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ANTI – ULCER ACTIVITY OF POLYHERBAL FORMULATION – RO12 ON EXPERIMENTALLY INDUCED ULCER IN RATS

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ABSTRACT

RO12, a polyherbal formulation, is a marketed drug which has been used in various gastrointestinal problems, however, there is no such clear documentation for antiulcer activity. So, the present study was carried out to test its antiulcer activity in rats using different models. Wistar rats were grouped into four, each group with 6 animals and injected with Normal Saline (control), Ranitidine 50 mg/kg (Standard drug), RO12 aqueous extract 200mg/kg and 400mg/kg (test drug) per orally in case of pylorus ligation model. In case of ethanol induced ulcer, misoprostol 10 mcg/kg was given as standard keeping everything else same as pylorus ligation method. Acute gastric ulceration in rats was produced by oral administration of ethanol and by pylorus-ligated technique. The results showed that in both pylorus ligation and ethanol induced ulcer models, the aqueous extract RO12 exhibited significant decrease ($P<0.001$) in ulcer index, ulcer score, free acidity, total acidity, pepsin content in pylorus ligation induced ulcer as well as in ethanol induced ulcer models. This study demonstrated that, RO12 polyherbal formulation possess significant antiulcer activity. The aqueous extract of RO12 possesses dose dependent antiulcer effect. Current studies confirmed the claimed antiulcer activity in selected doses (200mg/kg and 400mg/kg).

Key words: Polyherbal Formulation, RO12, Antiulcer, Pyloric ligation.

INTRODUCTION

Ulcer is a symptomatic gastrointestinal disorder resulting as a breach in the mucosal membrane of the alimentary tract, which passes through the muscularis mucosa into the submucosa or deeper Peptic ulcer diseases (PUD) most commonly occur when the linings of stomach, so called duodenal ulcer (DU) are corroding by the acid-peptic juices (Tham *et al*., 2001). Acid peptic diseases include hyperacidity, gastroesophageal reflux diseases (GERD), stress induced mucosal erosions and peptic ulcers (gastric as well as duodenal). The corrosive action of pepsin and hydrochloric acid on the mucosa of upper gastrointestinal tract is related to both gastric as well as duodenal ulcers. Peptic ulcer arises when the normal mucosal defensive factors (mucus, mucosal blood flow, formation of bicarbonate and PGE) are impaired or over powered by the aggressive factors (acid, pepsin, epithelial cell restoration) (Sharma *et al.*, 2007, Gilmann *et al*., 2006, Barar FSK, 2003). The other causes which leads to PUD include *Helicobacter pylori* (*H. pylori*) infection, long term and high dose of drugs like NSAID's, diseases like *Zollinger-Ellison syndrome* and other factors which includes various psychological factors like smoking, stress and alcohol consumption to larger extent. Incidence is higher in the third world and the life time risk of developing PUD is 10% (Peckenpaugh *et al*., 1997).

Various plants are being used in complementary and alternative medicines for management of gastric ulcer, because of their minimum toxicity and more effectiveness. According to practitioners of traditional medicine, a combination of herbs exhibits augmented therapeutic efficacy than a single herb (Toews *et al.*, 2005). The Poly herbal formulation RO12 is a marketed drug, which contains: *Glycyrrhiza glabra, Rosa damascena, Citrus aurantifolia, Aegles marmelos, Saccharum officinarium and Eletteria cardomum* as

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disclosed by the manufactures, Rumi herbals, Chennai, India. The antiulcer properties and antioxidant activity of *Glycyrrhiza glabra root* has been established (Bafna *et al.,* 2005, De B *et al*., 1997). The anti-ulcerogenic activity of *Rosa damascena* (Maleev *et al.,* 1972), *Aegles marmelos* (Shenoy *et al*., 2012), *Elleteria cardamom (Jafri et al., 2001)* were established. Some of these ingredients have shown to produce considerable increase in levels of endogenous antioxidant enzymes. Since no clear documentation of it possessing antiulcer activity has been established. Hence, the present study was considered worthwhile to investigate the anti-ulcer effects of RO12 polyherbal formulation along with its effect on the antioxidant enzymes to justify whether the formulation exerts an anti-ulcer activity.

