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**GJMR-B Classification :** *NLMC Code: WR 598, WI 310*



*Strictly as per the compliance and regulations of:*



# Anti-Ulcer Activity of *Rhus Coriaria* in Indomethacin and Water Immersion Restraint Induced Gastric Ulcer in Experimental Rats

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**Abstract-** The anti-ulcer activity of hydro alcoholic extract of *Rhus coriaria* Linn (HAERC) was investigated in Indomethacin and Water Immersion-induced restraint gastric ulcer in Wistar rats. The assessment was carried out by using ulcer index, ulcer score and histopathological studies of the specimens. HAERC at doses of 145 and 248 mg/kg given orally produced significant inhibition of the gastric lesions induced by Indomethacin and Water Immersion restraint method and the results were comparable to the standard treatment regime. We observed that *Rhus coriaria* Linn extract exhibits significant anti ulcer activity and thus supports the Unani claims about the drug.

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## I. INTRODUCTION

Peptic ulcer disease (PUD) is one of the most common gastro intestinal disorders, which causes a high rate of morbidity. An estimated 15,000 deaths occur each year because of PUD. The prevalence of duodenal ulcer is dominant in western population whereas gastric ulcer is more frequent in most Asian countries<sup>1</sup>. The lifetime prevalence of peptic ulcer disease is 5 to 10% with about equal prevalence in men and women. The incidence of ulcer increases with age because of excessive use of NSAIDs and the reduction in the tissue prostaglandins<sup>2</sup>. In India, PUD is common and the Indian pharmaceutical industries share 6.2 billion rupee and occupy 4.3% of the market share in consuming the antacids and antiulcer drugs<sup>3</sup>. Peptic ulcer which is usually an asymptomatic gastrointestinal disorder defined as a breach in the mucosa of the alimentary tract, which extends through the muscularis mucosa into the submucosa or deeper. Peptic ulcer disease commonly occurs when the linings of stomach or proximal duodenum are corroded by the acid-peptic juices which are secreted by the stomach cells<sup>4,5</sup>. Peptic ulcer is caused by *H. pylori* infection, long term and high doses of drugs such as non steroidal anti-inflammatory drugs (NSAIDs), diseases like Zollinger-Ellison syndrome, other factors such as smoking; emotional stress and excessive alcohol consumption

also may contribute. In Unani Medicine, gastric ulcer is known as *Qarah-e-Medah*. Unani scholars mentioned its causes as, *Khilte Haad* (hot and irritant humour), Fuzlat (waste products), *intake* of hot and spicy foods, excessive intake of rotten food, alcohol and hard fibrous diet, desensitization of internal surface of stomach which causes excessive gastric secretions, chronic gastritis and indigestion, prolonged stress and strains and unabsorbed gastric secretions. The modern approach to control gastric ulceration is to inhibit gastric acid secretion, to promote gastro protection, to block apoptosis and to stimulate epithelial cell proliferation for effective healing<sup>6</sup>. Hence, Conventional medicine treats Peptic ulcer by proton pump inhibitors (PPI), H2-receptor antagonist, antacids and antibiotics for *H. pylori*. However, there are reports of adverse effects and relapse in the long run<sup>7</sup> that lead people to find the alternative medications. Furthermore, many of these drugs do not fulfill all the *beneficial necessities*<sup>8</sup>. The clinical evaluation of these drugs showed development of tolerance and incidence of relapse and side effects that make their efficacy debatable. This has been the rationale for the development of new, safe antiulcer drugs. Herbal drugs can provide lead for the development of such antiulcer drugs because these drugs are considered safer in view of their natural ingredients. In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems of medicine. More than 13,000 plants have been studied during the last few years<sup>9</sup>.

