great university-of-Nagarjunasagar at andhra Rasayan medicine gives prime importance to herbal based formulations. The present study involves Madiphalrasayan (MR), one of the ayurvedic-polyherbal formulations, is a compound drug containing five ingredients. This medicine is traditionally used: Anorexia, Indigestion, mildcontipation, vomiting, abdominal pain, apitizer, for So far no scientific studies were carried out to evaluate its medicinal values. Therefore, an attempt had been made to validate its traditional claim for its anti ulcer properties by using the models of acute gastric lesions induced by ethamol induced and pylorus ligation induced in rats [6-8].

MATERIALS AND METHODS:

Materials:

The drug sample ayurvedic polyherbal formulation [Madiphalrasayan-sandu] was purchased from local drug store [8].

Methods:

Preliminary phytochemical screening:

The ayurvedic formulation MR was screened for the presence of various phytoconstituents for the presence or absence of various primary or secondary metabolites employing standard screening test Conventional protocol for detecting the presence of glycosides, saponins, flavonoids, tannins, fixedoilsandfats, terpinoids, alkaloids, carbohydrates [9-10].

Solubility tests:

To understand the solubility of the Ayurvedic formulation MR, the solubility studies were carried out by using various solvents, in this method; one part of the formulation was placed in narrow mouthed screw cap container and the each solvent was added in respective container with continuous shaking using thermostat shaker for 24 hours and found the solubility of the formulation [10].

Pharmacological methods:

Chemicals:

Ethanol, Aclainblue, Omeprozole, NaoH, sodiumacetate tri- Hcl, sucrose, Hcl, magnesiumchloride, Ehrlichreagent, topfersreagent, formalin, DMSO 0.5%, distilled water [11].

Equipment's:

Microscope, centrifuge, Uv-spectrophoto-meter, homogenizer, animal weighing balance, rota shaker [11, 12].

Apparatus:

Burette, pipette, conical flask, Petridis, surgical equipments [13].

Preparation of vehicle:

0.5gm of DMSO is dissolved in 100 ml of water to get 0.5% DMSO which is used as vehicle [14].

Preparation of standard:

0.04gm of Omeprazole is dissolved in 10 ml of vehicle [14].

Animals:

Wistar albino rats of either sex, weighing 150-200 g were used for the study. They were fed standard light cycle (12 h light, 12 h dark) Source of animals: MKM Enterprises. Hyderabad. Healthy albino Wister rats of either sex, housed in animal house from shri Vishnu college of pharmacy bhimavaram, were I selected and maintained under standard laboratory conditions of light at 23 ± 2 0C and 55 ± 5% R.H. The animal housing and handling were done in accordance with CPCSEA guidelines. The experiments were conducted as per the norms of Institutional Animal Ethics Committee (IAEC). The animals were given standard rat pellet feed and purified tap water. After one week of acclimatization, rats were randomly selected and grouped into different groups [14, 15].

Parameters:

Collection of gastric juice:

The stomach was excised carefully keeping the oesophagus closed, opened along the greater curvature and the gastric contents were removed.The gastric contents were collected in plain tubes and centrifuged at 3000 rpm for 5 min; the volume of the supernatant was expressed as ml /100 gm body weight. The mucosa was washed with saline and observed for gastric lesions using a dissecting microscope, ulcer score was determined [16].

Ulcer scoring:

After sacrificing the rat, stomach was removed and opened along the greater curvature, and washed it slowly under running tap water. Put it on the glass slide and observed under 10X magnification for

ulcer [16-18]. The ulcer scores are as follows.

0 = normal coloured stomach, 0.5 = red colouration, 1 = spot ulcers, 1.5 = hemorrhagic streaks2 = Ulcers ≥ 3 but ≤ 5, 3 = Ulcers >5

Mean ulcer score for each animal is expressed as Ulcer Index.

