

ANTI – ULCER ACTIVITY OF POLYHERBAL FORMULATION - RO7D ON EXPERIMENTALLY INDUCED ULCER IN RATS

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Summary

Plants which are the major source of drugs in Indian system of medicine have the advantage of little or no side effect. In Ayurvedic system of medicine, Polyherbal formulations were frequently used to enhance the activity or counteract the toxic effect of compounds. The ulcer protective potential of Polyherbal formulation – RO7D was assessed in pyloric ligation (Shay) rat ulcer model and ethanol induced ulcer model in rats. The Polyherbal Formulation – RO7D consists of eleven medicinal plants namely *Centella asiatica*, *Cassia auriculata*, *Cynodon dactylon*, *Rosa damascene*, *Myristica fragrans*, *Nelumbo nucifera*, *Hibiscus rosa-sinensis*, *Hemidesmus indicus*, *Glycyrrhiza glabra*, *Eclipta alba* and *Phyllanthus niruri*. Aqueous extract of RO7D, 100, 200, 400 and 800 mg/kg administered intra-peritoneally as a single dose showed dose dependent ulcer protective effects in the pyloric ligation rat ulcer model and ethanol induced ulcer model in rats. The Polyherbal Formulation – RO7D exhibited (P<0.001) significant decrease in ulcer index in both the model and significant decrease in the gastric volume in pyloric ligation rat ulcer model. The study indicates that extract RO7D has anti-ulcer activity and its anti-ulcer potential may be due to anti-secretory and cyto-protective activity.

Keywords: Polyherbal Formulation, RO7D, Anti – ulcer and Pyloric ligation

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Introduction

Gastric ulcers, one of the most widespread disease states, are believed to be due to an imbalance between acid and pepsin along with weakness of the mucosal barrier. There are many products used for the treatment of gastric ulcers, such as antacids, proton pump inhibitors or antihistaminic agents, but most of these drugs produce several adverse reactions. Thus, there is a need for more effective and less toxic anti-ulcer agents. Plants are some of the most attractive sources, and have been shown to produce promising results for the treatment of gastric ulcer (1). Even though single herbal is effective in the treatment of human ailments, but drugs with multiple mechanisms of protective action may be one way forward in minimizing tissue injury in human disease (2). It has been demonstrated that many drugs or formulations possess potent anti-oxidant actions and are effective in healing experimentally induced gastric ulcers. The herbal formulation derived from ayurveda, the traditional system of Indian medicine, has been found to have antiulcer properties. The anti-ulcer and anti-oxidant activity of *Glycyrrhiza glabra* root has been mentioned earlier (3,4). The anti-ulcer activity of *Centella asiatica* (5), *Rosa damascene*(6), *Hemidesmus indicus*(7) and *Eclipta alba*(8). RO7D, a poly herbal formulation contains *Centella asiatica*, *Cassia auriculata*, *Cynadon dactylon*, *Rosa damascene*, *Myristica fragrans*, *Nelumbo nucifera*, *Hibiscus rosa-sinensis*, *Hemidesmus indicus*, *Glycyrrhiza glabra*, *Eclipta alba*, *Phyllanthus niruri* as disclosed by the manufactures, Rumi herbals, Chennai, India. These herbal ingredients have a folkloric claim for their anti ulcer activity. However, till now scientific validation for such claim is lacking. An attempt was made in the present study to explore the anti-ulcer activity of the aqueous extract of polyherbal formulatin RO7D (AERO7D) using standard experimental models like shay rat procedure and alcohol induced ulcer model.

Materials and Methods

Preparation of Extract

The aqueous extract of polyherbal formulatin RO7D (AERO7D) was prepared in distilled water using a soxhlet apparatus, shade dried under reduced pressure using a rotatory flask evaporator. This extract was used for experimentation.

Animals

Male Wistar rats weighing between 150 – 220 gm were used for this study. The animals were obtained from animal house, Arupadai Veedu Medical College, Pondicherry, India. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of $24\pm 2^{\circ}\text{C}$ and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee and were in accordance with the guidelines of the CPCSEA.

Pyloric Ligation Induced Ulcer

The method of Shay rat ulcer was adopted (9). The animals were divided into six groups each consisting of six rats. Group 1 represented Control group of animals received normal saline (1ml/kg).

