www.jahm.in
(ISSN-2321-1563)

ORIGINAL RESEARCH ARTICLE- EXPERIMENTAL STUDY

ANTIULCER ACTIVITY OF RASA PARPATI AGAINST PYLORUS LIGATION INDUCED GASTRIC ULCER IN RATS

ANUROOPA H K[1] SUMAN[2] HANUMANTHACHAR JOSHI[3]

ABSTRACT

Background: Rasa Parpati is one of the Rasayoga having less toxicity, cost effective and high therapeutic value specially in GI disorders including ulcers. **Aim:** To evaluate the Antiulcer activity of Rasa Parpati by pyloric ligation method in Albino rats and to know its effect on gastric secretions. **Subjects and Methods:** The Anti ulcer activity of Rasa Parpati was investigated at different doses using Ranitidine as standard drug. The effect was studied by calculating the total number of ulcers, Ulcer index, its Percentage of inhibition and histological study and the effect on gastric secretions was studied by its Volume, pH, Total Acidity and Free acidity. **Results:** Rasa parpati have shown significant decrease in the number of ulcers and ulcer index and significant increase in % inhibition of ulcers as compared with control group. Histological studies revealed that ulcer control group exhibited severe damage of gastric mucosa, along with edema and leucocytes infiltration of submucosal layer compared to rats pre-treated with Rasa parpati which showed gastric mucosal protection, reduction or absence of edema and leucocytes infiltration of submucosal layer. There was significant reduce in the Volume, free acidity and Total acidity and increase in its pH. **Conclusion:** Rasa parpati possess significant Anti ulcer property which could be either due to cytoprotective action of the drug or by reducing the gastric secretion or by strengthening of gastric mucosa and thus enhancing mucosal defense

Key words: Rasa parpati, Gastric ulcer, Rasayana Kalpana, cytoprotection, Anti ulcer activity.

[1] Associate Professor, Dept. of Rasa Shastra & Bhaishajya Kalpana, [2]Associate Professor, Dept. of Dravya Guna, Govt. Ayurveda Medical College, Mysore (India)

[3]Professor, Dept. of Pharmacognosy, Sharada Vilas College of Pharmacy, Mysore-04 (India)

Corresponding Author Email id: dr.anuroopa@gmail.com Access this article online: www.jahm.in

INTRODUCTION:

Parpati Rasayana Kalpanas are the most popular among the processing of mercury and are widely used. Parapati Rasayanas have high therapeutic value, potent, less toxic medicine. Rasa parpati is a type of pota bandha[1] and It is a pharmaceutically prepared by homogenous mixture of purified parada and purified Gandhaka which is heated till molten, spread on the plantain leaf placed on the platform of cowdung and compressed by another plantain leaf to make it crisp, thin wafer. Parpati is indicated in Grahani, Aruchi, Amlapitta, Atisara, Rakta-pitta[2].

Peptic ulcer diseases are the disorders of the gastrointestinal system and are thought to occur due to damage to mucosa and deeper tissues due to acid and pepsin[3]. Peptic ulcers are common in the present day life of the industrialized and civilized world. There are many products used for the treatment of gastric ulcers, such as antacids, proton pump inhibitors or Antihistamine agents, but most of these drugs produces several adverse reactions. So to overcome this, it's a time to have a look on alternative medicines. Rasa parpati is mainly indicated in Gastro Intestinal disorders which is recommended for ulcerative colitis. However, there is no sceintific data available on anti ulcer activity. Hence the work has been undertaken to find out the safety and efficacy of Rasa parpati and to find out the anti ulcer property of Rasa parpati.

MATERIALS & METHODS:

Preparation of Rasa Parpati: Rasa parpati was prepared after procuring the genuine raw drugs and their methodological processing as per the classical reference in the teaching Pharmacy of GAMC, Mysore

Experimental study:

Acute Toxicity Study: The acute oral toxicity of Rasa parpati was performed as per OECD guidelines. Acute toxicity Study and Animal experimentation was done in Sharada vilas College of Pharmacy, Mysore. (Registration No.706/CPCSEA). The animals were given with standard rat pellets and tap water and individually placed in separate cages with wide-mesh wire bottoms to prevent coprophagy during the experiment. A total of 6 rats was fasted overnight (but was allowed water) prior to dosing. Food was withheld for a further 3 to 4 hours after dosing. The animals were observed for 48 hours after the administration of the suspension for the onset of clinical or toxicological symptoms. Mortality, if any, was observed over a period of 2 weeks. Throughout the experiments, all animals will be treated humanely according to the CPCSEA guidelines.

