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Evaluation of Anti-ulcer activity of hydro alcoholic extract of *Post Sumaq* (*Rhus coriaria* Linn.*)* in Ethanol induced Gastric ulcer in experimental Rats

Haqeeq Ahmad*, Abdul Wadud, Nasreen Jahan, Mudasir Khazir and Ghulamuddin Sofi
Dept of Ilmul Advia (Pharmacology), National Institute of Unani medicine, Bangalore-91, Karnataka, INDIA

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Abstract

The anti-ulcer activity of hydro alcoholic extract of Rhus coriaria Linn. was investigated in ethanol induced gastric ulcer in Wistar rats. The assessment was carried out by using ulcer index, ulcer score and histopathological studies of the specimens. The extract at doses of 145 and 248 mg/kg given orally produced significant inhibition of the gastric lesions induced by ethanol as compared to control groups and the results were comparable to the standard treatment regime. Present study indicated that Rhus coriaria Linn extract has substantial anti ulcer activity in experimental rats, thus it may favour the Unani claims about effectiveness of this drug in case of gastric ulcer disease.

Keywords: *Rhus coriaria*, ethanol induced ulcer model, ulcer index, *Post Sumaq*.

Introduction

The gastric ulcers being amongst the most common disease conditions world over, many causative factors are believed to be involved in its etiology. Mucosal ulcers may be caused by imbalance in protective and ulcer causing factors[1], potential injurious substances such as acid, pepsin, bile acids, food ingredients, bacterial products[2] and drugs. Other factors like inhibition of prostaglandin synthesis, diminished gastric blood flow[3] and gastric motility have also believed to play important role in causing ulcers. The drug therapy for gastric ulcers is aimed at preventing and opposing the effect of ulcer causing factors or provoking the effect of ulcer preventing factors like mucosal defences and blood flow of the gastric mucosa[4]. The aim of treating peptic ulcers is to relieve pain, heal the ulcer and prevention of ulcer recurrence. Currently there is no cost-effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product sources.

Rhus coriaria Linn (Family: Anacardiaceae) is among the important herbs used in traditional Unani medicine for various diseases of digestive system. There are a number of drugs used in Unani Medicine and folklore for the treatment of gastritis, gastric ulcer and associated disorders e.g. Tabasheer (*Bambusa arundinacea*), Aslussoos (*Glycyrrhiza glabra*), Dammul Akhawain (*Dracaena cinnabari*), Gule surkh (*Rosa damascena*), Zanjabeel (*Zingiber officinalis*), and Khatmi (*Althoea officinalis*) etc. as single drugs where as Qurs Tabasheer Qabiz, Sharbate Anar, Arqe Gulab, Rubbe Afsanteen and Jawarish Tabasheer etc. are used as compound formulations[5,6]. Some of the drugs described in Unani medicine and other traditional systems of medicine as being effective in gastritis and PUD have been investigated scientifically in different experimental models and showed promising results[7-9]. These findings are a proof of efficacy of Unani drugs and show potential of Unani Medicine to provide a variety of drugs effective in PUD through diverse mechanisms.

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

Material and Methods

The present study entitled was carried out in the department of Ilmul Advia, National Institute of Unani Medicine (NIUM), Bangalore. Before starting the experiment, the research protocol was submitted to Institutional Animal Ethics Committee (IAEC) of NIUM for ethical clearance. The protocol was approved vide Registration No.953/C/06/CPCSEA.

Plant Collection: The test drug *Sumaq* (*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.

Preparation and Dose of the Test Drug: The fruits of test drug were dried in shade, the *Post* (rind) was peeled off, and the dose was calculated from the therapeutic dose (5gm) of the drug as mentioned in classical Unani medical literature[11] by the conversion factor of 7[12], and 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)[13] 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.

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 ware 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±2^{0}C) under strict hygienic conditions. Standard food pellets (Hindustan Lever Ltd.) and tap water ad libitum was used.

