

Evaluation of Antiulcer Potential of Polyherbal Preparation Against Experimentally Induced Ulcers in Rats

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ABSTRACT

Ulcer, the gastrointestinal disorder characterized by presence of painful sores in the stomach and intestine. Ayurvedic formulations are in lead for their use by the people because of side effects of allopathic drugs. In the present study a formulation was prepared and it was evaluated for its antiulcer activity. It has showed an excellent antiulcer activity with effective antioxidant activities. It reduced the formation of ulcers in the stomach both in pylorus ligation and NSAID induced ulcers significantly when compared to that in control group animals. So, from this we can conclude that the polyherbal formulation has good antiulcer activity.

Keywords: Peptic Ulcer, Antiulcer activity, Pylorus Ligation.

INTRODUCTION

Peptic ulcer is characterized by the presence of ulcers in any portion of gastrointestinal tract (GIT) exposed to acid in sufficient concentration and duration¹. An ulcer is defined as a breach in the continuity of the epithelial lining of more than 5mm in diameter, with associated inflammation². Peptic ulcers develop when the balance between defensive factors and offensive factors is disrupted. In current medicine, the treatment includes triple therapy with H₂ receptor blockers + Proton pump inhibitors + antibacterial agent. There are many traditional systems of medicine in the world, each with different associated philosophies and cultural origins^{3,4}.

It was known that the plants *Glycyrrhiza glabra* (Fabaceae), *Emblca officinalis* (Phyllanthaceae) and *Morinda citrifolia* (Rubiaceae) were found to have antiulcer activity and it was reported. In the present study an attempt was made to prepare a polyherbal preparation of the plants using their parts like roots, fruits and leaves respectively to prove a better antiulcer activity^{5,6}.

MATERIALS AND METHODS

Collection of plant material

The plant material like roots of liquorice, fruits of emblica, and leaves of noni were collected from the local areas in and around Tirupati and in S.V. Ayurveda pharmacy, Tirupati. The plant material was authenticated by Dr. B. Sitaram, professor, S.V. Ayurvedic medical college, Tirupati. The plant material was collected and cleaned under tap water and it was shade dried.

Preparation of Poly Herbal Formulation

Based on the previous studies the formulation was made in the ratio of 1:2:1 of *Glycyrrhiza glabra* (Fabaceae), *Emblca officinalis* (Phyllanthaceae) and *Morinda citrifolia* (Rubiaceae).

Preparation of extract

The dried plant material was powdered coarsely and the aqueous extract was prepared by the method of decoction. Weight per ml calculations was done for the extract and it was used accordingly. The extract was subjected to phytochemical analysis.

Experimental animals

Male albino rats of Wistar strain (150-180gm) were used for the study. The animals were housed under standard conditions of temperature (23±1°C), relative humidity (55±1%), 12h/12h light/dark cycle and fed with standard rat pellet diet and water *ad libitum*. All the experiments were performed after obtaining Institutional Animal Ethics Committee clearance from Sri Padmavathi School of Pharmacy.

Acute toxicity studies

Acute toxicity studies were performed as per the OECD guidelines 423.

Antiulcer activity

The animals were grouped into nine groups (n=6) to verify the antiulcer activity.

Pylorus ligation induced gastric ulcers

The antiulcer activity of the polyherbal preparation was evaluated using the method of pylorus ligation.

Group 1 animals received distilled water which served as positive control. Group 2 animals served as disease control animals for pylorus ligation. Group 3 animals received standard drug ranitidine at the dose of 50mg/kg *p.o* and served as standard group. Group 4 & 5 animals received poly herbal extract at two different doses (200 & 400 mg/kg *p.o*). The treatment was given for 28 days. One hour after the administration of drug, under light anesthesia, the abdomen was opened and pylorus end was ligated without causing damage to the blood vessels. The stomach was replaced carefully and the animals were deprived of water

Table 1: Effect of AEPHF against pylorus ligation induced gastric ulcer in rats

S.No.	Group	pH of the gastric content	Free acidity (mEq/L)	Total acidity (mEq/L)	Ulcer index
1.	Normal	3.33±0.333	0.0±0.0	0.0±0.0	0.0±0.0
2.	Disease Control	2.33±0.66	89.333±8.192*	172.0±17.692*	13.200±3.300*
3.	Standard (Ranitidine 50mg/kg, p.o)	3±1.275	26.667±5.696**	40.666±12.34**	2.64±1.320**
4.	Test I (PHF 200mg/kg, p.o)	4±0.577	36.667±6.227**	98.33±10.398**	2.083±0.417**
5.	Test II (PHF 400 mg/kg, p.o)	6.333±0.667	0.0±0.0**	30.0±10.0**	0.833±0.417**

All the values were expressed as mean±SEM for six observations.

*= p<0.05 when compared to normal group.

**=p<0.05 when compared to disease control group.

Table 2: Effect of extract against aspirin induced gastric ulcer in rats

S.No.	Group	Ulcer index
1.	Normal	0.0±0.0
2.	Disease Control	4.112±0.030*
3.	Standard (Ranitidine 50mg/kg, p.o)	1.500±0.050**
4.	Test I (PHF 200mg/kg, p.o)	2.133±0.617**
5.	Test II (PHF 400 mg/kg, p.o)	1.011±0.0417**

All the values were expressed as mean±SEM for six observations.

*= p<0.05 when compared to normal group.