Freeacidity and Total acidity:

Centrifuge the gastric contents are centrifuged at 1000 rpm for 10 min, note the volume. Pipette out 1 ml of supernatant liquid and dilute it to 10 ml with distilled water. Note the PH of the solution with the help of PH meter. Titrate the solution against 0.01N NaoH using topfers reagent as an indicator. (It is Dimethyl-amino-azo-benzene with phenolphthalein and used for detection and estimation of hydrochloric acid and total acidity in gastric fluids) Titrate to end point when the solution turns to orange colour. Note the volume of NaoH which corresponds to free acidity. Titrate further till the solution regains its pink colour. Note the total volume of NaoH which corresponds to the total acidity [18-20]. Acidity (mEq/1/100 g) can be expressed as;

$$\text{Acidity} = \frac{Vol.of\ NaOH \times Normality \times 100}{0.1}\ \text{mEq/l/100 g.}$$

Determination of gastric wall mucus:

Gastric wall mucus was determined in ethanol -induced ulcer models according to the method of Corne et al. (1974). The glandular segments from stomachs were removed, weighed and incubated in tubes containing 1% Alcian blue solution (0.16Msucrose in 0.05M sodium acetate, pH 5.8) for 2 h. The Alcian blue the binding extract was centrifuged (100 g) for 10 min and the absorbency of supernatant was measured at 498 nm [21-23].

The quantity of Alcian blue extracted (gm /gm of glandular tissue) was then calculated.

Preparation of stomach tissue homogenate:

The stomachs were homogenized using ice cold Tris-HCl pH 8.2 (1 g in 10 mL) on ice. The homogenate tissues were centrifuged at 4500 g for 15 min at 4 °C, and then stored at −80 °C until they were used. In this study, the homogenate was analyzed in order to estimate hexosamine synthesis [23-25].

Determination of Total Hexosamine:

The concentration of hexosamine in stomach tissue was analysed according to the reported method of [28] with minor modifications. The hydrolyzation of stomach tissue was carried out using 6 M Hcl, and then neutralized using 6 M NaoH. Next, 0.5 ml of freshly prepared acetyl acetone was added to 0.5 ml of the neutralized samples, and the mixture was boiled at 100 °C for 15 min. Following cooling of themixture, 0.5 ml of Ehrlich reagent was added and the absorbance of colored chromogens was calculated at 530 nm using a UV spectrophotometer [26-28].

Acute toxicity studies:

Acute toxicity studies were carried out according to 420 OECD guidelines, swiss albino rats (150-200 g) were divided into five groups. The rats were fasted for 6 h with only access to water ad labium before experimental study. Group I, II, III and IV animals were administered various doses of Madiphalrasayana formulation i.e 0.1, 0.25, 0.50 and 0.75 ml/kg. Group V received 0.5% DMSO only. All the doses and vehicle were administered by oral route. The animals were observed carefully for toxic symptoms for 72 hours. [29-57].

STATISTICAL ANALYSIS OF DATA

Results were expressed as mean ± S.E.M. The statistical difference between the groups i n the term of the mean rate of ulcer healing was calculated in terms of ANOVA mean ± S.E.M. The difference was considered significant if $P< 0.05$ [29-57].

RESULTS

ACUTE TOXICITY STUDY:

In acute toxicity study, no mortality or toxicity was observed during the experimental period. The trial drug [MR] was considered safe orally up to the dose level of 0.75 ml / kg body weight. No major behavioural changes were noted during the study [29-57].

ETHANOL-INDUCED GASTRIC ULCER:

The results of this study were summarized in (**Table 3**). The administration of two doses of MR (1.35 ml / kg and 2.75 ml / kg

bodyweight) 1 hr later the administration of ethanol 1ml / 200gm produced a significant reduction ($p<0.05$) of ulcer index observed in MR 1 group, whereas highly significant reduction of ulcer index ($p<0.0001$) was noted in the higher dose treated groups (2.75 ml / kg body weight) as compared to the control group.

The standard drug Omeprazole also produced highly significant decrease in ulcer index as compared to the control ($p<0.0001$). MR at the dose level of 2.75 mg/kg has protected the gastric mucosa against ulcerogenic effect of Ethanol with manner [58-60].

