# PRECLINICAL INVESTIGATION OF A POLYHERBAL FORMULATION FOR ITS GASTRIC ANTI-ULCER BY ASPIRIN AND STRESS INDUCED ULCER MODEL IN ALBINO RATS

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**ABSTRACT**

**Background and Objectives:** Peptic ulcer is a condition where benign lesions of gastric or duodenal mucosa occur at a site where the mucosal epithelium is exposed to acid and pepsin, due to imbalance between offensive and defensive factors. Rumalaya-forte, the polyherbal formulation is clinically used as an anti-inflammatory and analgesic in the treatment of osteoarthritis, gout, frozen shoulder etc. As the individual constituents of Rumalaya-forte are reported to have antiulcer activity, the present study was undertaken to evaluate the antisecretory, antiulcer and free-radical scavenging properties of Rumalaya-forte in albino rats.

**Methods:** The gastric antiulcer activity of Rumalaya-forte was performed by using three different ulcerogenic models like cold-restraint and swimming stress, aspirin induced model. Rumalaya-forte was administered for 7 days and on 8$^{th}$ day 30 min prior to the induction of ulcers.

**Results:** The results indicate that Pretreatment with Rumalaya-forte showed significant ulcer protection against all three different ulcer induced model as compared to control. Thus, the ulcer protective and healing effects of Rumalaya-forte may be due to its effect on both offensive and defensive factors.

**Conclusion:** The present findings suggest that Rumalaya-forte has antisecretory, antiulcer and free-radical scavenging properties.

**Key words:** Antiulcer; Antisecretory; Free-radical scavenging; Rumalaya-forte;

## INTRODUCTION

Peptic ulcer is a condition where benign lesions of gastric or duodenal mucosa occur at a site where the mucosal epithelium is exposed to acid and pepsin. Gastric ulcers are caused due to imbalance between offensive and defensive factors of the gastric mucosa[1].

A number of factors such as stress, chemical agents (ethanol, tobacco etc.), bile salts, hyperosmolar NaCl, drugs (nonsteriodal anti-inflammatory agents), may lead the gastroduodenal ulcer causing damage of the mucosa by a complex biological process. The ulcer in the stomach or the duodenum seems to be an enigmatic interaction of several local changes and central nervous factors[2].

The stress due to modern life style (smoking, alcoholism, eating of spicy food and tension etc.), disease state and anxiety also contribute to the production of ulcer. In general, stress induced ulcers due to mucosal damage and ischemic condition are far more common than due to hyper secretion of gastric acid in cancer patients[3].

A number of antiulcer drugs like antisecretory drugs – $H_2$ receptor antagonists, antimuscarinic agents, proton pump inhibitors, and mucosal protective agents– carbenoxolone sodium, sucralfate and prostaglandin analogues are available which are shown to have side effects and limitations[1].

Though the above drugs to a certain extent have been successful in treating and controlling peptic ulcer still the treatment is unsatisfactory due to lack of complete information about etiology and pathophysiology of the disease. Drugs used in the treatment of peptic ulcer though decrease the morbidity and mortality but may produce many adverse reactions like impotence, gynocomastia and haemopoietic changes and in addition the reoccurrence rates are high[1].

There are many agents in alternative medicine, which have shown promising antiulcer activity without producing above-mentioned adverse reaction. The antiulcerogenic activity of many plant products is reported due to an increase in mucosal defensive factors rather than decrease in the offensive factors[4].

Since herbal preparations have proved to be advantageous over the synthetic drugs, recent trends have shifted towards the use of polyherbal formulation for the treatment of peptic ulcer[1,5] because polyherbal formulation inhibits acid secretion, formation of free radical and erosion of mucosa etc. by its individual ingredients or may be by its synergistic effects. Rumalaya-forte is a polyherbal formulation. It contains number of medicinal herbs that have tested individually for their anti-ulcer activity. Rumalaya-forte was reported earlier for osteoarthritis, cervical and lumbar spondylosis, arthralgia, gout, frozen shoulder and sprain but not yet reported for its antiulcer activity. Therefore the present attempt has been made to investigate gastric anti-secretory, anti-ulcer and free radical scavenging properties of Rumalaya-forte. Since it can be used to treat the patient for the above condition and relieve the patient from side effects of NSAID which is mainly associated with gastric ulcers after chronic administration.