*Unani physicians in the treatment of gastritis, gastric ulcer and associated disorders due to its stomachic, astringent, desiccant, styptic, sedative and coolant activities*<sup>10</sup> also use *Post Sumaq* (Fruit rind of *Rhus coriaria* Linn.) frequently. However, there is no scientific report regarding its efficacy in PUD. Therefore, the present study was carried out to examine the effect of *Post Sumaq* in gastric ulcer on animal model.

## II. MATERIAL AND METHODS

Institutional Animal Ethics Committee (IAEC) of NIUM approved the present study. The test drug *Sumaq*

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(*Rhus coriaria* Linn) was procured from local market of Bangalore, and was identified by Dr. H.B. Singh, Chief Scientist and Head of National Institute of Science Communication and Information Resources (NISCAIR) New Delhi, vide Reference No. NISCAIR/RHMD 2030/38.

### III. PREPARATION AND DOSE OF THE TEST DRUG

The fruits of test drug were dried in shade, the Post (rind) was peeled off, and its therapeutic dose (5gm) as in Unani medicine was used to calculate the dose for experiment<sup>11</sup>. Thus, dose was found to be 580 mg /kg. Since, the test drug was studied at two different doses; therefore a second dose was also calculated by the method of Miller and Tainter (1944)<sup>12</sup> and found to be 990 mg/kg. As the hydro alcoholic extract was used for the study, the dose of the extract was calculated with reference to the dose of crude drug after obtaining the 25% yield percentage of extract. The hydro alcoholic extract of the drug was used in the dose of 145mg/kg and 248mg/kg. Standard drug, Omeprazole (Manufactured in India by Dr Reddys Laboratories Ltd. Village Manuja Thana) was used in the dose of 20mg/kg.

### IV. ANIMALS

The study was carried out in healthy Wistar rats of either sex, weighing 150-250 gm. The animals were procured from Biogen Laboratory Animal Facility (Reg. No. 971/bc/06/CPCSEA), a registered breeder in Bangalore. They were acclimatized to the laboratory condition for 7 days before the experimental studies. The rats were housed in polypropylene cages under controlled conditions of light (12/12) and temperature (23±20C) under strict hygienic conditions. The animals were given Standard food pellets (Hindustan Lever Ltd.) and tap water ad libitum.

### V. INDUCTION OF GASTRIC ULCER

This test was carried out by the method described by Vogel<sup>13</sup> with minor modification in the treatment schedule. The animals were divided into 8 groups of 6 animals each. The animals in group I were administered with distilled water throughout study and served as Plain control and after 36 hours they were sacrificed while the animals in group II (after 24 hours of fasting) were treated with Indomethacin 20 mg/kg, once daily, orally for 5 days and served as negative control. The animals in group III, IV and V were treated with standard drug Omeprazole and hydro alcoholic extract of test drug in the dose 20 mg/kg, 145mg/kg and 248 mg/kg, respectively and served as pre-treated standard, Pre-treated test group A and Pre-treated test group B, respectively. These treatments were continued for five days; however, on 6th day after 24 hours of

fasting ulcer was induced by the administration of Indomethacin in the dose of 20 mg/kg, for the next five days. Food was withdrawn for two hours after Indomethacin administration. On 5th day after 12 hours of fasting, the animals were treated with the last dose of Indomethacin and after five hours of administration of Indomethacin, the animals including negative control were sacrificed. While in Post treated standard and test groups the animals were first kept on fasting for 24 hours and ulcer induced by the administration of Indomethacin in the dose 20 mg/kg, daily for 5 days, thereafter the animals were treated with standard and test drug for next 5 days in the same dose and same manner as described above. On 6th day after 12 hours of fasting, the animals were sacrificed.