Table 1: Qualitative Chemical Tests for Phyto Constituent

S. NO	Phyto constituents	Formulation [+] presence [-] absence
1	Alkaloids	+
2	Glycosides	+
3	Flavonoids	+
4	Terpenoids	+
5	Carbohydrates	+
6	Saponins	+
7	Phytosterols	-
8	Fixedoils and fats	+
9	Phenols	-
10	Tannins	+

Table 2: Solubility Tests

S. NO	Solvents	Solubility
1	Acetone	Insoluble
2	Benzene	Insoluble
3	Chloroform	Soluble
4	CCl4	Insoluble
5	Ethanol	Soluble
6	Methanol	Soluble
7	Petroleum ether	Insoluble
8	Propylene glycol	Soluble
9	Arachis oil	Soluble
10	Caster oil	Soluble
11	Sesame oil	Soluble
12	Coconut oil	Soluble
13	Hot water	Soluble

Table 3: Effect of MR formulation on uv-absorbance of gastric mucus layer, uv-absorbance of hexosomine, mean ulcer index, % protection Ethanol Induced Gastric Ulcer in Rats

Group	Treatment and Dose (mg/Kg)	Uv-absorption of Gastric mucus	Uv-absorption of hexosamine	Mean ulcer index	% Protection
I	Control	0.78±0.04***	0.62±0.05***	9.6 ±0 .6***	--
II	Standard	0.36± 0.20***	0.2±0.26*	3.0 ± 0.15	62.2%
III	MR1	0.46 ±0.03****	37±0.25***	3.7 ± 0.05**	67.2%
IV	MR2	0.40± 0.03***	32± 0.22*	3.2 ± 0.17	62.5%

*Results are mean ±S.E.M. (n=6) Statistical comparison was performed by using ANOVA ****P<0.0001 were considered statistically significant, highly significant and * P<0.05, significant when compared to control group.*