## METHODOLOGY

### I. ASPIRIN INDUCED GASTRIC ULCER MODEL IN RATS[6]

Albino Wister rats of either sex weighing between 150-200 g body weight were divided into 3 groups of 6 rats in each group.**Group 1:** Control (Aspirin-200mg/kg) **Group 2:** Ranitidine 30 mg/kg **Group 3:** Rumalaya-forte 161.5 mg/kg oral. Test animals were treated for 7 days with the test drug (Rumalaya-forte). After 7 days treatment animals were fasted for 24 h with free access to water. The control and the standard animals were also fasted for 24 h with free access to water. The test (Rumalaya-forte) and the standard (ranitidine) drug were given 30 min before the administration of aspirin (200mg/kg or 20mg/ml). After 4 h the animals were sacrificed by cervical dislocation and stomachs were incised along the greater curvature and examined for the ulcers. The ulcer index was scored by a person unaware of the experimental protocol. Statistical analysis was done by calculation of Mean ± SEM followed by ANOVA and Dennett's't' test. Percentage protection by the test and the standard drug was also calculated.

| Sl. No. | Stomach colors | Ulcer score |
|---|---|---|
| 1 | Normal color | 0 |
| 2 | Red color | 0.5 |
| 3 | Red spots | 1 |
| 4 | Hemorrhagic streaks | 1.5 |
| 5 | 3 > 5 ulcers | 2 |
| 6 | < 5 ulcers | 3 |

### II.COLD AND RESTRAINT STRESS INDUCED ULCER MODEL IN RATS[7]

Albino wister rats of either sex weighing between 150-200 g body weight were divided into 3 groups of 6 rats in each group. **Group 1:** Control
**Group 2:** Ranitidine 30 mg/kg
**Group 3:** Rumalaya-forte 161.5 mg/kg oral
Test animals were treated for 7 days with the test drug (Rumalaya-forte). After 7 days treatment animals were fasted for 24 h with free access to water. The control and the standard animals were also fasted for 24 h with free access to water. The test (Rumalaya-forte) and the standard (ranitidine) drug were given 30 min before the animals were subjected to stress. They are then Immobilized in stress cage and forced to remain in cold room (4 to $6^0$ C) for 3 h. The animals were sacrificed by blow on the head, stomachs were incised along the greater curvature and ulcer index was calculated by scoring the ulcer.

**III. SWIMMING STRESS INDUCED ULCER MODEL IN RATS**[8] Albino wister rats of either sex weighing between 150-200g body weight were divided into 3 groups of 6 rats in each group. **Group 1:** Control **Group 2:** Ranitidine 30 mg/kg **Group3:**Rumalaya-forte 161.5 mg/kg oral**.**Test animals were treated for 7 days with the test drug(Rumalaya-forte). After 7 days treatment animals were fasted for 24 h with free access to water. The control and the standard animals were also fasted for 24 h with free access to water. The test (Rumalaya-forte) and the standard (ranitidine) drug were given 30 min before the animals were subjected to stress. Thirty min later, they were placed inside a vertical cylinder filled with water up to a height of 15 cm (height 30 cm,diameter 15 cm). The temperature of the water was maintained at 20–25 °C. The rats were removed from the cylinder after 3 h and sacrificed. . Stomachs were incised along the greater curvature and ulcer index was calculated by scoring the ulcer

## RESULTS

The results of the above studies are discussed one by one in the following context.

### Results of Aspirin induced ulcer model

It is evident from the Table 26 and fig 18 and 19 that the ulcer index in control, Ranitidine and Rumalaya-forte is 5.756 ± 00.9197, 2.167 ± 0.4410, 2.583 ± 0.5388 respectively. The % protection of Rumalaya-forte was 62.42 % and for Ranitidine it was 55.53 %. The result is statistically significant (p value <0.005). From the above result, when compared with Ranitidine, Rumalaya-forte showed less effect on aspirin induced ulcer model.

