ORIGINAL RESEARCH PAPER

Ayurveda

ANTI-ULCER ACTIVITY OF RASA PARPATI IN RATS

KEY WORDS: Rasaparpati, Gastriculcer, Ethanol, Antiulcer

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ABSTRACT

Rasa Parpati is one of the mineral formulation indicated mainly in Gastro Intestinal disorders. In the present study, the Anti ulcer activity of Rasa Parpati was studied by calculating the total number of ulcers, Ulcer index, its Percentage of inhibition, histological study and by the estimation of catalase, GSH, and lipid peroxidation. 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, compared to rats pre-treated with Rasa parpati. Significant increase was observed in the levels of the antioxidant defense enzyme glutathione(GSH) in the gastric mucosa, whereas that of a lipid peroxidation was significantly decreased in rats pre-treated with Rasa parpati. Results indicate that Rasa parpati possess significant Anti ulcer property.

INTRODUCTION:

Parapati Rasayanas have high therapeutic value, potent, less toxic and cost effective medicine. Rasa parpati is a type of pota bandha[1] and It is a pharmaceutically prepared by homogenous mixture of purified parada(mercury) and purified Gandhaka(Sulphur) which is heated till molten, spread on the plantain leaf smeared with ghee and 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 is thought to occur due to damage of 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 and millions of people suffer from this disease globally. 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. However, there is no scientific data available on anti ulcer activity of Rasa parpati. Hence the work has been undertaken to find out the safety, efficacy and 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 was done as per the classical reference[4] in the teaching Pharmacy of GAMC, Mysore. The drug was made suspension in 2% of tween80 and administered orally to the rats in concentration of 11mg/kg, 22mg/kg and 44mg/kg body weight.

Experimental study: Acute toxicity Study and Animal experimentation was done in Sharada vilas College of Pharmacy, Mysore. (Registration No.706/CPCSEA). The study was performed as per the OECD guidelines

Acute Toxicity Study: A total of 6 rats was fasted overnight (but water was allowed) prior to dosing. Food was withheld for 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. No mortality and no signs of toxicity were found at the dose of 220mg/kg body weight of rasa parpati. Therefore considering the LD50 of rasa parpati more than 220mg/kg, three doses of 11mg/kg, 22mg/kg and 44mg/ kg was selected for the present study. Throughout the experiments, all animals were treated humanely according to the CPCSEA guidelines.

After Acute toxicity study, the prepared medicine Rasa Parpati is taken to evaluate antiulcer activity on albino rats by Ethanol induced and NSAID induced ulcer models.

Experimental design:

Ethanol induced ulcers: Randomly selected 30 animals were equally divided into five groups of six animals each as mentioned in table 1.

Table1: Experimental design

Group	Purpose	No.of Rats	Drug
1	Control	6	Tween80(2%)
2	Standard	6	Ranitidine hydrochloride(50mg/kg)
3	Trial I	6	Rasa Parpati(11 mg/kg wt)
4	Trial II	6	Rasa Parpati(22 mg/kg wt)
5	Trial III	6	Rasa Parpati(44 mg/kg wt)

Procedure : For the first seven days the control, trial and standard drugs were administered to the animals of respective groups according to the dose calculated based on their weight. Ranitidine hydrochloride was administered as standard in the dose of 50 mg/kg body weight[8]. On 7th day, all the animals were fasted overnight and provided only with tap water. Next day the control drug, standard drug and trial drug were given 2hour prior to the administration of ulcerogen i.e, Ethanol (1ml) orally[6]. 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 and washed with distilled water, then screened for gastric lesions and ulcers.

NSAID's induced ulcers:

The same procedure was followed and Indomethacin (100mg/kg) was administered to induce the ulcers[7,8].