The water immersion restraint induced gastric ulcer was done by the method of Hayaso and Takeuchi<sup>14</sup>. The animals in this model were also divided in to 8 groups of 6 animals each. The animals in Group I and II were treated with distilled water and were serve as Plain control and Negative control, respectively. While the animals in Group III, IV and V were treated with standard drug, and hydro alcoholic extract of the test drug in the dose of 20 mg/kg, 145mg/kg and 248mg/kg and served as Pre-treated standard, Pre-treated test group A and Pre-treated test group B, respectively. All the animals were treated in this way once daily for 5 days. They had free access to food and water during the treatment period. However, on 4<sup>th</sup> day they were kept on fasting for 12 hours with water ad libitum. On 5<sup>th</sup> day, 12 hour fasted rats were treated routinely and after one hour of treatment animals in Group I were sacrificed while in rest of the groups, ulcer was induced by water immersion restraint method. Then animals were sacrificed. The animals in Group VI, VII and VIII were also subjected to gastric ulceration in the same manner as mentioned above. After one hour of ulcer induction animals were treated with standard and test drug and served as *Post*-treated standard group (VI) and *Post*-treated test group A and group B (VII and VIII), respectively. All the animals were treated in this manner orally, once daily for five days. On 5<sup>th</sup> day, 12 hours fasted animals were treated routinely and after one hour of treatment, they were sacrificed. In all the above methods, the animals were sacrificed under The opent one anesthesia (40 mg/kg, IP). Stomach was removed from the body and opened along with the greater curvature, washed with fresh water and spread on cardboard with the mucous surface upwards. The mucosal surface was examined for ulceration with the help of magnifying lens (10 fold magnification) and scored by the method of Brzozowski et al<sup>15,21,22</sup>

### VI. ASSESSMENT OF EXTENT OF ULCERATION

The parameters viz. Ulcer score, ulcer index and reduction percentage in ulcer were taken to assess the

anti ulcerogenic effect. Histopathological studies were also carried out to determine the nature and amount of damage and the improvement after treatment.

## VII. STATISTICAL ANALYSIS

The observations in various groups were expressed as Mean  $\pm$  SEM. The ulcer score and index of various groups were compared with plain control group. The group comparison was analyzed by using ANOVA one way with Kruskal Wallis and Dunn's pair comparison test.

## VIII. RESULTS

Plain control (Group I), showed no pathological sign. In Group II (Negative control) where ulcer was induced by Indomethacin (20mg/kg) once daily for 5 days, the ulcer score was found to be  $1.08 \pm 0.27$ . The ulcer scores in pre-treated standard and test groups where the animals were treated with Standard drug &

test drug in low dose were found to be  $1.16 \pm 0.30$  &  $1.33 \pm 0.27$  respectively when compared to negative control showed non- significant result. In pre-treated test Group B (Group V), the test drug was given orally in the dose of 248mg/kg ulcer score was found to be  $0.66 \pm 0.27$  with respect to negative control, showed non- significant decrease. In *Post* treated standard group (Group VI). Ulcer score was found to be  $0.66 \pm 0.27$  (insignificant) with respect to negative control. In *Post* treated test group A (VII) it was observed  $1.08 \pm 0.27$  (insignificant). In *Post* treated test group B (VIII) score was found to be  $0.33 \pm 0.10$  (insignificant). The ulcer index in Negative control Pre and *Post* treated standard, test group A and B were found to be 1.25, 1.63, 1.80, 0.44, 0.17, 1.67 and 0.22 respectively and percentage of ulcer reduction in Pre and *Post* treated standard, test group A and B were observed -7, -19, 39, 39, 0, and 47, respectively when calculated with Negative control. (Table no 1)