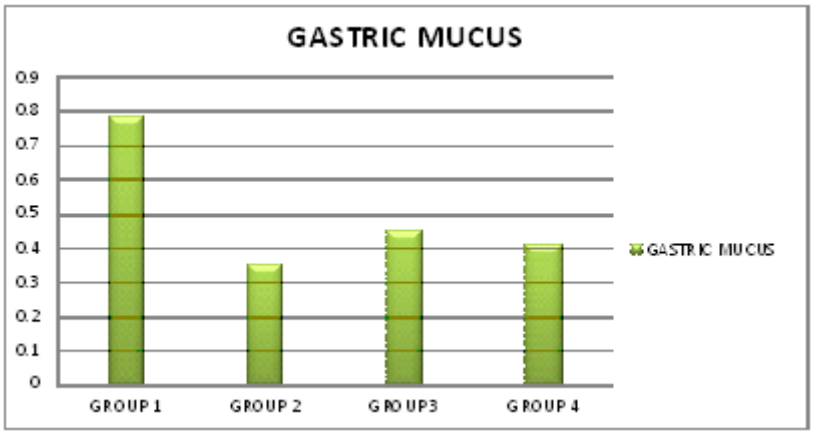


Figure 1: Effect of MR formulation on Uv-absorbance of gastric mucus layer

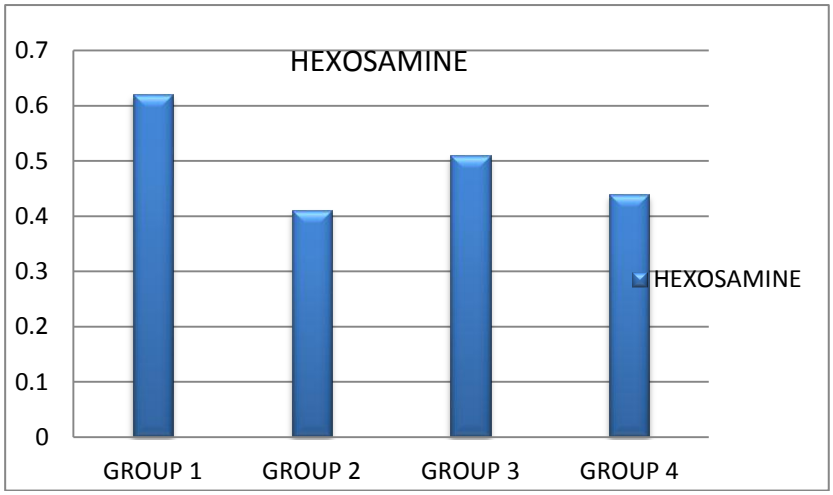


Figure 2: Effect of MR formulation on Uv-absorbance of hexosomine

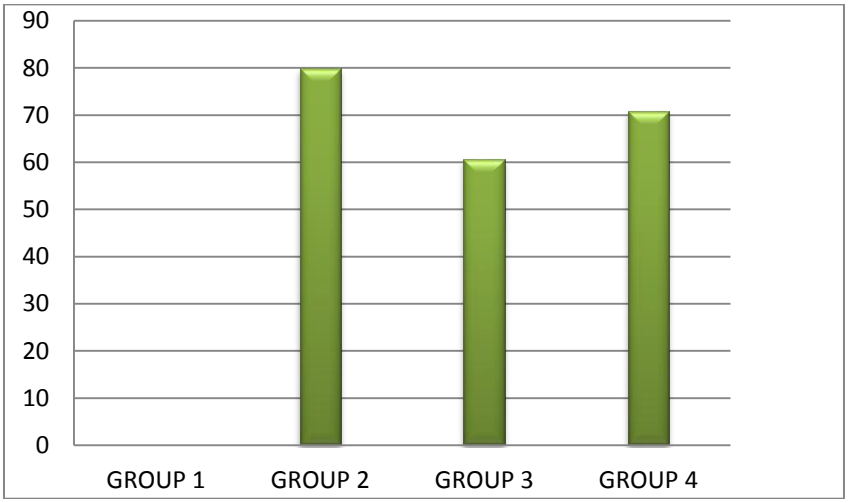


Figure 3: Effect of MR formulation on % protection Ethanol Induced ulcer

The result of pyloric ligation induced gastric ulcer model was summarized in (**Table 2**). In this method, MR at both doses (1.35 ml/kg and 2.75 ml / kg) produced a reduction in the ulcer score, gastric volume, free acidity, total acidity and raised gastric pH significantly in comparison with control produced significant reduction in total acid output as compared to control group 2.75 ml / kg was found to possess remarkable ulcer protective properties and almost exhibited similar effects as that of

omeprozole (20mg/kg) in reducing the gastric volume

PYLORUS- LIGATION INDUCED GASTRIC ULCER:

Table 4: Effect of [MR] against Pylorus Ligation Induced Gastric Ulcer in Rats

Group	Treatment and Dose (mg/Kg)	Gastric volume (ml)	pH	Free acidity (mEq/l)	Total acidity (mEq/l)	Mean ulcer index	% Protection
I	Control	8.4± 0.09****	2.6± 0.06****	45± 0.20****	84±0.94****	3.0± 0.15****	--
II	Standard	5.4± 0.1*	5.7±0.07	32±0.26*	64±0.76*	0.5±0.08	86.6%
III	MR1	6.0 ±0.2	5.4± 0.1	37±0.25****	66±0.76****	1.0±0.08****	71.8%
IV	MR2	5.3± 0.1	5.6± 0.07****	32± 0.22*	63±0.41*	0.8± 0.16	83.5%

Results are mean ±S.E.M. (n=6) Statistical comparison was performed by using ANOVA ****P<0.0001 were considered statistically significant,highly significant and * P<0.05, significant w hen compared to control group

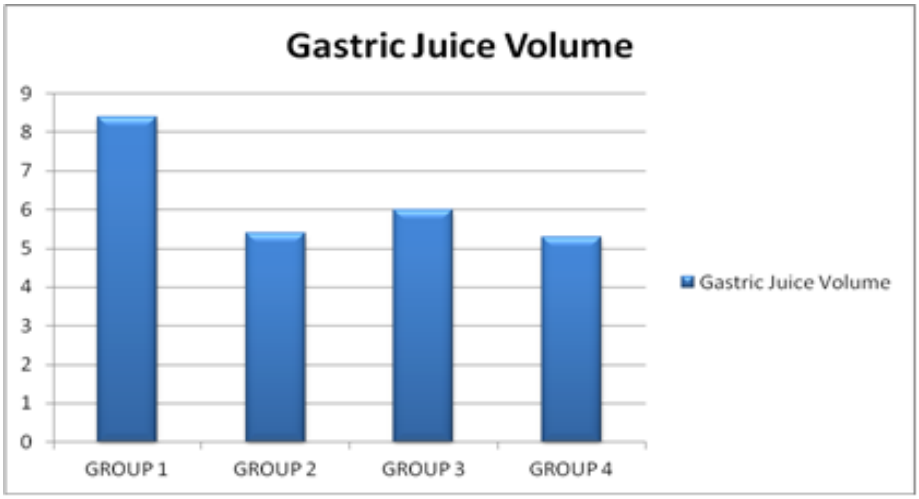


Figure 4: Effect of MR formulation gastric juice volume on pylorus ligation gastric ulcer in rats

Comparable to the standard drug of Omeprazole. The percentage inhibition of ulcer was 86.6 %, 71.8 % and 83.5 % produced by the treatment of standard drug Omeprazole, trial drug MR at dose level 1.35 ml / kg and 2.75 ml/kg respectively The stomach of rats of control group, standard, MR 1 (1.37 ml / kg) and MR 2 (2.75 ml / kg) which appeared to have beneficial ulcer protective effects of standard and trial drugs.