Gastric lesions were counted and mean ulcerative index was calculated[5] 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:

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

U_N- Average number of ulcers per animal; U_S – Average number of score ; **Up** – Percentage of animals with ulcers

Percentage inhibition of ulceration was calculated as

Percentage inhibition of ulceration =

(UI of control- UI of test) X 100

UI of control

Histopathological studies:

Gastric tissue preserved in 10% formalin was sent to K. R. Lab, Mysore for Histopathological studies and and the tissue preserved in normal salinefor the estimation of catalase, GSH, and lipid peroxidation.

Statistics applied:

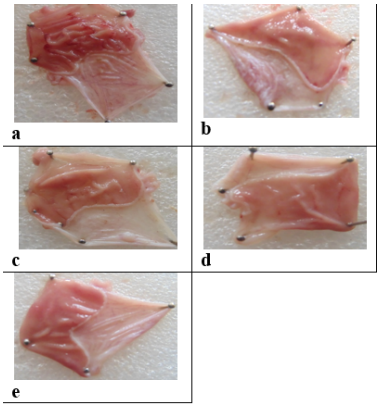
The results are expressed in mean ± SEM and one- way ANOVA followed by Tukey's post-hoc test was done for statistical analysis. The differences between means were considered stastically significant when the p value was less than 0.05.

OBSERVATIONS AND RESULTS:

Ethanol induced ulcers:

Gross evaluation of gastric lesions: In the present study, it had been observed that ethanol produced smaller area of gastric ulceration with spot ulcers and streaks in the control group. The gastric mucosa was appeared to be slightly damaged with red coloration, less spot ulcers and few hemorrhagic streaks in the stomach of rats pre treated with ranitidine and rasa parpati(11mg/kg, 22mg/kg and 44mg/kg). However the mucosal damage was negligible with a continuous epithelial surface in standard and trial groups. It was also observed the flattening of mucosal folds in standard and trial groups(figure1).

Figure 1: Effect of Rasa Parpati on the macroscopic appearance of gastric mucosa in Ethanol-induced rats(a) control (b) standard (c) rasa parpati 11mg/kg (d) rasa parpati 22mg/kg (e) rasa parpati 44mg/kg



The results showed that rats pre-treated with ranitidine and Rasa Parpati in all the 3 doses(Trial group) had significantly(p <0.0001) suppressed the formation of the ulcers and decreased the mean ulcer index in a dose dependent manner when compared to rats pre treated with only vehicle Tween 80(control group)(table 2)

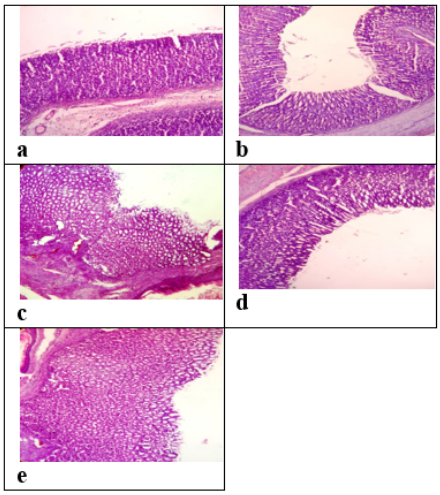
Table 2 Effect of Rasa Parpati on ulcer index and its % of inhibition in Ethanol-induced rats

GROUP	ULCER INDEX	% OF INHIBITION
Control	10.91 ± 0.06	--
Standard	5.22 ± 0.06	52.14 + 0.59
Trial 1	6.99 ± 0.07	35.9 + 0.62
Trial 2	6.9 ± 0.02	36.59 + 0.18
Trial 3	6.895 ± 0.03	36.59 + 0.34

Histological study

Histological observation of group 1 specimen show sloughing of mucosa with ulcer formation, altered mucosal architecture with disordered orientation of glands and mild to moderate inflammation due to ulcer formation. Group 2 specimen shows predominantly normal orientation of gastric glands with mild to minimal erosion present with no significant alteration in lining glands. Group 3 specimen shows mild to moderate discontinuation of mucosa showing ulcer. Mild inflammation and congestive zones were seen. Group 4 specimen shows mild to moderate distortion of mucosa. Mildly altered mucosa and mild inflammation was seen. Group 5 specimen shows predominantly normal to mildly hyperplastic mucosa with mild distortion. Mild inflammation and congestive zones was observed (Figure 2).