**Table 1 :** Effect of Hydro alcoholic extract of *Post Sumaq* on Indomethacin induced restraint gastric ulcer

Groups	Treatment	ADU(Mean $\pm$ SEM)	%RU	Ulcer index	%Reduction
Group I Plain control	D W	$0.08 \pm 0.08$	17%	0.01	94
Group II Negative control	D W + IM 20mg/kg dissolved in CMC	$1.08 \pm 0.27$	100%	1.25	----
Group III Pre-treated Stand	Omeprazole 20 mg/ kg + IM 20mg/kg Dissolved in CMC	$1.16 \pm 0.30$	100%	1.63	-7
Group IV Pre-treated test A	<i>Post Sumaq</i> 145mg/kg+ IM 20mg/kg dissolved in CMC	$1.33 \pm 0.27$	100%	1.80	-19
Group V Pre-treated test B	<i>Post Sumaq</i> 248mg/kg IM 20mg/kg dissolved in CMC	$0.66 \pm 0.27$	67%	0.44	39
Group VI <i>Post</i> -treated Stand	IM 20mg/kg. dissolved in CMC+ omeprazole 20 mg/kg	$0.66 \pm 0.27$	50%	0.17	39
Group VII <i>Post</i> -treated test A	IM 20mg/kg. dissolved in CMC + <i>Sumaq</i> 145 mg/kg	$1.08 \pm 0.27$	100%	1.67	0
Group VIII <i>Post</i> -treated B	IM 20mg/kg. dissolved in CMC + <i>Post Sumaq</i> 248 mg/kg	$0.33 \pm 0.10$	67%	0.22	47

(N=6 in each group. DW = Distilled water, IM = Indomethacin, CMC= Carboxy, methyl cellulose, %RU =Percentage of rats with ulceration, ADU = Average degree of ulceration)

Ulcer score in Negative control was found to be significantly increased ( $p < 0.01$ )  $1.16 \pm 0.21$  when compared to plain control. The ulcer score in pre-treated standard, test group A and test group B, score was found to be  $0.91 \pm 0.27$ ,  $0.66 \pm 0.16$ , and  $0.83 \pm 0.21$  respectively. No significant reduction was observed when compared to negative control. While in *Post*-treated Group VI, Group VII and Group VIII first ulcer was graded and ulcer score was found to be  $0.41 \pm 0.08$ ,

$0.75 \pm 0.25$  and  $0.75 \pm 0.25$  respectively, but no significant reduction was observed when compared to negative control. The ulcer index in Negative control, Pre and *Post* treated standard, test group A and B was observed 1.42, 0.76, 0.67, 0.83, 0.35, 0.76, and 0.83 respectively and percentage of ulcer reduction was found to be 45, 60, 50, 75, 55, and 55 respectively. (Table no 2).

**Table 2 :** Effects of hydro alcoholic extract of *Post Sumaq* on Water-immersion induced restraint gastric ulcer

Groups	Treatment	ADU(Mean $\pm$ SEM)	%RU	Ulcer index	%Reduction
Group I Plain control	D.W	$0.08 \pm 0.08$	17%	0.01	93



<b>Group II</b> Negative control	D.W+ Ulcer induction	1.16±0.21*	100%	1.42	---
<b>Group III</b> Pre-treated Stand	Omeprazole 20 mg/ kg + Ulcer Induction	0.91±0.27	83%	0.76	45
<b>Group IV</b> Pre-treated test A	<i>Post Sumaq</i> 145 mg/kg+ Ulcer induction	0.66±0.16	100%	0.67	60
<b>Group V</b> Pre-treated test B	<i>Post Sumaq</i> 248mg/kg + Ulcer induction	0.83±0.21	100%	0.83	50
<b>Group VI</b> <i>Post-treated</i> Stand	Ulcer Induction + Omeprazole 20 mg/kg	0.41±0.08	83%	0.35	75
<b>Group VII</b> <i>Post-treated</i> test A	Ulcer induction + <i>Post</i> <i>Sumaq</i> 145 mg/kg	0.75±0.25	83%	0.76	55
<b>Group VIII</b> <i>Post-treated</i> B	Ulcer induction. + <i>Post</i> <i>Sumaq</i> 248 mg/kg	0.75±0.25	83%	0.83	55

(N=6 in each group. Test used Kruskal Wallis test with Dunn, pair comparison test,  
N= 6\*  $p < 0.05$  with respect to plain control, D.W= Distilled water)