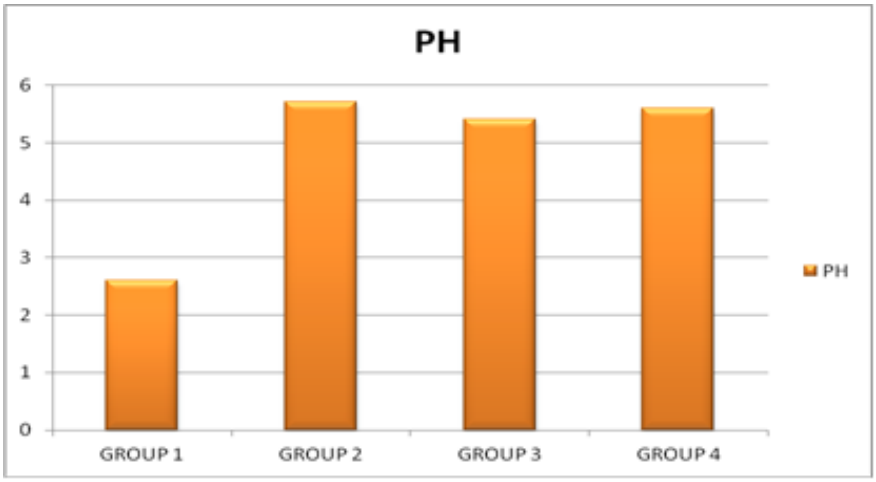


Figure 5: Effect of MR formulation on pH in pylorus ligation Induced ulcer model

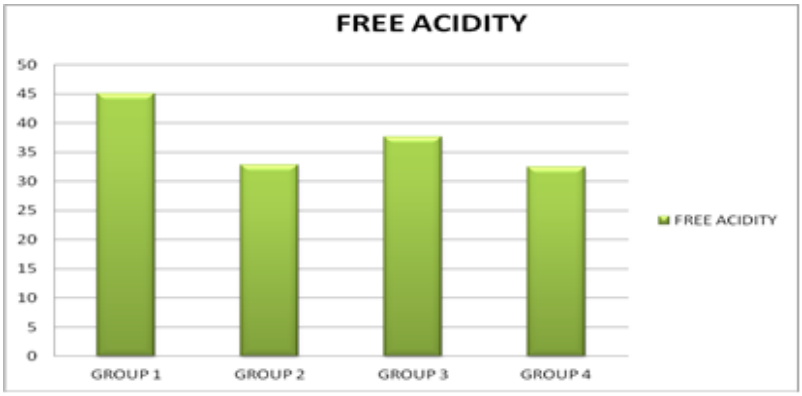


Figure 6: Effect of MR formulation free acidity in pylorus ligation Induced ulcer model