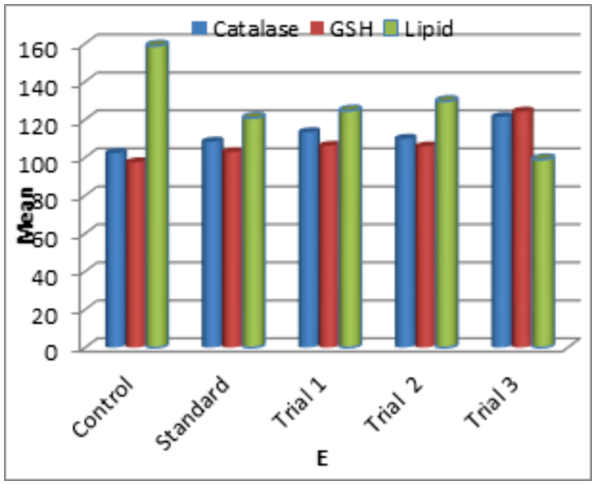
Figure 2 Histology of stomach in Ethanol-induced rats(a) control (b) standard (c) rasa parpati 11mg/kg (d) rasa parpati 22mg/kg (e) rasa parpati 44mg/kg



Study on Catalase, GSH and Lipid peroxidation

Ethanol increased the lipid peroxidation and reduced catalase and GSH in the control group. Treatment with Ranitidine(standard) and Rasa parpati(trial drug) in all the 3 doses significantly(p <0.001) reduced the lipid peroxidation and the increase in the catalase enzyme was insignificant (p< 0.178), but there was significant(p <0.003) increase of the GSH enzyme(figure 2)

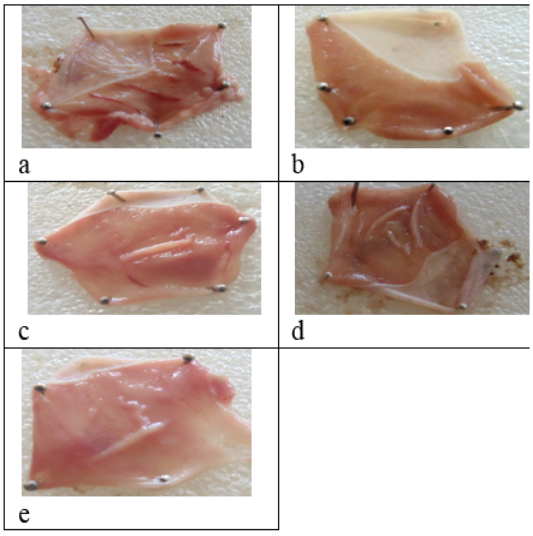
Figure 3 Effect of Rasa Parpati on Catalase, GSH and Lipid peroxidation in Ethanol-induced rats



Indomethacin induced ulcers:

Gross evaluation of gastric lesions: In the present study, it had been observed that indomethacin produced smaller area of gastric ulceration with spot ulcers and streaks in the control group. The gastric mucosa was appeared to be slightly damaged with red coloration, less spot ulcers and few hemorrhagic streaks in the stomach of rats pre treated with ranitidine and rasa parpati(11mg/kg, 22mg/kg and 44mg/kg). However the mucosal damage was negligible with a continuous epithelial surface in standard and trial groups. The flattening of mucosal folds was also observed in standard and trial groups (figure 4).