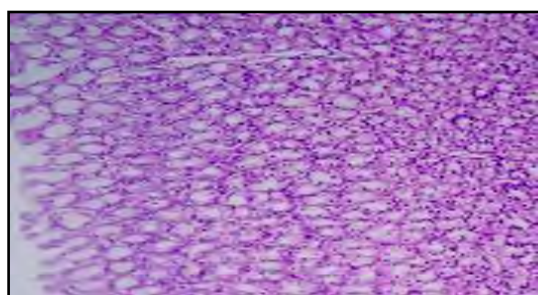
## IX. DISCUSSION

Gastric ulceration has long been viewed as the disease of stress, hence central nervous system may also play role in production of ulcer by causing hyperacidity. The techniques of restraint in albino rats provide a model for the study of stress induced gastric ulceration. Water immersion induced restraint gastric ulcer model is suitable to see anti stress effect of drugs. Therefore, the test drug was also evaluated by using this model. In Water Immersion Induced restraint gastric ulcer model the test drug was found both *precautionary and therapeutic in pre treated and Post treated test Groups at both dose level but the result was statistically non significant*. The histopathological findings are also in consonance. The findings indicate that the test drug does not possess anti anxiety properties and the same has not also been mentioned in Unani classics. However, it is clear from the result that the test drug has preventive & curative effect only at higher dose. Phytochemicals in *Rhus coraria* are ellagic acid, gallic acid isoquercitrin, myricitrin, myricetin, quercetin,

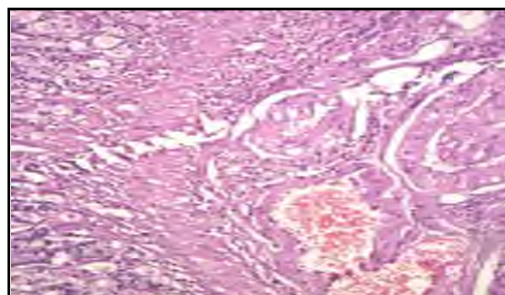
quercitrin and tannic acid and flavinoids. Flavonoids protect the gastrointestinal mucosa from lesions produced by experimental ulcer models and different necrotic agents. Several mechanisms of action may be involved in this protective effect. Quercetin has an anti secretory mechanism of action. However, the most important mechanism of action responsible for the antiulcer activity of flavonoids is the antioxidant properties. Tannins are gastro protective which are present in the drug in sufficient amount<sup>16,17</sup>.

As per the Unani theories it seems that the drug may have acted by temperament, as the *Mizaj* of the test drug is cold whereas that of diseases is hot<sup>18,19,20</sup>. But the anti ulcer mechanism cannot be just understood by *Mizaj* or Phytochemicals only as in case of herbal drugs only one or two or more Phytochemicals are not responsible for action. A number of chemicals and other interventions play the role in exerting actions and the ultimate effect is the cumulative effect. Further study is needed to establish the mechanism of anti ulcer effect of *Post Sumaq*.

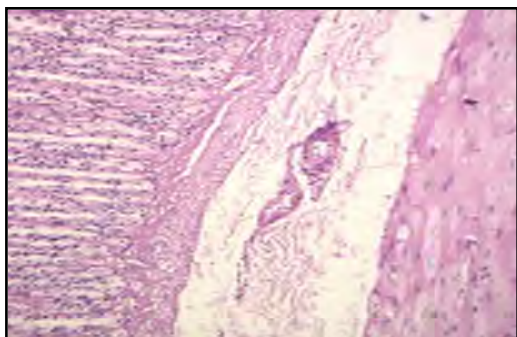
*Histopathological slides of different groups are shown below (Indomethacin ulcer model)*