MATERIALS AND METHODS

Drug

RO12 a polyherbal formulation was gifted by Rumi Herbal Research Institute Private Limited, Chennai. The ingredients of RO12 were authenticated by the research Laboratory of Rohini Global Marketing (P) Ltd.

Preparation of Extract RO12

The aqueous extract of polyherbal formulation RO12 was prepared in distilled water using a soxhlet apparatus, evaporated under reduced pressure using a rotatory flask evaporator..

Preliminary phytochemical group test

The preliminary phytochemical group test of the aqueous extract of RO13 was performed by the standard methods to see the various chemical constituents. (Tyler *et al.*, 1993, Plummer DI, 2002)

Experimental Animals

Male Wistar rats (150 – 200g) were used for this study. The animals were procured from Sri Venkateshwara Enterprises No.4304, 13th main, 1st cross, subramanyamnagar, Bangalore, INDIA. The animals were placed at random and allocated to treatment groups in polypropylene cages provided with paddy husk as bedding. Animals were maintained under standard temperature of 24±2^{o}C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were given free access to standard commercial pelleted rat chaw and water *ad libitum*. All the protocols and the experimental procedures used in this study were reviewed by the Institutional Animal Ethics Committee and were in accordance with the guidelines of the CPCSEA (Ref. No.461/01/C/CPCSEA).

Acute toxicity studies

The acute toxicity of RO12 polyherbal formulation was determined as per the OECD guideline no. 423 (Acute toxic class method). It was observed that all doses up to 2000mg/kg were safe, so 1/5th (400mg/kg) and 1/10th (200mg/kg) of safety dose of RO12 polyherbal formulation were selected as high dose and low dose respectively for this study.

EXPERIMENTAL DESIGN

Pyloric Ligation Induced Ulcer

The method of Shay rat ulcer was adopted. The rats were divided into 4 groups(n=6). GROUP A: Normal animals treated with vehicle; GROUP B: Standard Ranitidine (50 mg/kg); GROUP C: Low dose of RO12 (200 mg/kg); GROUP D: High dose of RO12 (400mg/kg). The animals were fasted for 24 hours before pylorus ligation with free access to water *ad libitum* by placing those individually in cages to avoid caprophagy and cannibalism. All animals were administered normal saline (1 ml/rat p.o.) twice daily. After the pretreatment period of 1 h, the animals were anaesthetized with anesthetic ether, the abdomen was opened by midline incision below the xiphoid process to avoid damage to the pylorus or damage to its blood supply precaution was taken. The animals were deprived of food and water during the postoperative period and animals were sacrificed six hours after pylorus ligation by the over dose of ether anesthesia animals were sacrificed six hours after pylorus ligation.

The content of the stomachs were collected and centrifuged after stomachs being isolated. The volume of the gastric juice output was measured and this was used for determination of free acidity, total acidity (Hawk *et al*., 1947), pepsin content (Debnath *et al.,* 1974) and total proteins (Lowry *et al*.,1951). The stomach samples were scanned using a computer scanner after being cut open along the greater curvature. The total mucosal area and total ulcerated area was measured using public domain image processing and analysis program developed at National Institute of Health, USA.

Ethanol induced ulcers

Male wistar rats of 150-200 g were selected and weighed and marked for identification. The rats were divided into 4 groups (n=6). GROUP A: Normal animals treated with vehicle only; GROUP B: Standard (misoprostol 100 µg/kg, *p.o.*); GROUP C: Low dose of RO12 (200mg/kg); GROUP D: High dose of RO12 (400mg/kg). Ethanol (90%) was administered to induce ulcer. The animals were fasted for about 36 hours before ethanol was administered. The standard drug (Misoprostol 100 µg/kg p.o.) or the RO12 extracts (200 mg/kg & 400 mg/kg p.o.) were administered one hour before ethanol administration. To all the animals Ethanol (90%) was administered at a dose of 1ml/200gm rat and after one hour all the animals were sacrificed, stomachs were

isolated and ulcer index was determined as mentioned above (Brzozozowski *et al.,* 1998).