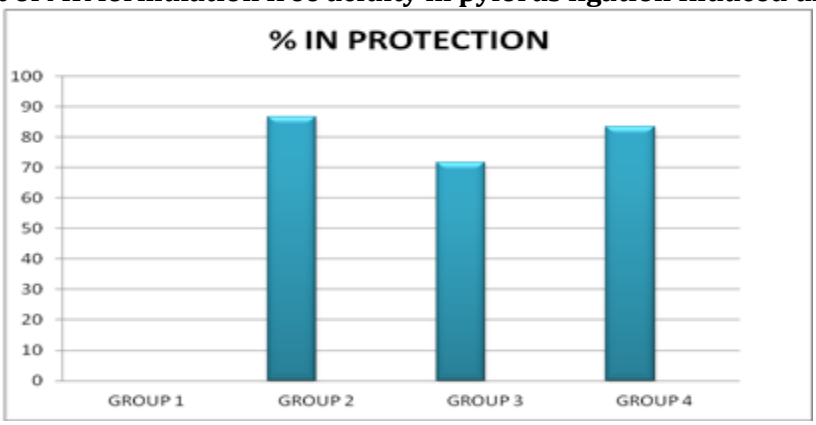
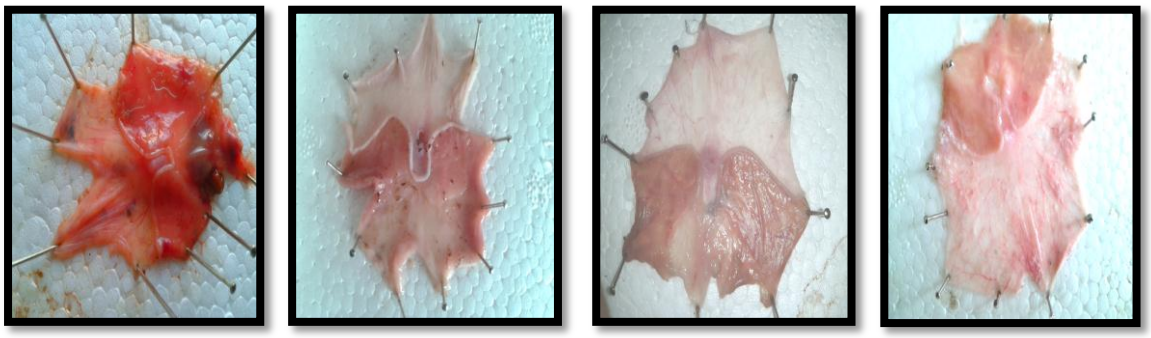
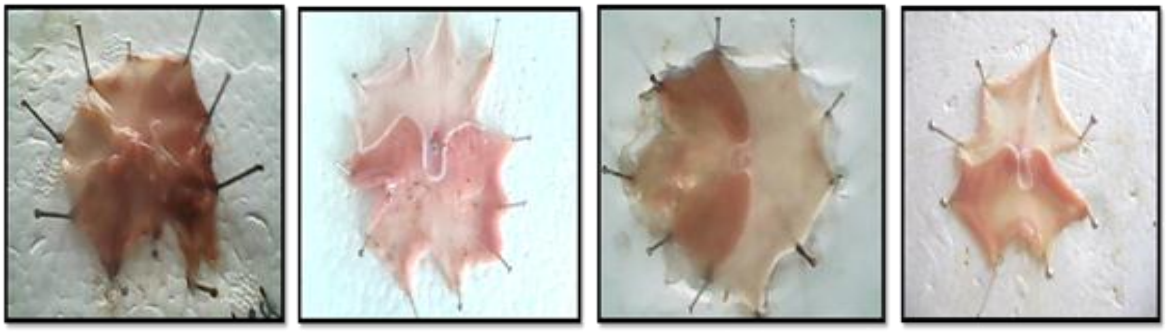


Figure 7: Effect of MR formulation % inhibition in pylorus ligation Induced ulcer



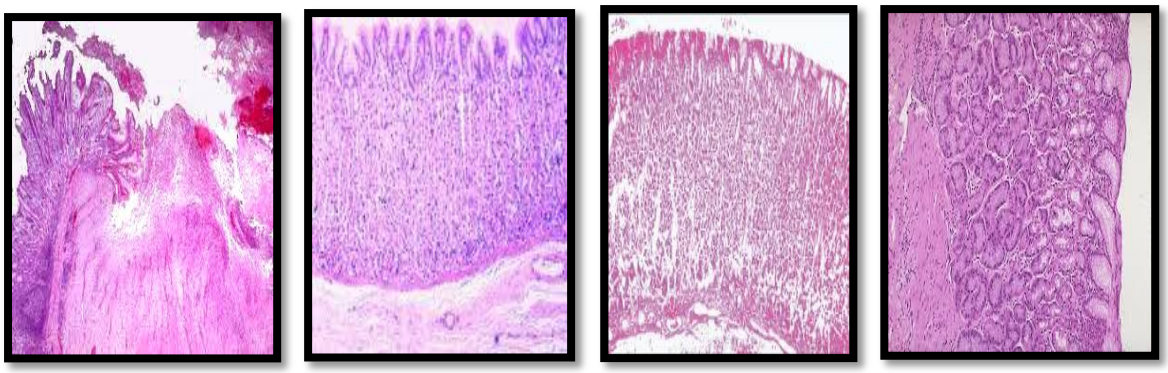
Microscopic appearance of the gastric mucosa in Pyloric Ligation induced ulcer models:

Control	Standard	Test-1	Test-2
Figure 8	**Figure 9**	**Figure 10**	**Figure 11**



Microscopic appearance of the gastric mucosa in ethnolinducedulcer

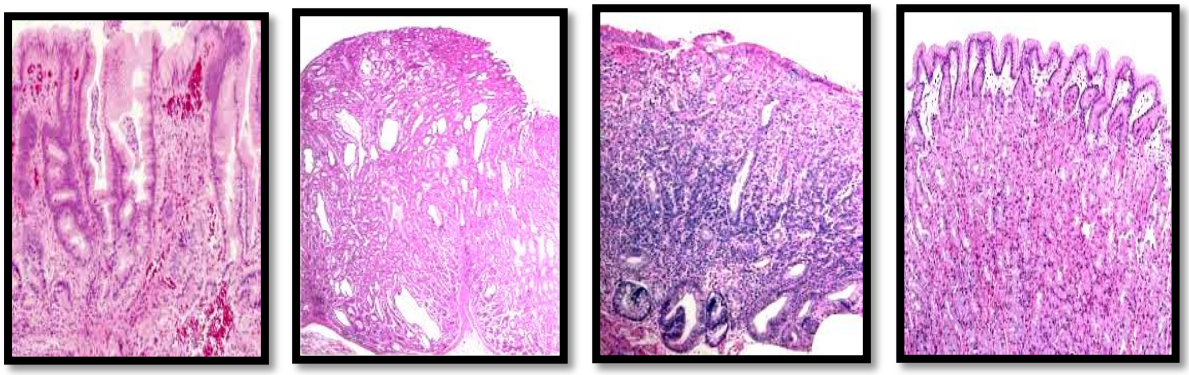
Models: Control	Standard	Test-1	Test-2
Figure 12	**Figure 13**	**Figure 14**	**Figure 15**



Histopathology results of MR formulation in pyloric ligation induced ulcers:

Control	Standard	Test-1	Test-2
Figure 16	Figure 17	Figure 18	Figure 19

Control rat stomach showing severely eroded gastric mucosa haemorrhagic streaks in histamine ulcer induction Rat stomach showing fairly protected gastric mucosa with Omeprazole Pyloric Ligation induced gastric ulcers. Rat stomach showing a protected epithelium due to MR formulation of (2.75ml/kg) in pyloric ligation induced gastric ulceration.



Histopathology results of madiphalrasayana formulation extract of in ethanol induced ulcers : Control Standard Test-1 Test-2

Figure 20	Figure 21	Figure 22	Figure 23

Control (ethanol): superficial ulceration of gastric mucosa near gastro oesophagus junction with erosion,1/3rd to slightly deep are found.Standard (Omeprazole):ulcer not seen in picture, superficial erosions of surface epithelim with shallow ulceration of mucosa MR formulation : only small sized erosions of surface epithelium/no ulcer.

DISCUSSION

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanisms [1-6]. To regain the balance, different therapeutic agents including plant extracts and formulations may be used [7-14]. MR is one such herbal drug formulations used in the present study primarily to evaluate the anti-ulcerogenic in pylorus ligation and ethanol induced ulcers in rats [15-25]. The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid [26]. Pylorus ligation induced ulcers are due to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier [27]. These factors are associated with the development of upper Gastrointestinal

damage including lesions, ulcers and life threatening perforation and hemorrhage [28-31].

Madiphal rasayan formulation anti ulcerogenic activity was studied in ethanol induced gastric mucosal damage model in albino rats. This model was chosen because ALCOHOL abuse is the main exogenous cause of refractory peptic ulcer constituting Gastric ulcer is known as damage of themucosal integrity of the stomach, and duodenum defect produced due to active inflammation [32-41]. Some noxious agents like (acid, pepsin, bile acids, pancreatic enzymes,drugs and bacteria) attacking on the gastroduodenal mucosa by a host of integrity ismaintained by an intricate system that providesmucosal defense and repair Mucus bicarbonatelayer formed an intricate biologic system,surface epithelial cells and a rich sub mucosalmicro-circulatory bed which providesbicarbonate ions which neutralize the acidgenerated by parietal cell section (HCl), duringremoving toxic metabolic, the adequate supplyof micronutrients and oxygen is supplied bymicrocirculatory bed [42-48].