Experimental design and Gastric ulcer induction: This test was carried out by the method of Robert et al[14] with minor modification in treatment schedule. The animals were divided in to 8 groups of 6 animals each. The animals in group-I served as plain control and received distilled water throughout study. After 36 hours of fasting, they were sacrificed, while the animals of group III, IV and V were treated with standard drug Omeprazole (III group), hydro alcoholic extract of test drug (IV and V group) in the dose of 20mg/kg, 145mg/kg and 248mg/kg respectively, daily orally for 5 days. These groups served as Pre-treated standard, Pre-treated test group A and Pre-treated test group B, respectively. On 6th day after 36 hours of fasting (Coprophagy was prevented during fasting by putting the animals in cages grating on the floor), ulcer was induced in the animals of group II, III, IV and V by the administration of 1ml /200gm, of Ethanol (90%) orally. Group II that was treated with ethanol only, served as negative control. After one hour of ethanol administration, the animals were sacrificed under theopentone anesthesia (40 mg /kg IP). while in post- treated standard and test groups first ulceration was induced in the same manner after 24 hours of fasting , thereafter the animals were treated with standard and test drug in the dose of 20mg/kg, 145mg/kg and 248mg/kg respectively, for 5 days. Thereafter, on 6th day after 12 hours of fasting, the animals were sacrificed.

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.

Ulcer score: Normal stomach (score=0), Red coloration (0.5), Spot ulcer (1), Hemorrhagic streak (1.5), ulcers (2), Perforation (3).

The average degree of single ulceration (ADU) for each group was determined by adding together the degree of single ulceration (DSU) and dividing it by the number of animals. Based on percentage of rats with ulceration (%RU), the ulcer index was calculated by the following formula[15].

$$\text{Ulcer index} = \frac{(ADU)(\%RU)}{100}$$

ADU- Average degree of single ulceration, % RU- Percentage of rats with ulceration, The percentage of ulcer protection was determined by the following formula.

$$\% \text{ Protective} = \frac{\text{Control mean ulcer index-test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Statistical analysis: The data was analysed by using Graph pad software. The observations in various groups were expressed as Mean ± SEM. The ulcer score and index of various groups were compared with negative control group. The group comparison was analysed by using ANOVA one way with Kruskall Wallis and Dunn's pair comparison test. The difference of mean was considered significant at $p<0.05$.

Results and Discussion

Plain control (group I), showed no pathological sign. Ulcer score in Negative control (group II), where ulcer was induced by single dose of 1ml Ethanol, was found to be 1.58±0.08, ($P<0.01$) when compared to Plain control. While the ulcer scores in pre-treated standard group (Group III) in which the animals were treated with Omeprazole was found to be 0.58±0.08 which was statistically not significant when compared to negative control. But in pre-treated test group A (Group IV), in which the test drug given in the dose of 145 mg/kg, ulcer score was significantly decreased to 0.50±0.12 ($p<0.05$). In pre-treated test group B (Group V) in which the test drug was given in the dose of 248mg/kg, the ulcer score was found to be 66±0.44.While in case of post-treated animals, ulcer score in standard group was found to be 0.66±0.27 that was not- significant with respect to Negative control. In post-treated group A (VII) animals were treated with test drug *Post Sumaq* ulcer score was found to be 0.83±0.30 which was non-significant with respect to Negative control. In post-treated group B (group VIII) ulcer score was found to be 0.33±0.16 which showed significant decrease ($p<0.05$) with respect to Negative control. The ulcer index in Negative, Pre and Post-treated standard, test group A and B were found to be 1.91, 0.58, 0.49, 0.97, 0.44, 0.92, and 1.58, respectively and percentage of ulcer reduction in Pre and Post- treated standard, test group A and B were 63, 68, -5, 58, 47 and 79, respectively when calculated with Negative control. The results are summarized in table 1 and figure 1.

Ethanol produces necrotic lesion in the gastric mucosa by its direct toxic effect, reducing the secretion of bicarbonates and production of mucus[4] .The products of 5-lipoxygenase pathway may also play a key role in the development of ulcer induced by irritant agents[2]. Ethanol produces a marked contraction of the

circular muscles of rat fundic strip. Such a contraction can lead to 'mucosal compression' at the site of the greatest mechanical stress, i.e. at the crests of mucosal folds leading to necrosis and ulceration[1]. So, Ethanol induced gastric ulcer method was applied to see ulcer preventive effect of the test drug.

In Ethanol induced gastric ulcer, In pre-treated test group A (Group IV), the test drug reduced ulcer score which was found to be 0.50±0.12 ($p<0.05$) and reduced by 68% , thus showed significant result and indicated that the test drug is preventive to irritation caused by Ethanol. This effect may be due to *Mugharri* (protective covering formation) property of the test drug. In post-treated group A (VII), ulcer score was found to be 0.83±0.30 and reduced by 47% but was found non- significant. In post-treated group B (VIII), ulcer score was found to be 0.33±0.16 ($p<0.05$) and was reduced by 79% which showed significant decrease. This indicates that the test drug is curative at higher dose. Histopathological results also confirmed the effect.