Group 2-5 received AERO7D in doses of 100, 200, 400 and 800mg/kg, and groups 6 served as reference control, received ranitidine (30mg/kg). All the test drugs were administered through intra peritoneally to rats. After the test drug administration, the rats were kept for 18 h fasting and care was taken to avoid coprophagy. The animals were anaesthetized with anaesthetic ether. The abdomen was opened by a small midline incision below the xiphoid process; pylorus portion of stomach was slightly lifted out and ligated. Precaution was taken to avoid traction to the pylorus or damage to its blood supply. The stomach was placed carefully in the abdomen and the wound was sutured by interrupted sutures. Four hours after pylorus ligation the rats were sacrificed and the stomach was removed. The stomach was then incised along the greater curvature and observed for ulcers. The number of ulcers was counted using a magnifying glass and the diameter of the ulcers was measured using a vernier caliper and expressed as ulcer index (4).

Ethanol-Induced Gastric Ulcers (5)

The animals were divided into six groups each consisting of six rats. Group 1 represented Control group of animals received normal saline (1ml/kg). Group 2-5 received AERO7D in doses of 100, 200, 400 and 800mg/kg, and groups 6 served as reference control, received ranitidine (30mg/kg). The test drugs were administered for 3 days through intra peritoneal route to rats. On day 3, for 48 h fasting rats the last dose was administered, 60 min prior to induction of gastric ulcers by oral administration of ethanol 1ml/200gm of rat. The animals were sacrificed and examined for gastric ulcers 60 min later. Ulcer index was scored as described earlier (4).

Statistical Analysis

The values were expressed as mean \pm SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' - test. P values <0.05 were considered significant.

Results

Pyloric Ligation Induced Ulcer

The effects of AERO7D at various doses were studied in pylorus ligated gastric ulcer model in rats were shown on table 1. AERO7D at 100, 200, 400 and 800 mg/kg given i.p, as a single dose showed dose dependent protective effect against gastric ulcer induced by pyloric ligation. AERO7D at a doses of 400 and 800 mg /kg inhibited ulcer formation and gastric volume significantly ($p<0.01$ and $p<0.05$, respectively), but failed to do so for at 100 and 200mg/kg. Ranitidine, the reference anti-ulcer agent, significantly ($p<0.001$) inhibited the ulceration and gastric volume induced by pyloric ligation. The effects were remarkable at doses of 800 mg/kg as compared to those in the vehicle treated groups.

Ethanol Induced Ulcer

The effects of AERO7D at various doses were studied in ethanol induced ulcer in rats were shown on table 2. In alcohol induced ulcer model, ranitidine 30mg mg/kg significantly reduced the ulcer index whereas AERO7D inhibit the ulcer index significantly at the doses 800, 400mg/kg ($p<0.001$) and 200mg/kg ($p<0.05$). The anti-ulcer effect of AERO7D was comparable with that of reference drug ranitidine.

Table 1: Effect of AERO7D on the ulcer index and gastric volume of pyloric ligation induced ulcer in rats.

Drug Treatment	Ulcer Index	Gastric Volume (ml)
Control (Normal Saline)	72.33±3.40	11.6±0.27
Ranitidine (30mg/kg)	23.83±0.65***	3.9±0.08***
AERO7D (100mg/kg)	72.50±2.38	10.9±0.41
AERO7D (200mg/kg)	68.5±4.65	10.5±0.41
AERO7D (400mg/kg)	59.17±2.42*	7.18±0.30*
AERO7D (800mg/kg)	45.33±6.12**	5.2±0.38**

Values are presented as mean ± SEM (n = 6)

***P<0.001, **P<0.01 and *P<0.05 Vs control

Table 2: Effect of AERO7D on the ulcer index of ethanol induced ulcer in rats.