The finding of present study demonstrated that MR significantly protected against mucosal damage induced by ethanol and curative ratios Ethanol induced both long ulcers and petechial lesions with-in a shorttime, which makes this technique suitable for screening experiments for investigation of antiulcer drugs. The genesis of ethanol-induced gastric lesion is of multifactorial origin with the decrease in gastric mucus amount also it is associated with significant production of free radicals leading to cases of peptic ulcer [49-50]. ETHANOL produce a spectrum of injury to the gastric mucosal and form haemorrhages and petechiae to erosions and ulcers which inturn causes damage to cell and cell membranes the animals treated with Madiphalrasayan formulation was found to be devoid of ulcerogenic potential [52].

The above discussion shows that MR the herbal formulation is said to produce beneficial antiulcer activity. In conclusion, to our knowledge, this study provides for the first time evidence that showed gastroprotective effect of MR formulation against ethanol induced ulcer.In our study formulation significantly reduced the ulcers induced by ethanol and results were comparable to omeprazole [53-60].

The antiulcer property of MR in pylorus ligation model and ethanol induced model is evident from its significant reduction in free acidity, total acidity, number of ulcers and ulcer index gastric mucusol estimation,total hexosimine estimation. MR treated animals significantly inhibited the formation of ulcers in the pylorus ligated rats and also decreased both the concentration and increased the pH,and increased the gastric wall mucus and protein content of the gastric mucosa so it is suggested that formulation can suppress gastric damage induced by aggressive factors [61-64].

Ethanol-induced ulcer is mediated through tissue damaging radicals, which are produced from the conversion of hydroperoxyl to hydroxy fatty acids, which leads to cell destruction. The hydroperoxyl fatty acids are generated from the degeneration of mast cells and generalized significantely increase the cell damage (Van Kolfschten et al., 1983). So MR significantly inhibit the celldamage. Omeprazole the proton pump inhibitor play an important role in the reduction of gastric volume and total acidity and thus perform a cytoproective effect From references it is observed that By comparing the effect of various clinical agents on healing of ulcers induced by Ethanol, We observed that among different anti-secretory and cytoprotective agents, omeprazole was found to be most effective drug. Omeprazole produced highest protection of 86.6% followed by misoprostol, ranitidine and sucralfate. These inducing methods of gastric lesions are rapid and convenient way of polyherbal for formulations antiulcer potency and cytoprotection in macroscopically and microscopically visible lesions [65].

The preliminary photochemical analysis of MR showed the presence of flavonoids, triterpenoids, carbohydrates, alkaloids, proteins, aminoacid, volatileoil, glycosides, sapponins and tannins. The significant increase in the antiulcer activity of MR could be attributed to the presence of flavonoids ,proteins , aminoacids,volitileoils,

tannins, saponin glycosides and alkaloid compounds. Flavonoids are among the cytoprotective materials for which antiulcerogenic efficacy has been extensively confirmed [66-74]. It is suggested that, these active compounds would be able to stimulate mucus, bicarbonate and the prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen. So the antiulcer activity of MR may be attributed to its flavonoids content [75].

The results of the present study suggest that the polyherbal ayurvedic formulation of MR may be beneficial in the treatment of gastric lesions. So it possess anti secretory and mucus formation action [76-79]. Further studies to identify the active moieties and elucidation of the mechanism of action are recommended.

CONCLUSION

It can be summarized that the MR formulation possess the antiulcer activity against the Pyloric ligation and Ethanol induced gastric ulceration animal model of rats. Peptic ulcer is the most common disease. Many pharmaceutical chemical drugs are there in market to treat the ulcer, but they are having lot of adverse effects. In the present theory, using Traditional ayurvedic herbal formulations have proved that these are the effective alternatives for chemical drugs. Among the two doses (1.35 and 2.75 ml / kg) of 2.75ml /kg, MR formulation produces significant antiulcer activity. Formulation produces significant anti-ulcer activity which comparable with that of standard drug Omeprazole. The high dose of the formulation show better activity compared to low dose of the formulation showed better activity in pyrolic ligation induced than in Ethanol induced ulcers. The Anti-ulcer activity of MRl formulation is having significant activity in animals models used, as compared to the standard drug Omeprazole.

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