Figure 4: Effect of Rasa Parpati on the macroscopic appearance of gastric mucosa in Indomethacin-induced rats(a) control (b) standard (c) rasa parpati 11mg/kg (d) rasa parpati 22mg/kg (e) rasa parpati 44mg/kg



The results showed that rats pre-treated with ranitidine and Rasa Parpati in all the 3 doses(Trial group) had significantly($p < 0.0001$) suppressed the formation of the ulcers and decreased the mean ulcer index in a dose dependent manner when compared to rats pre treated with only vehicle Tween 80(control group)(table 3)

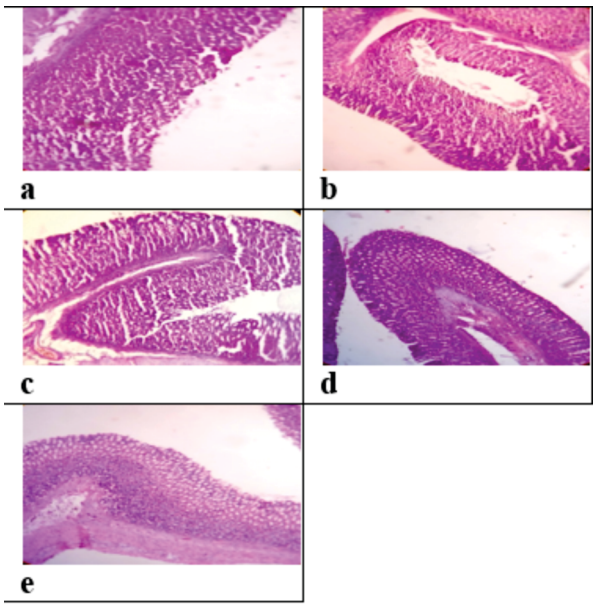
Table 3 Effect of Rasa Parpati on ulcer index and its % of inhibition in Indomethacin-induced rats

GROUP	ULCER INDEX	% OF INHIBITION
Control	10.76 + 0.05	--
Standard	5.20 + 0.03	51.67 ± 0.24
Trial 1	7.00 + 0.05	34.91 ± 0.49
Trial 2	6.90 + 0.05	35.84 ± 0.51
Trial 3	6.90 + 0.05	35.84 ± 0.46

Histological study

Histological observation of group 1 specimen show sloughing of mucosa with ulcer formation with mucosal and submucosal congestion. There was surface erosion with inflammation. Group 2 specimen shows predominantly normal orientation of gastric glands. No or minimal inflammation seen with no ulcer formation. Group 3 specimen shows mild to moderate discontinuation of mucosa showing ulcer. Superficial erosions with mild disorganization of mucosa was observed. Group 4 specimen shows mild to moderate distortion of mucosa. Moderate disruption of surface epithelium was seen. Group 5 specimen shows predominantly normal to mildly hypeplastic mucosa with mild distortion and mild inflammation and congestive zones was seen (Figure 5) .

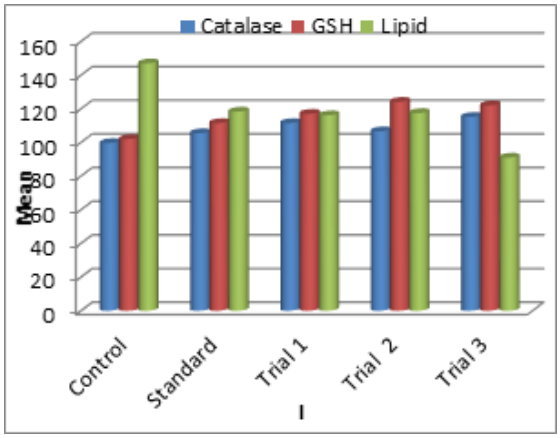
Figure 5 Histology of stomach in Indomethacin-induced rats(a) control (b) standard (c) rasa parpati 11mg/kg (d) rasa parpati 22mg/kg (e) rasa parpati 44mg/kg



Study on Catalase, GSH and Lipid peroxidation

Indomethacin increased the lipid peroxidation and reduced catalase and GSH in the control group. Treatment with Ranitidine(standard) and Rasa parpati(trial drug) in all the 3 doses significantly($p < 0.001$) reduced the lipid peroxidation and the increase in the catalase enzyme was insignificant ($p < 0.459$), but there was significant($p < 0.003$) increase of the GSH enzyme(figure 6)

Figure 6 Effect of Rasa Parpati on Catalase, GSH and Lipid peroxidation in Indomethacin-induced rats



DISCUSSION:

The acute toxicity study of rasa parpati shows no toxicity or mortality in rats. This test revealed that the drug is safe and has no toxicity when administered orally up to the dose of 220mg/kg.