**Ulcer index Table 1. (Control)**

| Sl. No. | Body weight | Ulcer index | % Ulcer protection |
|---|---|---|---|
| 1 | 165 | 3.5 | |
| 2 | 200 | 6.0 | |
| 3 | 170 | 10.0 | 0.00% |
| 4 | 155 | 5.5 | |
| 5 | 160 | 4.5 | |
| 6 | 175 | 5.0 | |
| Mean ± SE | | 5.756±0.9197 | |

**Table 2. (Ranitidine)**

| Sl. No. | Body weight | Ulcer index | % Ulcer protection |
|---|---|---|---|
| 1 | 160 | 1.5 | |
| 2 | 175 | 0.5 | |
| 3 | 200 | 3.0 | 62.42% |
| 4 | 185 | 2.5 | |
| 5 | 155 | 2.0 | |
| 6 | 170 | 3.5 | |
| Mean ± SE | | 2.167±0.4410 | |

**Table 3. (Rumalaya-forte)**

| Sl. No. | Body weight | Ulcer index | % Ulcer protection |
|---|---|---|---|
| 1 | 180 | 0.5 | |
| 2 | 170 | 3.0 | |
| 3 | 160 | 4.5 | 55.36% |
| 4 | 175 | 2.5 | |
| 5 | 190 | 3.0 | |
| 6 | 150 | 2.0 | |
| Mean ± SE | | 2.583±0.5388 | |

**Table 4. Ulcer index of Rumalaya-forte VS Raniidine (ANOVA)**

| Sl. No. | Treatment | Ulcer index (mean ± SEM) | % Ulcer protection |
|---|---|---|---|
| 1 | Control | 5.756±0.9197 | 0.00% |
| 2 | Ranitidine | 2.167±0.4410 | 62.42% |
| 3 | Rumalaya-forte | 2.583±0.5388 | 55.36% |
| F, df value | | 8.659, (2/17) | |
| P value | | <0.005 | |

**ULCER INDEX**

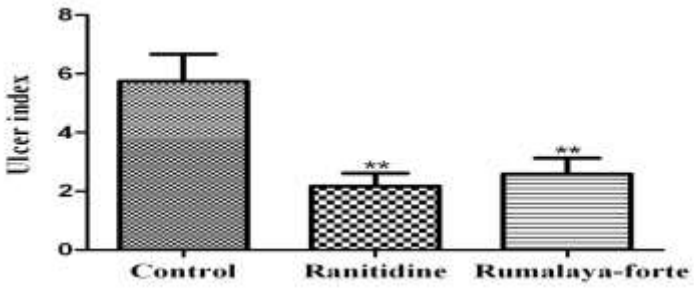



**Fig.1. All values are expressed as Mean ± S.E.M.**
***p<0.05, **p<0.01, ***p<0.001, when compared to control.**

**% ULCER PROTECTION**

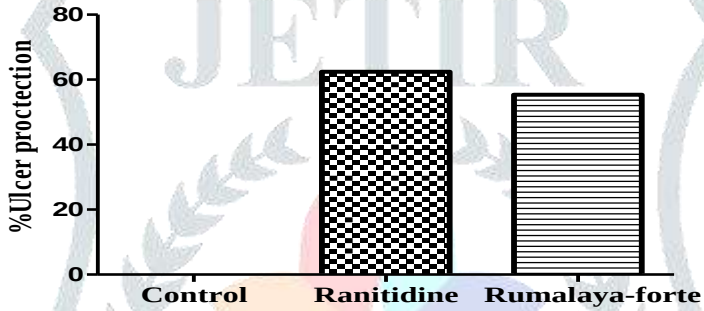
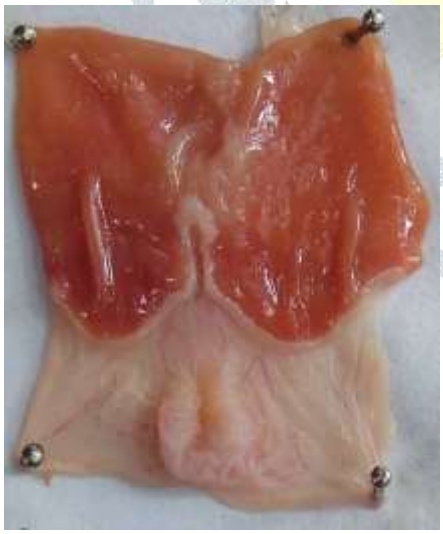
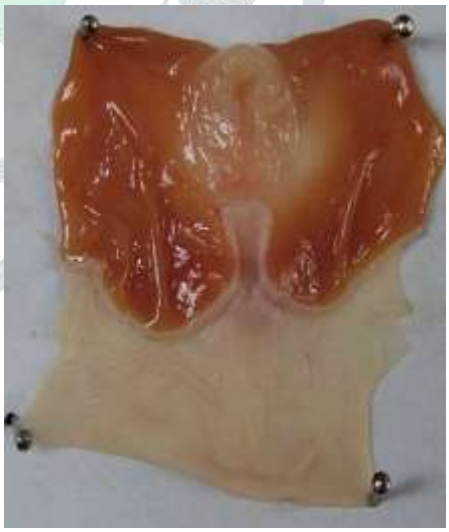