Histopathological studies

Sections of tissue from stomachs were examined histopathologically to study the anti-ulcerogenic activity of RO12 polyherbal formulation. The gastric samples were fixed in 10% buffered formalin for 24 hr and afterwards processed using a tissue processor. The processed tissues were embedded in paraffin blocks and about 5-µm thick sections were cut using a rotary microtome. These sections were stained with hematoxylin and eosin. The slides were then examined microscopically for pathomorphological changes such as congestion, hemorrhage, edema and erosions (Galighar *et al.,*1971)

Statistical Analysis

The values were expressed as Mean ± SEM. Data was analysed using one way ANOVA followed by Tukey-kramer post hoc multiple comparison test using Graph pad InStat version 3.00. The results were analyzed by the one- way ANOVA. P value of <0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Pyloric Ligation Induced Ulcer

Aqueous extract of RO12 showed a considerable reduction in ulcer index and ulcer score when compared to control ($p<0.001$) and the high dose (400mg/kg) significantly reduced the ulcer index when compared with that of the low dose (200mg/kg) but ulcer score was reduced significantly by the low dose when compared with that of the high dose ($P<0.01$) as shown in table 1.

The low dose of aqueous extract of RO12 (200 mg/kg, *p.o*.) showed a significant reduction in free acidity ($p<0.01$) and total acidity ($p<0.001$), total protein with $P<0.001$, and pepsin content with $P<0.01$ as shown in table 2 and the high dose of (400 mg/kg, *p.o*.) also produced reduction in total acidity ($p<0.001$) and free acidity ($p<0.001$) when compared to control. The total protein content was increased in higher dose (400mg/kg) than in lower dose (200mg/kg). The high dose significantly reduced the total, free acidity, and pepsin content when compared to low dose ($P<0.01$, $P<0.05$) as shown in table 2.

Ethanol Induced Ulcer

Aqueous extract of RO12 showed a considerable reduction in ulcer index and ulcer score when compared to control ($p<0.001$) as shown in Table 3. The high dose of aqueous extract of RO12 (400 mg/kg, *p.o*.) showed a significant reduction in ulcer index as well as ulcer score than the low dose (200 mg/kg, *p.o*.) as given in Table 3. The high dose of RO12 showed reduction in ulcer index and ulcer score comparable to that of a standard ($P<0.05$).

Table 1. Efficacy of RO12 in pyloric ligation induced gastric ulcer on ulcer index and ulcer score in rats

GROUP	TREATMENT	ULCER INDEX	ULCER SCORE
Group 1 (Control)	Normal Saline(1 ml/kg)	0.54±0.017	2.308±0.24
Group 2 (Standard)	Ranitidine(50 mg/kg)	0.198±0.002***	0.285±0.016***
Group 3 (Low Dose)	RO12 (200 mg/kg)	0.285±0.016**	1.106±0.043***
Group 4 (High Dose)	RO12(400 mg/kg)	0.208±0.007***	1.778±0.031**

All the values are Mean ± SEM n=6. ***P<0.001, **P<0.01, *P<0.1 compare Vs control(group 1). Data was analysed using one way ANOVA and Tukey Test.

Table 2. Efficacy of Ro12 in pyloric ligation induced gastric ulcer on free acidity, total acidity. Total protein content and pepsin content in rats

GROUP	TREATMENT	FREE ACIDITY (meq/l)	TOTAL ACIDITY(meq/l)	TOTAL PROTEIN	PEPSIN CONTENT
Group 1 (Control)	Normal Saline (1 ml/kg)	79.96±0.44	100.46±0.522	4.0529±0.03	0.170±0.001
Group 2 (Standard)	Ranitidine(50 mg/kg)	33.59±0.253***	47.9±1.635	2.44±0.22***	0.057±0.002***
Group 3 (Low Dose)	RO12 (200 mg/kg)	60.103±0.326**	82.25±0.465**	6.55±0.095***	0.125±0.002**
Group 4 (High Dose)	RO12(400 mg/kg)	46.89±0.273***	68.86±0.23***++	5.35±0.169***+	0.116±0.002**

All the values are mean ± SEM n=6. ***P<0.001, **P<0.01, *P<0.1 compare Vs control (group 1). Data was analysed using one way ANOVA and Tukey Test.