**=p<0.05 when compared to disease control group.

Table 3: Effect of extract on biochemical parameters of gastric juice in pylorus ligation induced gastric ulcers in rats

S.No.	Group	Pepsin activity (mg tyrosine liberated/ml)	Total carbohydrates (mg/ml)	Proteins (mg/ml)	TC:P
1.	Normal	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
2.	Disease Control	4.291±0.156**	5.754±0.103**	7.677±0.452**	0.666±0.015**
3.	Standard (Ranitidine 50mg/kg, p.o)	1.377±0.871**	10.678±1.412**	0.935±0.539**	11.420±1.777**
4.	Test I (PHF 200mg/kg, p.o)	2.8775±0.922**	8.851±0.149**	2.015±1.661**	13.898±11.526**
5.	Test II (PHF 400mg/kg, p.o)	0.469±0.361**	12.960±3.476**	0.625±0.048**	20.736±1.732**

All the values were expressed as mean±SEM for six observations.

*= p<0.05 when compared to normal group.

**=p<0.05 when compared to disease control group.

after post operative stage. 19hrs after surgery, all the animals were sacrificed and stomachs were excised, dissected and ulcer score was noted⁷.

From gastric contents, the following parameters were determined, that is, free and total acidity, pepsin activity, total carbohydrates, and protein content.

Centrifuge the gastric content at 1000 rpm for 10 minutes. 1 ml of the supernatant liquid was and diluted to 10 ml with distilled water. Then gastric contents titrated with 0.01 N sodium hydroxide using Tofer's reagent and phenolphthalein as an indicator to determine free and total acidity. The result was expressed as mEq / litre. The acidity is calculated by using the formula:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1} \times 100$$

NSAIDs induced ulcers

Aspirin was used to induce the ulcers. Aspirin at a dose of 200mg/kg *p.o.* was used.

Group 6 animals served as disease control animals aspirin induced ulcers. Group 7 animals received standard drug ranitidine at the dose of 50mg/kg *p.o.* and served as standard group. Group 8 & 9 animals received poly herbal extract at two different doses (200 & 400 mg/kg *p.o.*). The treatment was given for 28 days. On the last day of treatment, 4 hrs after administration of aspirin, the animals were sacrificed and the stomach was incised. The stomach

Table 4: Effect of aqueous extract on *in vivo* anti-oxidants (pylorus ligation induced ulcers)

S.No.	Group	SOD (U/mg protein)	CAT (uM consumed/mg protein)	H ₂ O ₂	GSH (ug of GSH / mg protein)	MDA (nM of MDA/mg protein)
1	Normal	12.400±2.200	32.733±16.538		30.509±0.686	1.660±0.128
2	Control	3.406±1.505**	7.75±0.347**		0.742±0.313**	2.418±0.216**
3	Standard (Ranitidine 50mg/kg, p.o)	17.441±2.780**	35.103±7.819**		9.493±5.530**	1.533±0.221**
4	Test I (PHF 200mg/kg, p.o)	7.026±1.837**	38.883±3.328**		4.068±1.371**	2.033±0.221**
5	Test II (PHF 400 mg/kg, p.o)	47.666±0.333**	43.18±4.433**		13.82±2.060**	1.341±0.197**

All the values were expressed as mean±SEM for six observations.

*= p<0.05 when compared to normal group.

**=p<0.05 when compared to disease control group.

Table 5: Effect of aqueous extract on *in vivo* anti-oxidants (aspirin induced ulcers)

S.No.	Group	Superoxide dismutase (U/mg protein)	Catalase H ₂ O ₂ (uM consumed/mg protein)	Reduced glutathione (ug of GSH / mg protein)	Lipid peroxidation (nM of MDA/mg protein)
1	Normal	12.400±2.200	32.733±16.538	30.509±0.686	1.660±0.128
2	Control	1.44±0.05	10.60±1.2	11.76±0.51	3.45±0.216
3	Standard (Ranitidine 50mg/kg, p.o)	9.62±0.11	24.81±3.6	20.65±2.9	1.9±0.061
4	Test I (PHF 200mg/kg, p.o)	7.51±0.09	19.3±3.18	19.51±1.371	2.111±0.09
5	Test II (PHF 400 mg/kg, p.o)	9.81±0.133	26.9±3.433	25.62±±1.760	1.841±0.097

All the values were expressed as mean±SEM for six observations.

*= p<0.05 when compared to normal group.

**=p<0.05 when compared to disease control group.

was observed for ulcers. Mucosal scrapings were used for the estimation of oxidative stress⁸.

RESULTS

The Phytochemical studies of aqueous extract of the poly herbal formulation revealed the presence of carbohydrates, proteins, glycosides, flavanoids and tannins. The extract showed no signs of mortality during acute toxicity studies till 2000mg/kg *p.o*. The present study revealed that the aqueous extract has reduced the ulcerations induced by aspirin and pylorus ligation models.

DISCUSSION

The preliminary phytochemical tests revealed the presence of various phytoconstituents. the aqueous extract showed the significant antiulcer activity by decreasing the gastric volume in pylorus ligation method and also by reducing the formation of ulcers in both pylorus ligation and NSAID induced ulcer model in test groups when compared to that in control group animals. The extract reduced the ulcer index and protected the animals from affecting to ulcers. The extract also increased the mucous secretion in pylorus ligation method in test animals. The pepsin activity and the total protein content in the gastric secretion were reduced and the carbohydrate content was increased in the animals

treated with PHF extract. This showed the effective mucous formation of the extract. Also the pH of the gastric content was drastically increased indicating the decreased acidity in the stomach in the same. As a result, the pepsin activity was also reduced. Even the total and free acidity was also reduced. The antioxidant levels were highly elevated in the test group animals which can be indicated by the activities of SOD, Catalase, GSH and lipid peroxidation activities. The aqueous extract of the polyherbal formulation of *Glycerhiza*, *Embllica* and *Morinda* has shown the effective antiulcer activity when compared to that in control.

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