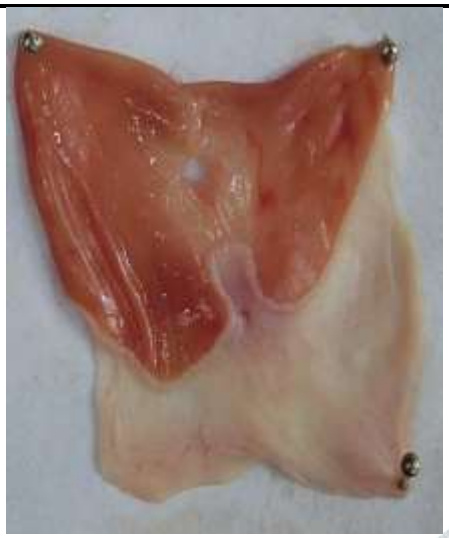

**Fig.2**



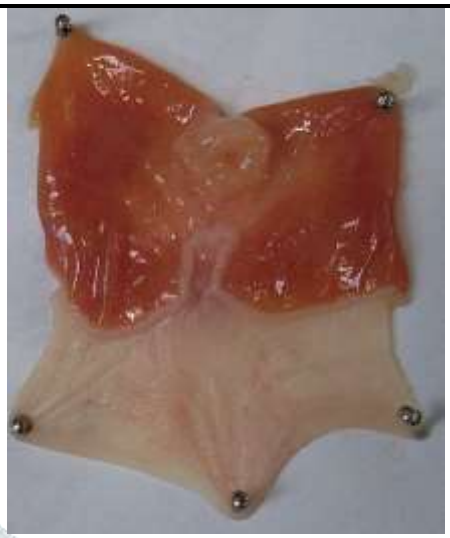
**Normal**



**Control**



Ranitidine



Rumalaya-forte

**Fig.3.Photographs of rat stomach in Aspirin induced ulcer model.**

**Results of cold restraint stress induced ulcer model**

It is from Table 30 and fig 21 and 22 the ulcer index in control, Ranitidine and Rumalaya-forte is 3.50 ± 0.6325, 1.413 ± 0.4902, 1.33 ± 0.2472 respectively. The % protection of Rumalaya-forte was 61.92 % and for Ranitidine it was 59.52 %. The results are statistically significant (p value<0.001). From the above result, when compared with Ranitidine, Rumalaya-forte showed more effect on cold restraint induced ulcer model.

**Ulcer index Table 5. (Control)**

| Sl. No. | Body weight | Ulcer index | % Ulcer protection |
|---|---|---|---|
| 1 | 190 | 3.0 | |
| 2 | 180 | 4.5 | |
| 3 | 200 | 3.0 | 0.00% |
| 4 | 180 | 1.5 | |
| 5 | 170 | 6.0 | |
| 6 | 200 | 3.0.0 | |
| Mean ± SE | | 3.500±0.6325 | |

**Table 6. (Ranitidine)**

| Sl. No. | Body weight | Ulcer index | % Ulcer protection |
|---|---|---|---|
| 1 | 180 | 1.5 | |
| 2 | 190 | 0.5 | |
| 3 | 170 | 2.0 | 59.52% |
| 4 | 185 | 3.5 | |
| 5 | 175 | 0.5 | |
| 6 | 190 | 0.5 | |
| Mean ± SE | | 1.417±0.4902 | |