The Phytochemicals of *Rhus coraria* like ellagic acid, gallic acid isoquercitrin, myricitrin, myricetin, quercetin, quercitrin and tannic acid may have a role in its antibacterial, antispasmodic, antiviral, astringent, hepatoprotective, protisticide, analgesic, anti ulcer, and antioxidant effects. Flavonoids are protective for gastrointestinal mucosa against its lesions produced experimentally. Several mechanisms of action may be involved in this protective effect. Quercetin has an anti secretary mechanism of action. However the important factor responsible for the antiulcer activity may be the antioxidant property of flavonoids. Tannins which also show gastro protective activity are present in the drug in sufficient amount[16,17].

Table-1
Effects of hydro alcoholic extract of *Post Sumaq* on Ethanol induced Gastric ulcer

Groups	Treatment	ADU(Mean±SEM)	%RU	Ulcer index	%Reduction
Group I Plain control	Distilled Water	0.08±0.08	17%	0.01	95
Group II Negative control	Distilled Water + Absolute ethanol 1ml/rat	1.58±0.08**	100%	1.91	0.0
Group III Pre-treated Stand.	Omeprazole 20 mg/ kg + Absolute ethanol 1ml/rat	0.58±0.08	100%	0.58	63
Group IV Pre-treated test A	*Post Sumaq* 145mg/kg+ Absolute ethanol 1ml/rat	0.50±0.12*	83%	0.49	68
Group V Pre-treated test B	*Post Sumaq* 248mg/kg +Absolute ethanol 1ml/rat	1.66±0.44	83%	0.97	-5
Group VI Post-treated Stand.	Absolute ethanol 1ml/rat+ Omeprazole 20 mg/kg	0.66±0.27	67%	0.44	58
GroupVII Post-treated test A	Absolute ethanol 1ml/rat+ *Post Sumaq* 145 mg/kg	0.83±0.30	67%	0.92	47
Group VIII Post-treated B	Absolute ethanol 1ml/rat + *Post Sumaq* 248 mg/kg	0.33±0.16*	100%	1.58	79

N=6in each group. Test used Kruskall Wallis test with Dunn' pair comparison test, * $p<0.05$ w.r.t. Negative control, ** $p<0.01$ w.r.t. plain control, %RU =Percentage of rats with ulceration, ADU = Average degree of ulceration.

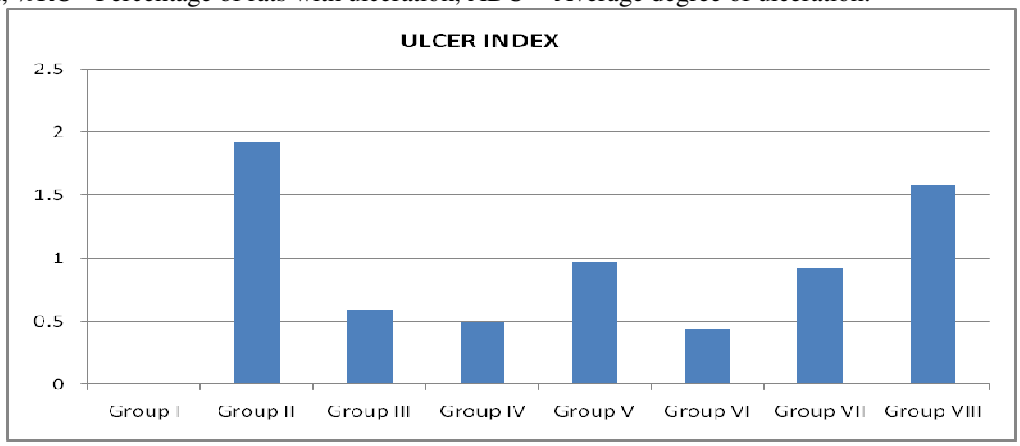
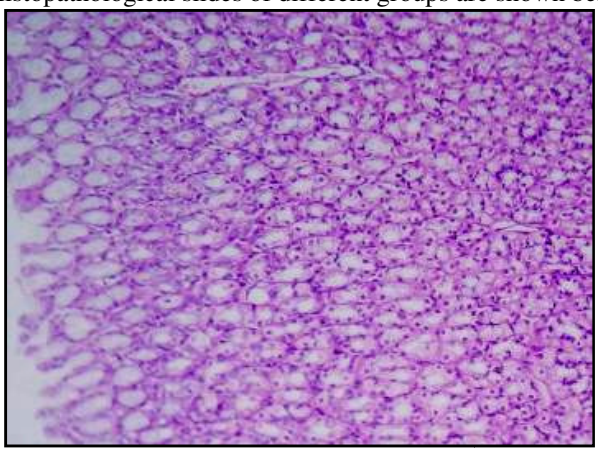
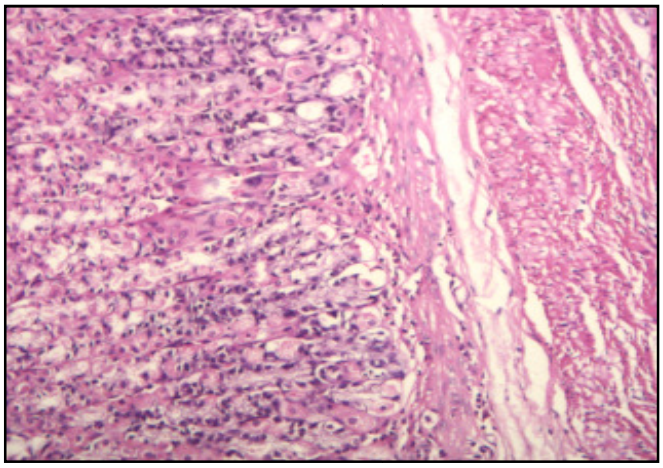