Fixation and Preparation of Rat Dose was done based on Paget and Barner's surface

area ratio i.e. Rat dose / kg body wt = 0.018 x Human therapeutic dose(250mg) x 5

Table1: Experimental design

Group	Purpose	Number of Rats	Drug
Group I	Control	6	Tween80(2%) (1ml)
Group II	Standard	6	Ranitidine hydrochloride(27mg/kg)
Group III	Trial I	6	RP11 mg/kg
Group IV	Trial II	6	RP22 mg/kg
Group V	Trial III	6	RP44 mg/kg

Procedure: For the first seven days, each rat of control group was administered with 1ml of 2%Tween80, standard group with Ranitidine hydrochloride(27mg/kg), 5.4mg of drug dissolved in 1 ml of 2%Tween80, Trial 1 group with 11mg/kg of Rasa parpati(2.2 mg of drug dissolved in 1 ml of 2%Tween80), Trial 2 group with 22mg/kg of Rasa parpati,(4.4 mg of drug dissolved in 1 ml of 2%Tween80), Trial 3 group with 44mg/kg of Rasa parpati(8.8 mg of drug dissolved in 1 ml of 2%Tween80). The animals were fed with standard rat feed and tap water. On 7^{th} day night all the animals were fasted overnight and provided only with tap water. Next day the control drug, standard drug and trial drug were given 2 hours prior to the Pylorus ligation. After 4 hours, animals were sacrificed by cervical dislocation. Then the abdomen is opened by taking incision through abdominal skin and muscles. The organs were identified and the stomach was elevated. The external surface was studied for hemorrhage, congestion and perforation. Then the stomach was dissected by separating adherent organs. Stomach was cut along the greater curvature, gastric juice was collected and washed with distilled water. Then screened for gastric lesions and ulcers.

Gastric lesions were counted and mean ulcerative index was calculated as follows**:**

0 = Normal colored stomach

0.5 = Red coloration

1 = Spot ulcers

1.5 = Hemorrhagic streaks

2 = Ulcers ≥ 3 but ≤ 5

3 = Ulcers > 5

Ulcer index was measured by using formula:

$\mathbf{U_I}$ (ulcer index) = $\mathbf{U_N + Us + Up}$ X 10^{-1}

$\mathbf{U_N}$**-** Average number of ulcers per animal; $\mathbf{U_S}$ **–** Average number of score ; **Up –** Percentage of animals with ulcers

Percentage inhibition of ulceration was calculated as follows:

Percentage inhibition of ulceration = (UI of control - UI of test)X100/UI of control

The gastric mucosa was washed with 3 ml of lukewarm distilled water and collected in graduated centrifuge tubes. The gastric juice

and washings were homogenised and centrifuged for 5 min. The gastric juice was estimated to evaluate Gastric Volume, pH, total acidity and free acidity [3].

Histo-pathological studies: Gastric tissue preserved in 10% formalin was sent to K. R. Lab, Mysore for Histopathological studies

Statistics applied: The results are expressed mean ± SEM and one- way ANOVA followed by Tukey's post-hoc test was done for statistical analysis. The differences between means were considered statistically significant when the p value was less than 0.05.

OBSERVATIONS AND RESULTS:

Acute Toxicity Study:

An Acute toxicity study was carried out in which the animals were treated with Rasa parpati at the dose of 440mg/kg and were kept under observation for 14 days. All the animals remain alive and did not manifest any significant visible signs of toxicity. There were no abnormal signs, behavioral changes, body weight changes or macroscopic finding at any time during the observation period. From these results it is concluded that the Rasa parpati is quite safe even at its higher dose.

Gross evaluation of gastric lesions: It had been observed that rats pre-treated with ranitidine(standard group) and Rasa Parpati(RP11mg/kg, RP22mg/kg and RP44mg/kg) had significantly suppressed the formation of the ulcers compared to rats pre-treated with only Tween 80(control group). The effect was observed in dose dependent manner.