**Table 7. (Rumalaya-forte)**

| Sl. No. | Body weight | Ulcer index | % Ulcer protection |
|---|---|---|---|
| 1 | 160 | 2.0 | |
| 2 | 170 | 1.5 | |
| 3 | 150 | 1.0 | |
| 4 | 180 | 0.5 | 61.92% |
| 5 | 165 | 1.0 | |
| 6 | 155 | 2.0 | |
| Mean ± SE | | 1.333±0.2472 | |

**Table 8. Rumalaya-forte VS Ranitidine (ANOVA) of cold-restrained stress induced ulcer model.**

| Sl. No. | Treatment | Ulcer index (mean ± SEM) | % Ulcer protection |
|---|---|---|---|
| 1 | Control | 3.500±0.6325 | 0.00% |
| 2 | Ranitidine | 1.417±0.4902 | 59.52% |
| 3 | Rumalaya-forte | 1.333±0.2472 | 61.92% |
| F, df value | | 6.445,(2/17) | |
| P value | | <0.001 | |

**ULCER INDEX**

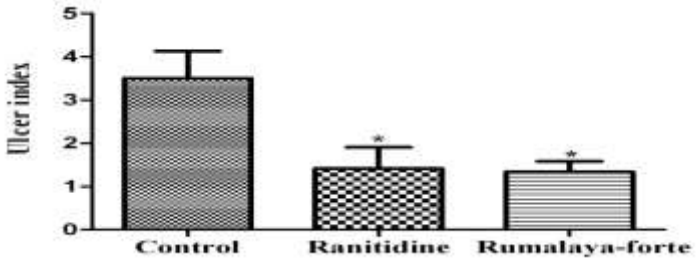



**Fig.4. All values are expressed as Mean ± S.E.M.**

***p<0.05, **p<0.01, ***p<0.001, when compared to control.**

**% ULCER PROCTECTION**

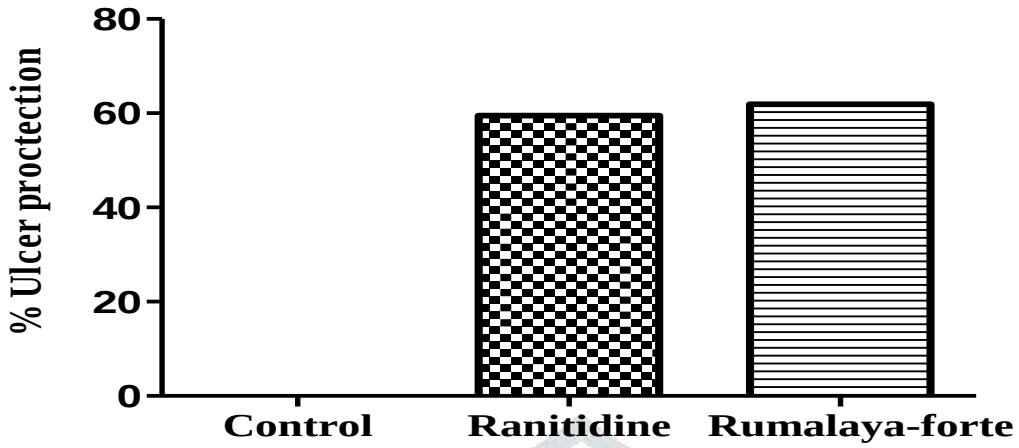
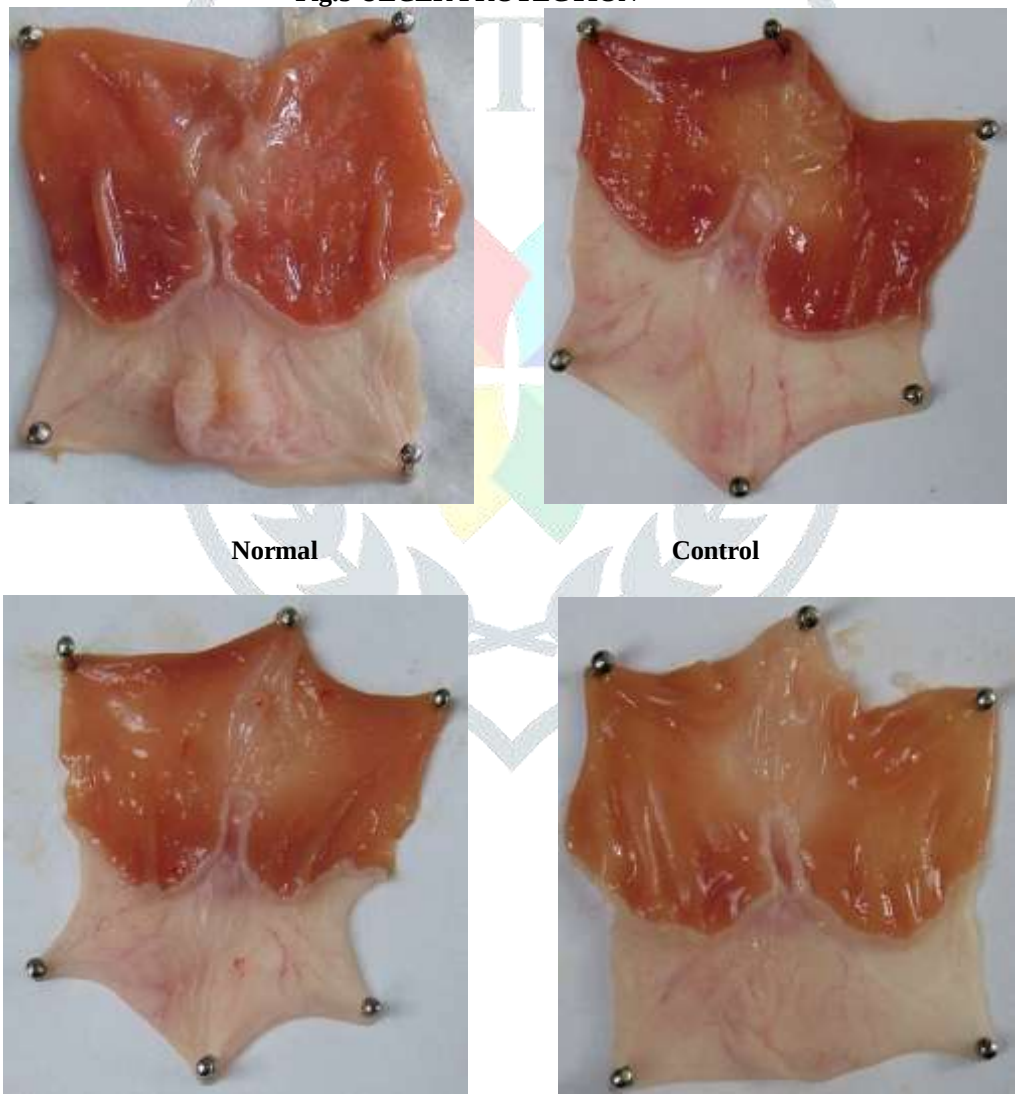