Figure-1
Effect of Hydro alcoholic extract of *Post Sumaq* on Ethanol induced Gastric ulcer

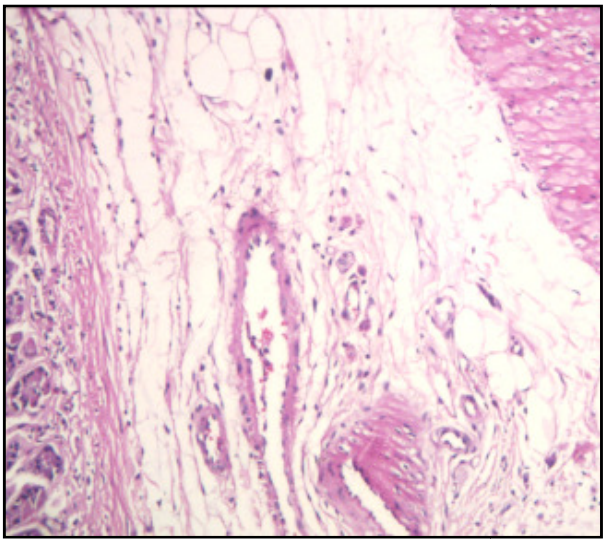
Histopathological slides of different groups are shown below



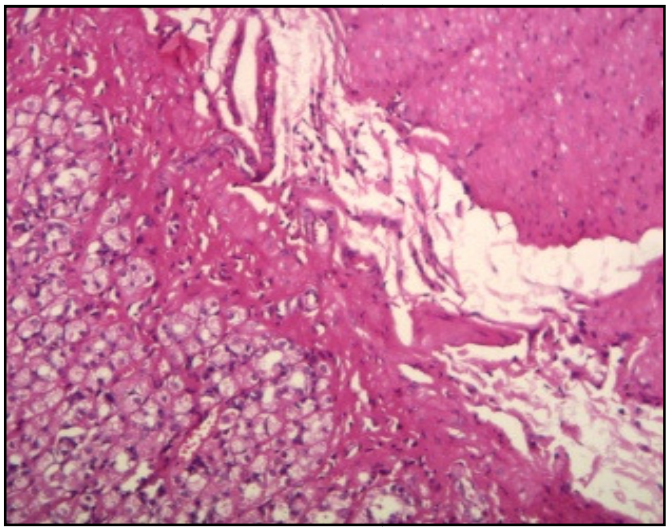
Group-I: Normal mucosa



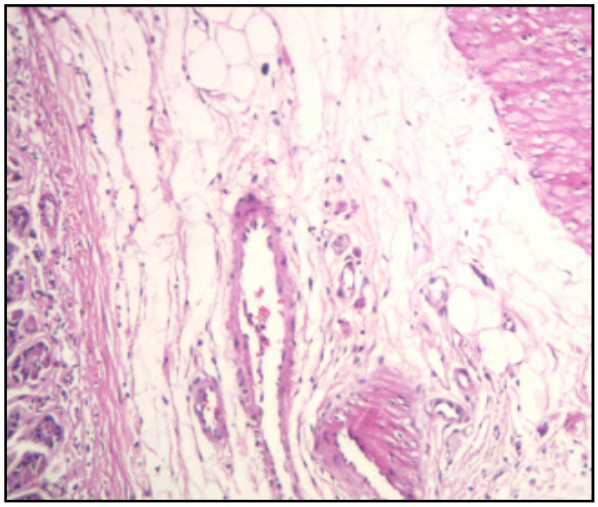
Group-IV: Necrotic changes



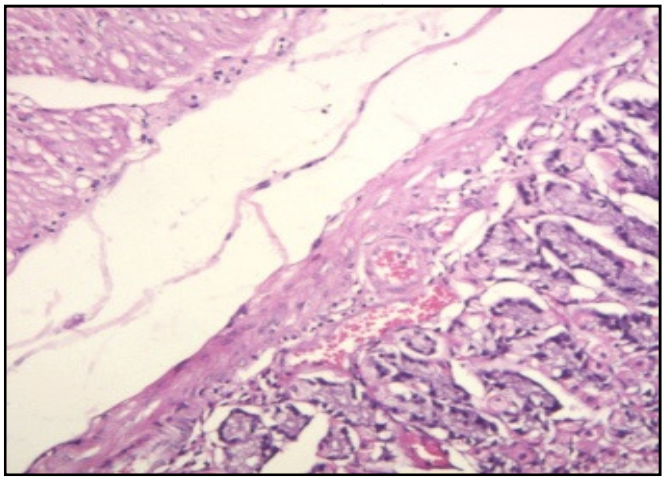
Group-II
Congestion necrosis, erosion and ulceration



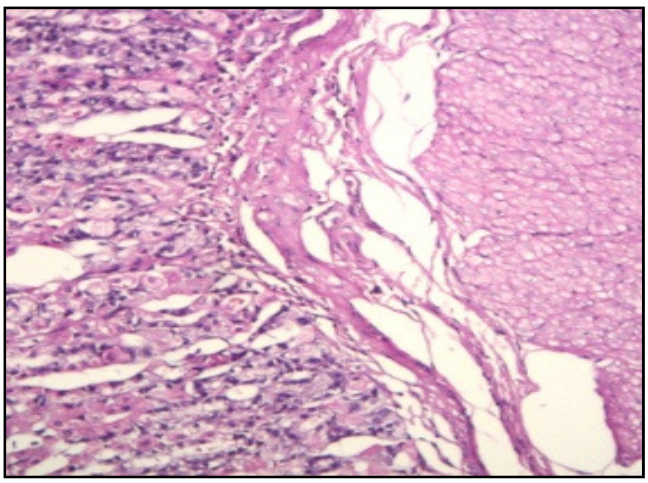
Group V: Necrosis and inflammatory changes



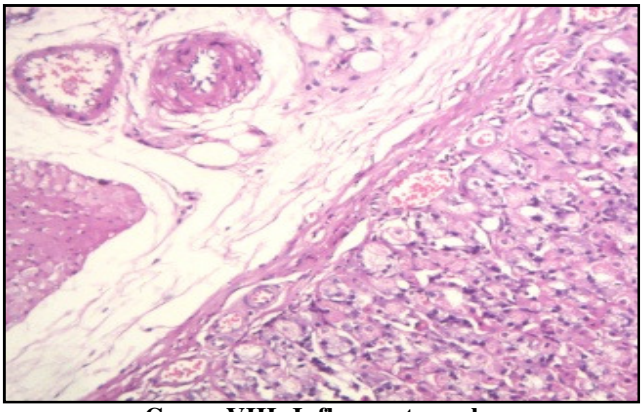
Group-III: Congestion, necrosis and erosion



Group VI: Congestion, necrosis and inflammatory changes



Group VII: Necrotic changes



Group VIII: Inflammatory changes

Conclusion

According to Unani physicians the causes of gastric ulcer are *Khilte Haad* (Irritant and corrosive humour) *fuzlat* (Waste materials which accumulate in the stomach and get infected), *Nawazil* (Descendants which get purulent), intake of hot and spicy foods, excessive use of alcohol, prolong stress and strain, and chronic gastritis and indigestion. Additionally, *Mizaj* (temperament) of drugs and diseases is an important concept of Unani medicine kept in mind when treating a disease and usually drug of opposite *Mizaj* to disease is used. When viewed from this point, 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[18-21]. But the anti ulcer mechanism cannot be just understood by Mizaj or Phytochemicals only as in case of herbal drugs multiple phytochemicals are 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*.

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