Table 3. Efficacy of RO12 in ethanol induced gastric ulcer in rats

GROUPS	TREATMENT	ULCER INDEX	ULCER SCORE
Group 1 (control)	Normal saline (1 ml/kg)	1.028±0.002	6.4±0.239
Group 2 (Standard)	Misoprostol (10 mcg/kg)	0.157±0.008***	1.26±0.024***
Group 3 (Low Dose)	RO12 (200 mg/kg)	0.273±0.016***	2.27±0.024***
Group 4 (High Dose)	RO12 (400 mg/kg)	0.204±0.007***	2.71±0.037***

All the values are mean ± SEM n=6. ***P<0.001, **P<0.01, *P<0.1 compare Vs control (group 1). Data was analysed using one way ANOVA and Tukey Test.

DISCUSSION

Ulcer formation is currently viewed as an interactive process resulting from an imbalance of aggressive acid-pepsin and defensive mucosal factors causing a break in the line of gastrointestinal mucosa. Because of multifactorial etiopathogenesis of mucosal damage and there is now an evidence that the gastric mucosa can increase its resistance to injury when challenged repeatedly with many agents, including ethanol, acid, alkali, hyperosmolar solution, bile acids and non-steroidal anti-inflammatory drugs and over a periods of time ranging from a few minutes to several weeks. Various factors that have been implicated in the pathogenesis of gastric ulcers are an increase in gastric acid secretion, pepsin activity and oxidative stress in the gastric mucosa, and decrease in mucous and bicarbonate secretion (Wallace *et al.*, 1996, Granger *et al.*, 1986, Tandon *et al.*, 2004,Hung CR, 2005). The polyherbal formulation ROI2 is composed of extracts of *Glycyrrhiza glabra* , *Citrus aurantifolia*, *Aegles marmelos*, *Elleteria cardamom, Rosa damacena*. Of these *Glycyrrhiza glabra*, *Citrus aurantifolia* , *Aegles marmelos* have been shown to exhibit antiulcer properties (Bafna *et al*., 2005, Maleev *et al.* 1972, Shenoy *et al*. 2012. The antioxidant properties of *Elleteria cardamom and Glycyrrhiza glabra* (De B *et al*., 1997) were investigated and found to possess free radical properties. Some of these ingredients have shown to produce considerable increase in levels of endogenous antioxidant enzymes.

In studies using various models, the animals treated with RO12 showed ulcer protective effects as observed from significant decrease in acute ulcers induced by pylorus ligation, ethanol and cysteamine. Pylorus-ligated ulcers may be due auto digestion of gastric juice, decrease mucosal blood flow and break down of mucosal barrier (Sohn *et al*., 2003). Ethanol induced gastric ulcers have been widely used for the evaluation of gastro protective activity. Ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radicals. The incidence of ethanol induced ulcers is predominant in the glandular part of stomach. It was reported to stimulate the formation of leukotriene C4 (LTC_4), mast cell secretory products and reactive oxygen species resulting in the damage of rat gastric mucosa (Obi *et al*., 2000). It has been found that oxygen derived free radicals are implicated in the mechanism of acute and chronic ulceration in the gastric mucosa and scavenging these free radicals can play an appreciable role in healing these ulcer (Umamaheshwari *et al*., 2007). In pyloric ligation model, RO12 prevented the ulcer formation and decreased the gastric secretion; this may be due to its anti-secretory activity. The anti-ulcer activity of RO12 in ethanol induced ulcer model may be due to increase in mucus and bicarbonate secretion.

On comparing the two models it was seen that RO12 and standard drugs afforded more protection against development of ulcers in pylorus ligation and ethanol induced ulcer models. The present investigation established the anti ulcer activity of R012 in pylorus ligation, ethanol induced ulcer and it may be due to its anti secretary, muco-protective, increase blood circulation or bicarbonate production.

CONCLUSION

In conclusion, the present study showed that the antiulcer activity of the test compound was perhaps a result of the interplay between its antisecretory, cytoprotective and the antioxidant properties. These findings suggest the potential for use of RO12 as an adjuvant in the antiulcer therapy.

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