**Fig.5 ULCER PROTECTION**



**Normal** **Control**

**Ranitidine** **Rumalaya-forte**

**Fig.6.Photographs of rat stomach in Cold-restrained stress induced ulcer model.**

**RESULTS OF SWIMMING STRESS INDUCED ULCER MODEL:**

It is from Table 34 and fig 24 and 25 the ulcer index in control, Ranitidine and Rumalaya-forte is 10.33 ± 1.842, 3.66 ± 0.7379, 2.833 ± 0.0.6412 respectively. The % protection of Rumalaya-forte was 72.58% and for Ranitidine it was 64.51 %. The results are statistically significant (p value<0.0001). From the above result, when compared with Ranitidine, Rumalaya-forte showed more effect on swimming stress induced ulcer model.

**Ulcer index**

**Table 9. (Control)**

| Sl. No. | Body weight | Ulcer index | % Ulcer protection |
|---|---|---|---|
| 1 | 160 | 16.5 | |
| 2 | 170 | 5.5 | |
| 3 | 180 | 8.0 | 0.00% |
| 4 | 170 | 12.0 | |
| 5 | 160 | 14.0 | |
| 6 | 180 | 6.0 | |
| Mean ± SE | | 10.33±1.842 | |

**Table 10. (Ranitidine)**

| Sl. No. | Body weight | Ulcer index | % Ulcer protection |
|---|---|---|---|
| 1 | 190 | 5.5 | |
| 2 | 170 | 2.0 | |
| 3 | 190 | 3.5 | 64.51% |
| 4 | 180 | 6.0 | |
| 5 | 170 | 1.5 | |
| 6 | 160 | 3.5 | |