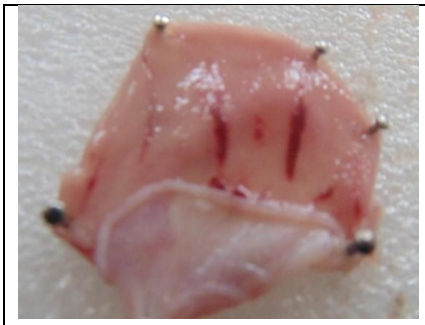

Figure 1

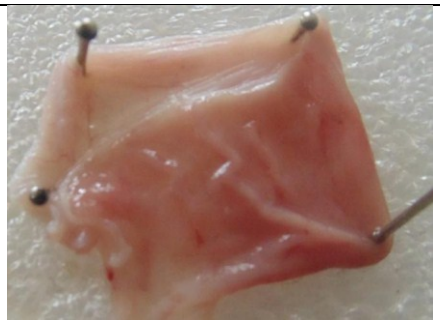

Figure 2

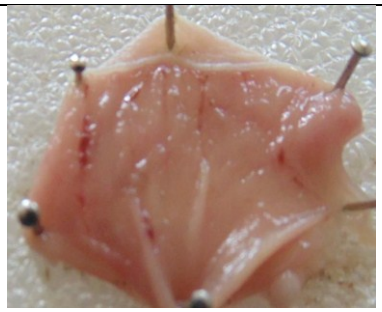

Figure 3

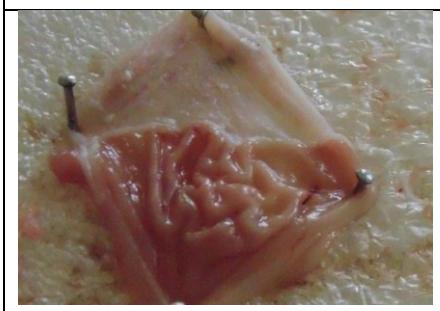

Figure 4

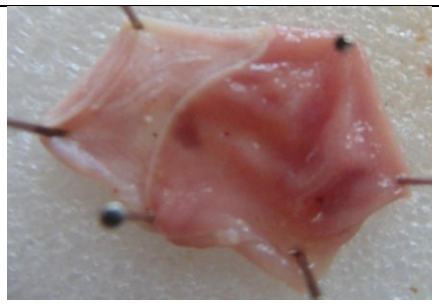

Figure 5

In control group **(Figure 1),** bleeding spot ulcers and haemorrhagic streaks were observed with red coloration. In ranitidine treated animals **(Figure2)**, the gastric mucosa

was appeared intact in the stomach with negligible gastric mucosal damage. RP11mg/kg (**Figure 3)** shows superficial erosions and a few ulcers with mild disorganization of mucosa RP22 mg/kg & RP44mg/kg (**Figure 4 & 5**) shows mild superficial erosions, slight disorganization of the mucosa with no appreciable inflammation.

Table2: Effect of Rasa Parpati on ulcer index and its % of inhibition in pylorus ligated rats

GROUP	ULCER INDEX	% OF INHIBITION
Control	10.83 ± 0.06	--
Standard	5.27 ± 0.09	51.37 ± 0.81
Trial 1	6.98 ± 0.11	35.57 ± 0.97
Trial 2	6.9 ± 0.07	36.26 ± 0.66
Trial 3	5.21 ± 0.06	51.91 ± 0.57
ANNOVA(F)	821.85	940.288
significance	.000	.000

Values are expressed as Mean ± SEM, $p<0.001$, as compared with ulcerated control using one way ANNOVA.

Ulcer index showed significant dose dependent reduction in the rats pretreated with RP11mg/kg, RP22mg/kg and RP44mg/kg. It indicated 35.57% gastroprotection at RP11mg/kg, 36.26% gastroprotection at RP22mg/kg and 51.91% gastroprotection at RP44mg/kg as compared with ulcerated control. The results indicate that the higher dose of RP i.e.44mg/kg was more effective in protecting ulcers in pylorus ligated rats. However, the ulcer index showed significant ($p<0.001$) reduction as compared with ulcerated control.

Histo-pathological studies: Histological observation showed comparatively extensive damage to the gastric mucosa with inflammation in the control group than the standard and trial groups. In the trial groups, the effect was dose dependent.