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Although, in most of the cases the etiology of the ulcer is unknown, it is generally accepted that they are a result of an imbalance between the aggressive factors of acid and pepsin and maintenance of mucosal integrity through endogenous defensive mechanism[9,10].

Ethanol is commonly used for inducing ulcers in experimental rats[9,11]. Ethanol cause depletion of gastric mucus content and increased expression of inflammatory

mediators and vascular permeability. The pathogenesis involves the generation of reactive oxygen species(ROS)[6,12]. In the present study, rasa parpati significantly increased the level of antioxidant enzyme GSH provides the first line defense system against reactive oxygen species induced gastric mucosal damage. Though the level of catalase was not significantly increased, the values were within the normal level which signifies there was no injury caused by that enzyme. Further there was significant decrease in the level of lipid peroxidation which supports the cytoprotective effect of rasa parpati.

Non-steroidal anti inflammatory agent like indomethacin is known to induce ulcers during the course of anti-inflammatory therapy[7,8]. Here, the ulcer formation is by inhibition of cyclooxygenase that prevents prostaglandin biosynthesis[13], which in then inhibits the release of mucus, a defensive factor against gastrointestinal damage[14]. It was observed in the present study that Rasa parpati showed significant reduction of the mucosal damage in the indomethacin induced ulcer model. These results suggest the possible involvement of prostaglandins and/or mucus in the antiulcer effect of rasa parpati. Use of leaves in the preparation of Rasa parpati which are highly rich in chlorophyll gets absorbed into formulation and exerts therapeutic effect on gastrointestinal tract. Properties of chlorophyll, one of the best anti oxidants able to neutralize the negative effects of free radical in the body.

Oxygen derived free radicals cause tissue injury through lipid peroxidation. Oxygen handling cells have different systems like peroxidases and catalases which are able to protect them against the toxic effects of oxygen derived free radicals[14,15]. In the present study, the result displayed that the rasa parpati significantly decreased the level of lipid peroxidation in gastric tissue compared to control group. The decrease in the level of lipid peroxidation and increase in the activities of free radical scavenging enzymes GSH in drug treated gastric mucosa compared to control group, suggest the drugs ability to protect the gastric mucosa against free radical mediated tissue injury.

It is also observed in the study, a flattening of the mucosal folds, which suggests the gastro protective effect of the Rasa parpati might be attributed to a decrease in gastric motility. It is reported that Changes in gastric motility have been implicated in the development and prevention of experimental gastric lesions[11]. The relaxation of the circular muscles may protect the gastric mucosa through a flattening of the folds. This flattening increases the mucosal area exposed to necrotizing agents and reduces the volume of the gastric irritants that come into contact with the regular crest[16]. Such action has been postulated to play a role in cytoprotective effect of prostoglandin[17]. This suggests the cytoprotective action of the Rasa parpati. 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 mucus layer in gastric mucosa and prevent gastric wall mucus depletion.

In the present study, it was observed that Rasa parpati showed significant reduction in the total number of ulcers in both the models. The result suggests that Rasparpati having a significant gastroprotective effect in a dose dependent manner, the dose of 22mg/kg and 44mg/kg were found to be more potent

CONCLUSION:

Rasa parpati produces significant autiulcer activity in both Ethanol and NSIAD induced ulcers in rats. According to the present finding, gastroprotective effect of Rasa Parpati in prevention of ulcers might be due to one or a combination of production of prostaglandins in the stomach, cytoprotection, antioxidant action on mucosal prostaglandin by enhancing the gastric mucosal defense.

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