**Table 11 (Rumalaya-forte)**

| Sl. No. | Body weight | Ulcer index | % Ulcer protection |
|---|---|---|---|
| 1 | 180 | 5.0 | |
| 2 | 160 | 2.0 | |
| 3 | 160 | 3.0 | 72.58% |
| 4 | 150 | 0.5 | |
| 5 | 170 | 2.5 | |
| 6 | 180 | 40 | |
| Mean ± SE | | 2.833±0.6412 | |

**Table 12. Rumalaya-forte VS Ranitidine (ANOVA) in swimming stress induced ulcer model.**

| Sl. No. | Treatment | Ulcer index (mean ± SEM) | % Ulcer protection |
|---|---|---|---|
| 1 | Control | 10.33±1.842 | 0.00% |
| 2 | Ranitidine | 3.667±0.7379 | 64.51% |
| 3 | Rumalaya-forte | 2.833±0.6412 | 72.58% |
| F, df value | | 11.65,(2/17) | |
| P value | | <0.0001 | |

**ULCER INDEX**

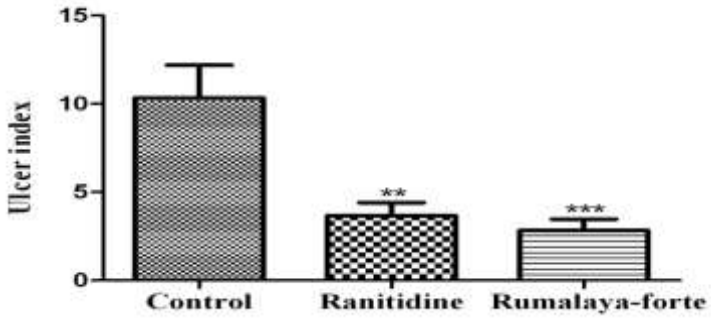



**Fig.7. All values are expressed as Mean ± S.E.M. *p<0.05, **p<0.01, ***p<0.001, when compared to control.**

**% ULCER PROTECTION**

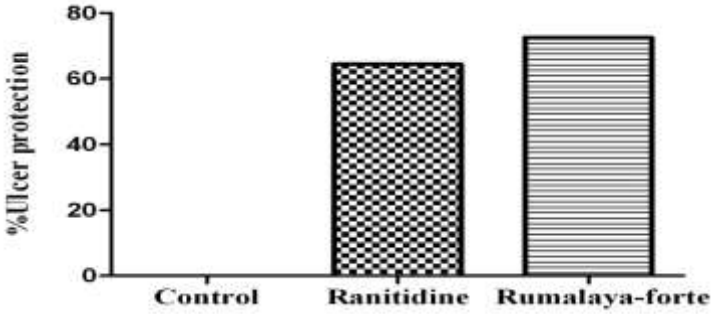
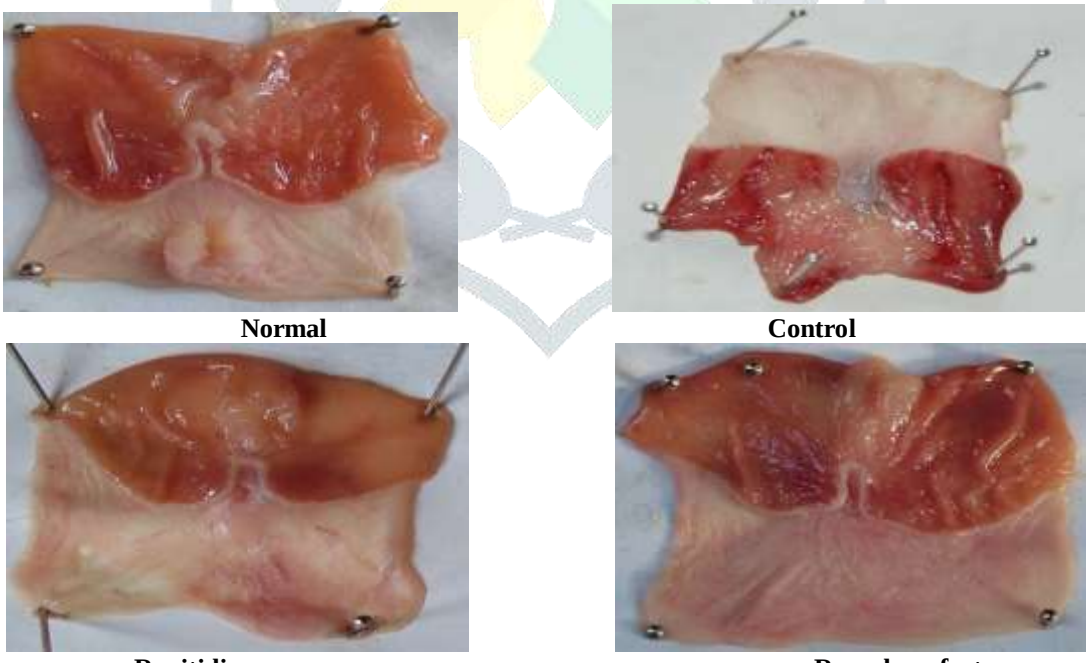