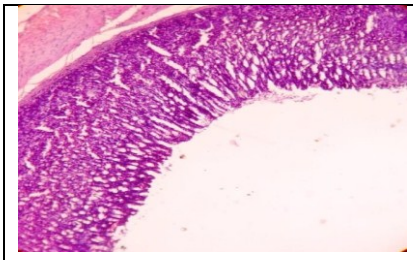

Figure 6

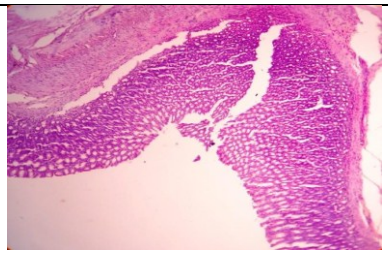

Figure 7

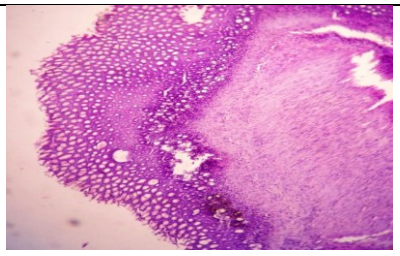

Figure 8

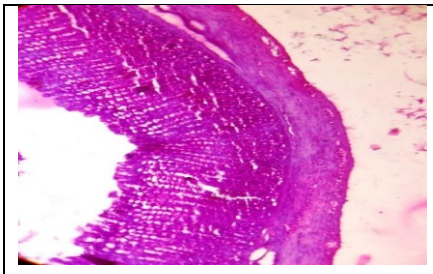

Figure 9

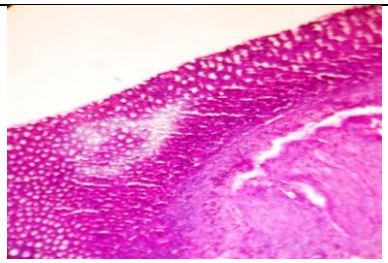

Figure 10

Sections studied from different control specimen bits shows sloughing of mucosa with ulcer formation, altered mucosal architecture with disordered orientation of glands and mild to moderate inflammation due to ulcer formation(**Figure 6)**. Multiple sections studied from the standard drug specimen shows predominantly normal orientation of gastric glands ,mild to minimal erosion present with no significant alteration in lining glands and no or minimal inflammation seen with no ulcer formation(**Figure 7)**. Sections studied from multiple mucosal bits of trial 1 shows mild to moderate discontinuation of mucosa showing ulcer with layering of mucosa and fibrous tissue, mild inflammation and congestive zones seen (**Figure 8)** . Multiple sections from specimen of gastric mucosa of trial 2 shows mild to moderate distortion of mucosa with mild inflammation(**Figure 9)**. sections of trial 3 shows predominantly normal to mildly hyperplastic mucosa(**Figure 10).**

Table 3: Effect of Rasa Parpati on Gastric volume, pH, Total and free acidity of gastric content in pylorus ligated rats

Sl. No.	Group	volume	pH	Total acidity (meq/L/100gm)	Free acidity (meq/L/100gm)
1	Control	5.85 ± 0.09	2.29 ± 0.06	84.40 ± 0.46	51.63 ± 0.31
2	Standard	3.85 ± 0.09	4.06 ± 0.12	54.61 ± 0.26	32.73 ± 0.62
3	Trial 1	4.15 ± 0.29	3.51 ± 0.17	67.85 ± 1.87	41.58 ± 0.92
4	Trial 2	3.87± 0.11	4.03 ± 0.20	59.48 ± 1.71	37.74 ± 0.72
5	Trial 3	3.82 ± 0.28	3.92 ± 0.26	58.97 ± 1.53	37.23 ± 1.12
	ANNOVA(F)	19.290	17.747	77.224	82.121
	significance	.000	.000	.000	.000

Values are expressed as Mean ± SEM, $p<0.001$, as compared with ulcerated control using one way ANNOVA

Gastric content estimation showed that the Gastric volume, Total acidity and Free acidity were significantly($p<0.001$) decreased and pH was significantly($p<0.001$) increased in

the standard and trial groups when compared to control group

DISCUSSION:

The finding of the present study demonstrated that Rasa Parpati possess antiulcer activity against the ulceration caused by pylorus ligation. In pylorus ligated rats, gastric acid is associated with severe ulceration of the rat gastric mucosa[4]. The activation of vagus – vagal reflux by stimulation of pressure receptors in the antral gastric mucosa is believed to increase gastric acid secretion[5]. Pylorus ligation causes accumulation of acid and pepsin, which leads to auto digestion of gastric mucosa and ulceration[6]. The digestive site of accumulated gastric juice and interference of gastric blood circulation is responsible for ulceration[7] .