Drug Treatment	Ulcer Index
Control (Normal Saline)	71.5±1.77
Ranitidine (30mg/kg)	6.0±0.68***
AERO7D (100mg/kg)	69.7±0.88
AERO7D (200mg/kg)	58.2±1.05*
AERO7D (400mg/kg)	27.0±1.16***
AERO7D (800mg/kg)	13.8±1.38***

Values are presented as mean ± SEM (n = 6)

***P<0.001, **P<0.01 and *P<0.05 Vs control

Discussion

AERO7D showed ulcer protective effects as observed from significant decrease in acute ulcers induced by pylorus ligation and ethanol. Ulcers due to pyloric ligation are due to increased accumulation of gastric acid and pepsin leading to auto digestion of gastric mucosa (6). In the ethanol induced gastric ulceration model, ethanol produces necrotic lesions by direct necrotizing action which in turn reduces defensive factors, the secretion of bicarbonate and production of mucus (7). In pyloric ligation model, AERO7D prevented the ulcer formation and decreased the gastric secretion; this may be due to its anti-secretory activity. The anti-ulcer activity of AERO7D in ethanol induced ulcer model may be due to increase the mucus secretion.

The present investigation establishes the anti ulcer activity of AERO7D in pylorus ligation and ethanol induced ulcer model and it may be due to its anti secretory, muco protective, increase blood circulation or bicarbonate production. The effectiveness of AERO7D as anti ulcerogenic could be due to its various actions on offensive and defensive factors. Hence further investigation is required on offensive and defensive factors in the gastric juice and mucosa to locate its exact mechanism of action.

References

- 1) Srinivasan D, Ramaswamy S, Sengottuvelu S. Protective effect of polyherbal formulation on experimentally induced ulcer in rats. *Pharmacologyonline*. 2008; 1: 331-350.(1)
- 2) Barry H. Antioxidant effects, a basis for drug selection. *Drugs*. 1991; 42: 569.(2)
- 3) Bafna PA, Balaraman R. Anti-ulcer and anti-oxidant activity of Pepticare, a herbomineral formulation. *Phytomedicine*. 2005; 12: 264-270.
- 4) De B, Maiti RN, Joshi VK, Agrawal VK, Goel RK. Effect of some Sitavirya drugs on gastric secretion and ulceration. *Ind J Exp Biol*. 1997; 35: 1084–1087.
- 5) Sairam K, Rao CV, Goel RK. Effect of *Centella asiatica* Linn on physical and chemical factors induced gastric ulceration and secretion in rats. *Indian J Exp Biol*. 2001;39;137-142.
- 6) Maleev A, Neshtev G, Stoianov S and Sheikov N. The ulcer protective and antiinflammatory effect of Bulgarian rose oil. *Ekspimentalna K.T.L. Sina. I. Morfologi*. 1972; 11: 55–60 (article in Bulgarian). (6)
- 7) Anoop A, Jegadeesan M. Biochemical studies on the anti-ulcerogenic potential of *Hemidesmus indicus* R.Br. var. *indicus*. *J Ethnopharmacol* 2003;84:149-156.
- 8) Manonmani S, Vishwanathan VP, Subramanian S, Govindasamy S. Biochemical studies on the antiulcerogenic activity of cauvery 100, an ayurvedic formulation in experimental ulcers. *Ind J Pharmacol*. 1995; 27: 101 – 105.
- 9) Shay M, Kamarov SA, Fels D, Meraaze D, Grueinstein H, Siple H. A simple method for the uniform production of gastric ulceration in the rat. *Gastroenterol*. 1945; 5: 43–61.(9)
- 10) Suzuki Y, Hayashi M, Ito M, Yamagami I. Antiulcer effects of 40-(2-carboxyethyl) phenyl trans-4-aminomethyl cyclohexane carboxylate hydrochloride (Cetraxate) on various experimental gastric ulcers in rats. *Jpn. J. Pharmacol*. 1976; 26: 471–480.(10)
- 11) Suleyman H, Ackay C, Altikayanak K. The effect of nimesulide on indomethacin and ethanol induced gastric ulcer in rats. *Pharmacol Res*. 2002; 45: 155–158.(11)
- 12) Goel RK, Bhattacharya SK. Gastroduodenal mucosal defense and mucosal protective agents. *Ind. J. Exp. Biol*. 1991; 29: 701–714.
- 13) Marhuenda E, Martin MJ, Alarcon de la Lastra C. Antiulcerogenic activity of aescine in different experimental models. *Phytother Res*. 1993; 7: 13–16.