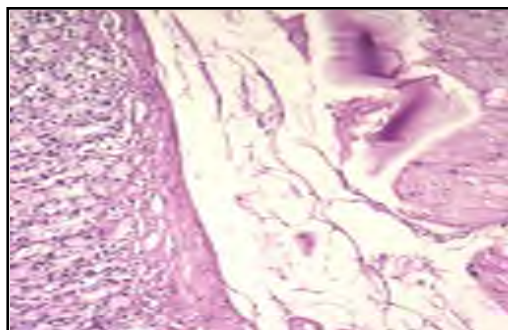
*Group I*: Normal mucosa inflammation and ulceration



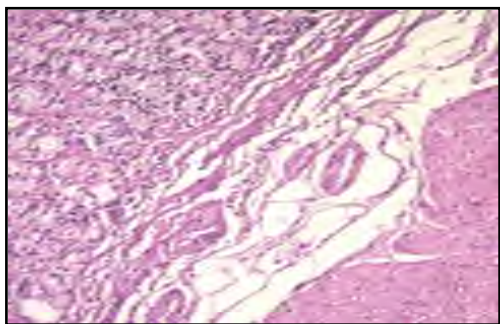
*Group II*: Congestion, necrosis,



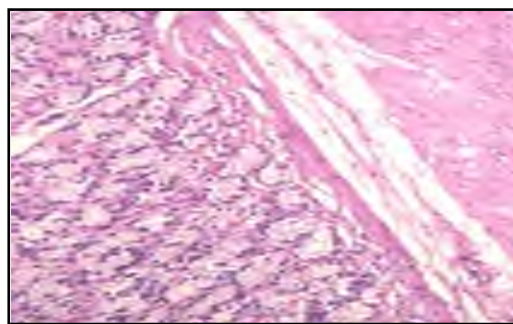
*Group III :* Oedema, necrosis, inflammation and ulceration



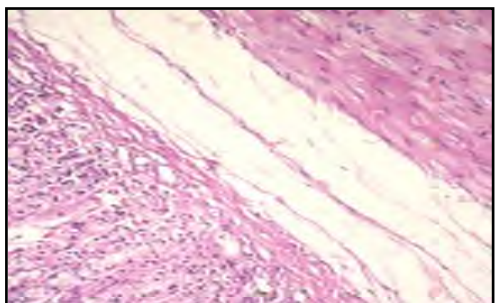
*Group IV :* Oedema, necrosis and ulceration



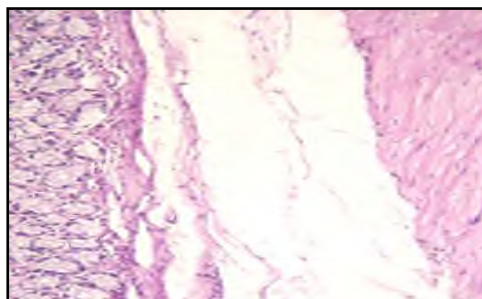
*Group V :* Inflammatory changes



*Group VI :* Inflammatory changes

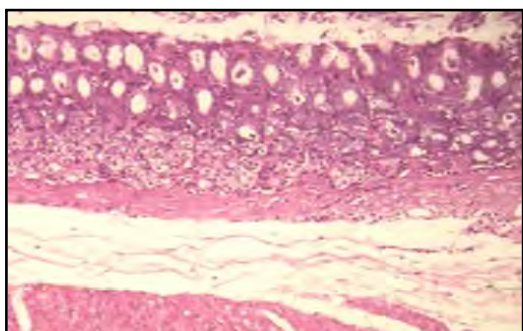


*Group VII :* Oedema, necrosis, inflammation and ulceration

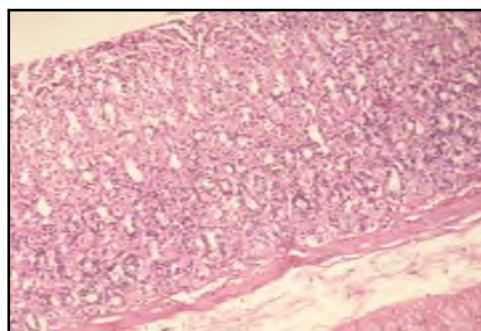


*Group VIII :* Inflammation and ulceration

*Histopathological slides of different groups are shown below (Water immersion-induced restraint ulcer model)*