It is evident from the present result that Rasa Parpati has potent ulcer protective activity at a dose of 11, 22 and 44mg/kg, but at the dose of 22mg/kg and 44mg/kg it was more effective. There was significant decrease in ulcer index, gastric volume, total acidity and free acidity of the gastric content in Rasa Parpati treated rats and ranitidine treated rats. There was significant increase in the pH of gastric content in the pylorus ligated rats. According to the present findings, gastroprotective effect of Rasa Parpati in prevention of ulcers might be due to reduction of gastric acid secretions in the stomach. The results suggest that Rasa Parpati having a significant gastroprotective effect in a dose dependent manner.

Probable mode of action of rasa parpati:

Rasa Parpati is an unique mercurial preparation where the melted homogeneous mixture of Purified Mercury and Purified Sulphur poured over the plantain leaf placed over the platform of cowdung and pressed. Biliary products present in the Gomaya gets absorbed into the heated Kajjali through the patra media and thus helps to regulate the biliary ailments. It is believed to provide protection against gastric mucosal damage through inhibition of gastric acid and stimulation of mucus secretion. One possible mechanism by which the gastric mucosa is protected by Rasa Parpati involves the reinforcement of the mucosal barrier resistance, generated by a protective coating. This protective effect of Rasa parpati preserves the mucosal layer in gastric mucosa and prevent gastric wall mucus depletion.

Pylorus ligation is an important procedure in determining the changes happening in the ulcer formation with respect to the gastric content. In pylorus ligation, the digestive effects of accumulated gastric juice and interference with the gastric blood circulation are responsible for induction of ulceration. H2 receptor antagonists block H2 receptor on parietal cell surface, therefore

decrease acid secretion of basal with reduction of volume of gastric juice in pylorus ligated rats. In the present study it is evident that Rasa parpati inhibited formation of ulcer in stomach, also reduce the volume of gastric juice, free acid and total acids, it is therefore most likely that the Rasa parpati acted through the same mechanism as H2 receptor antagonists.

Rasa parpati effect may be mediated through single or a combined effect of cytoprotection, anti secretory, antioxidants action on mucosal prostaglandin and H2 receptor antagonist mechanism.

CONCLUSION:

Rasa Parpati possess antiulcerogenic property. It provides protection against gastric mucosal damage induced by pylorus ligation.

Financial aid:

The author express gratitude to the Rajiv Gandhi University Of Health Sciences, Bengaluru for the financial support of the research project under the university order no. RGU:Adv.Res.:Proposal-AY-261:2015-16

Date:07-01-2016

REFERENCES:

1. Ashok D Satpute, Rasaratna sammuchaya of Vagbhatacharya, chapter 11, verse no. 65, 1st edition, Mysore; Chetan Prakashana ;1990; 100

2. Kashinath shastry , Rasatarangini of Sri Sadananda Sharma, chapter 6, verse no.135-142,11th edition, Delhi; Motilal Banarasi Das; 2004, 129-131.

3. Prasan R Bhandari, Pharmacology for medical undergraduates;1st edition, The health sciences Publisher,New Delhi;2018;307

4. Jainu M, Mohan kV and Devi CSS Antiulcerogenic and ulcer healing effect of solanum nigrum on experimental ulcer models : possible mechanism for the inhibition of acid formation. Journal of Ethanopharmacology. 2006: 104:156-163

5. Patel AV, Santani DD and Goyal RK. Antiulcer activity and mechanism of action of magaldrate in gastric ulceration models in rat. Indian J Physiology and Pharmacology. 2000;44:350-354.

6. bharati S, Wahane VD, Kumar VL, Protecttive effect of Calotropis procera latex extracts on experimentally induced gastric ulcers in rat, J Ethnopharmacol.2010;127:440-4.(pubmed)

7. Al-Harbi MM, Qureshi S, Raza M, Ahmed MM, Afzal M, Shah AH. Gastric antiulcer and cytoprotective effect of Commiphora molmol in rats. Jouranal of Ethanopharmacology. 1997:55:141-150.

Cite this article as: Anuroopa H K, Suman, Hanumanthachar Joshi. Antiulcer activity of Rasa Parpati against Pylorus ligation induced Gastric ulcer in Rats, *J of Ayurveda and Hol Med (JAHM)*.2018;6(5):52-59

Source of support: Nil

Conflict of interest: None Declared