**Fig.8**



**Normal** **Control**

**Ranitidine** **Rumalaya-forte**

**Fig.9Photographs of rat stomach in Swimming induced ulcer model.**

**DISCUSSION**

**COLD-RESTRAINT AND SWIMMING STRESS INDUCED ULCER MODEL** Ulcers due to stress are both due to physiological and psychological factors[9]. Stress causes an ischemic condition in the gastric mucosa by activation of parasympathetic and sympathetic nervous system resulting in vasoconstriction, which in turn causes free radical generation[10]. Further stress has also been found to inactivate mucosal prostaglandin synthase by accumulating $H_2O_2$, which in turn inhibits the synthesis of prostaglandins known to favors the generation of oxygen species.

Rumalaya-forte caused a significant inhibition in stress-induced gastric lesions. With regards to stress models, it has been reported that stress-induced gastric lesions develop as a result of multifactorial impairment of mucosal defense system, disturbance of gastric mucosal

microcirculation, stimulation of vagal nerve, which increases gastric secretion and gastric motility. Apart from peripheral events, central mechanisms including vagal overactivity motility have also been considered for the pathogenesis of stress ulcers. Based on the results of this study, it could be suggested that inhibition of acid hypersecretion, increase in gastric mucus secretion or alterations in gastric mucosal blood flow, might be involved in the protection afforded by Rumalaya-forte in these models[11].Also the results suggest that in the cold stress and swimming models pretreatment with Rumalaya-forte significantly suppressed the ulcer index as compared to control. This effect appears may be due to its Antioxidant properties or vagolytic effect[12].

**ASPIRIN INDUCED ULCER MODEL**

Aspirin which is a prostaglandin synthase inhibitor is responsible for producing ulcers by preventing secretion of mucin and bicarbonate and impaired mucosal blood flow[13]. It is quite clear that indomethacin/aspirin inhibits COX-1 thereby inhibits the prostaglandin synthesis, consequently lipooxygenase pathway is enhanced liberating leukotrine and these leukotrines reported to have a role in ulcerogenesis. In addition there are some evidences that NSAIDs may induce ulcer by causing the back diffusion of $H^+$ ion in to mucosal cells[14]. Rumalaya-forte contains *Boswelia serrata* which have boswellic acids as active constitutes. Boswellic acid is a known for its leukotriene inhibition. Antiulcer action of Rumalaya-forte indicate a possible local increase in synthesis of cytoprotective prostaglandin, inhibition of leukotrienes and gastric mucosal permeability to H+ and Na+ ions by boswellic acid[15].So this may be reason for significant reduced ulcer index in Rumalaya-forte treated as compared to control group.

**CONCLUSION**

It is demonstrated that Rumalaya-forte is an anti-ulcer agent and not having a single but multiple mechanisms. The anti-ulcer data of Rumalaya-forte signifies that it might be acting by its antioxidant action, suppression of acid hypersecretion and pepsin, increasing the gastric mucosal resistance, local synthesis of cytoprotective prostaglandins and inhibiting the leukotriene synthesis. Hence, it is right to state here that Rumalaya-forte, which is used as a therapeutic agent for osteoarthritis, cervical and lumbar spondylosis, arthralgia, gout, frozen shoulder and sprain, has an anti-ulcer potential. Still further investigation has to be done in this direction.

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