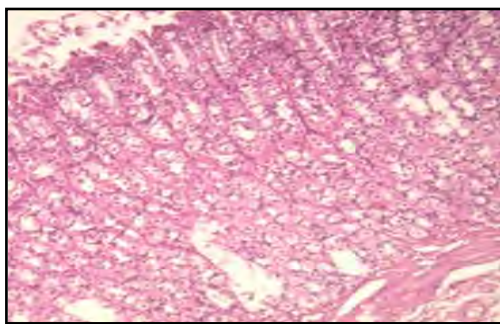


*Group I :* Necrosis, inflammation and ulceration

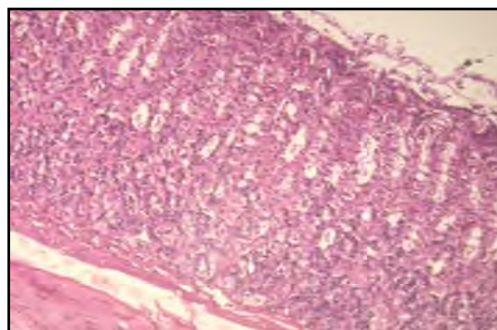


*Group II :* Necrosis and inflammation

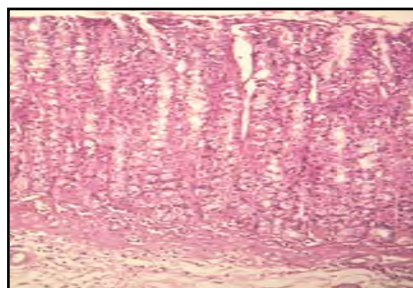




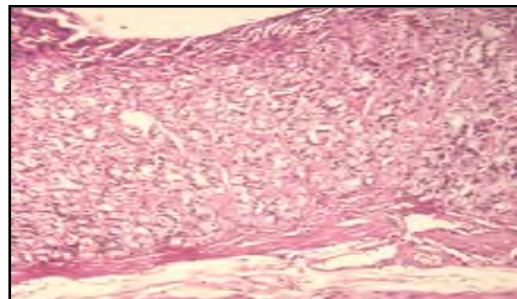
*Group III :* Necrosis and inflammation



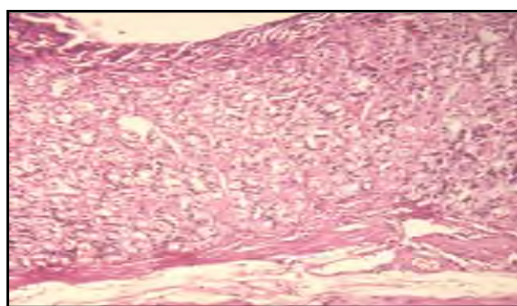
*Group IV :* Necrosis and inflammation



*Group V :* Necrosis and inflammation



*Group VI :* Inflammatory changes



*Group VII :* Inflammatory changes

## X. CONCLUSION

Results of different experimental models revealed Post *Sumaq* to be a promising antiulcerogenic drug. The test drug possesses curative effect at higher dose against Indomethacin induced gastric ulcer. In Water Immersion-induced restraint gastric ulcer model the effect was less prominent therefore it can be concluded the test drug does not possess anti anxiety effect as this model produces ulcer due to stress. This is also evident from the literature that Post *Sumaq* is not used as an anti anxiety. The preventive effect of the test drug was more pronounced. This also validates the claim that herbal drugs are more preventive in nature. The drug is more effective at higher dose; therefore the dose of Post *Sumaq* should be revised after toxicity studies.

## XI. ACKNOWLEDGEMENT

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