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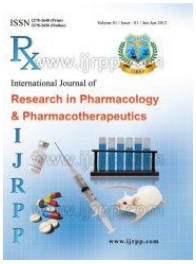

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Antiulcerogenic Activity on Aqueous Crude Extract of *Emblica Officinalis Gaerth in* Pylorus Ligated (Shay) Rats

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ABSTRACT

The complex and multifactorial pathogenesis of peptic ulcer has been studied over several decades, and results from an imbalance of aggressive gastric luminal factors acid and pepsin and defensive mucosal barrier function. Several environmental and host factors contribute to ulcer formation by increasing gastric acid secretion or weakening the mucosal barrier. Among environmental factors, smoking, excessive alcohol use, and drug use are most often quoted but none of them, apart from non-steroidal anti-inflammatory drugs (NSAID) use, were identified as an individual ulcerogenic agent. Emotional stress and psychosocial factors are frequently identified as important contributors to ulcer pathogenesis. Although stress cannot be neglected as a contributing factor, convincing evidence for it being the sole cause of duodenal ulcer is scarce. Aim of the present study is to evaluate the antiulcer activity of aqueous extract of *Emblica officinalis* (EO) on Pylorus ligated (Shay) rats model for ulcer. Additionally the muco protective effect also studied by aspirin-induced ulcerogenesis in pylorus ligated rats as the aqueous ethanolic extract reported as muco protective.

Keywords: *Emblica officinalis*, Peptic Ulcer, Aspirin and Experimental Rats

INTRODUCTION

Peptic ulcer disease embraces both gastric and duodenal ulcers and has been a major threat to the world's population over the past two centuries, with a high morbidity and substantial mortality. Epidemiological data for this disease and its complications have shown salient geographical variations in incidence and prevalence. Development of ulcer disease and death from it has been associated with the birth of urbanization and was interpreted as a birth-cohort event with the peak of disease in those born during the late 19th century [1-2]. Our understanding of the disease changed greatly with the discovery of *Campylobacter pyloridis* (renamed

Helicobacter pylori in 1989 because of a revised taxonomic classification) in 1982 by Warren and Marshall. This discovery switched the notion from an acid-driven disease to an infectious disease, opening a huge area for intensive research that resulted in the understanding of previously suggested mechanisms of pathogenesis. The fall of the acid dogma in peptic ulcer disease, which had found its undisputed acceptance during and after the introduction of histamine H2-receptor antagonists, led to the present therapeutic principle. Maintenance acid suppressive therapy for duodenal ulcer, which followed decades of dominance of surgical interventions (subtotal gastric resections, several forms of vagotomy), was replaced with a short-term antibiotic regimen targeting eradication of *H pylori* infection [3-4]. *H pylori* eradication as cure of peptic ulcer received its full recognition when the Nobel Prize for Medicine and Physiology was awarded to Warren and Marshall in 2005. This recognition has not, however, closed the chapter on peptic ulcers. The management of ulcer disease and its complications remains a clinical challenge. Additionally, non-steroidal anti-inflammatory drugs (NSAIDs) and low-dose aspirin are an increasingly important cause of ulcers and their complications even in *H pylori*-negative patients [5-6]. Other rare causes of ulcer disease in the absence of *H pylori*, NSAIDs, and aspirin also exist [7].

MATERIALS AND METHODS

Collection of Plant Material

Emblica officinalis (EO) was obtained from Papampatti Village of Coimbatore District, Tamil Nadu, India. the plant was Identified and Authenticated by Dr.G.V.S. Murthy, Scientist F & Head of Office Botanical Survey of India, Southern Regional centre Coimbatore.

Preparation of Crude Extract

Mature fruits were collected, dried and coarsely powdered. 250gm of powdered plant material were extracted with double distilled water in Soxhelt Apparatus. The aqueous extract of *Emblica officinalis* (EO) was obtained from department of Pharmacognosy, Karpagam College of Pharmacy, Coimbatore. The extract was suspended in double distilled water using Carboxymethylcellulose (CMC) (0.1%) just before administration to the animals.

Phytochemical evaluation

The aqueous crude extract of *Emblica officinalis* (EO) was subjected to the phytochemcial analysis and the same used for anti ulcer activity [8-9].

Experimental animals

Wistar albino rats of either sex (home bred) aged 7–8 weeks and weighing 150–200 g, were obtained from the Experimental Animal House, Karpagam University, Coimbatore. The animals were fed standard diet chow and water ad libitum and were maintained under standard conditions of humidity (55±5%), temperature (22±2 ◦C) and light (12-h light/12-h dark cycle). The rats were randomly assigned to different control and treatment groups. This study was approved by institutional animal ethical committee (IAEC), under the guidelines of (CPSCEA) Committee for the Purpose of Control and Supervision of Experimental for Animals.

Chemicals

Aspirin (Sigma), Double Distilled Water (DDW), Carboxymethylcellulose(CMC), Sodium Hydroxide, and Sodium Chloride (Merck) were used.

Statistical tools

Statistical analysis was carried out by GraphPad InStat DTCG through one way annova followed by Dunnett's Test, *P<0.05,**P<0.01,***P<0.001 was considered as a significant.

EXPERIMENTAL DESIGN

Antiulcerogenic activity

Pylorus ligated (Shay) rats

The animals were fasted for 36 h with access to water ad libitum before the pylorus was ligated under ether anesthesia, care being taken not to cause bleeding or to occlude blood vessels [10]. Emblica extract (200 and 400 mg/kg body weight) and Ranitidine were administered immediately after pylorus ligation by intraperitoneal injection. The animals were sacrificed 6 h after the pylorus ligation [11]. The stomach was excised carefully keeping the esophagus closed, opened along the greater curvature and the luminal contents were removed. The gastric contents were collected in a beaker and centrifuged at 1000 rpm for 10 min. The samples were analyzed for gastric volume, pH, free and total acidity as the sample subjected to analysis for titratable acidity

against 0.01N NaOH to pH 7. Each stomach was examined for lesions. The mucosa was flushed with saline and stomach pinned on a frog board and scored [12].

Score the ulcers as below

0 = Normal coloured stomach
0.5 = Red coloration
1 = spot ulcers
1.5 = haemorrhagic streaks
2 = ulcers > 3 but < 5
3 = ulcers>5

Mean ulcer score of each animal is expressed as ulcer index.

Acidity (meq/1 per100g) can be expressed as:

$$\text{Acidity} = \frac{\text{Volume of NaOH x Normality x 100}}{0.1} \quad \text{(meq/l per100g)}$$

Compare the gastric volume, acidity and ulcer index of control, extract and the animal treated with ranitidine.

Aspirin-induced ulcerogenesis in pylorus ligated rats

In aspirin-induced ulcerogenesis in pylorus ligated rats, aspirin is administered at a dose of 200 mg/kg orally in a suspension prepared in 1% CMC with water 1h prior to pyloric ligation (time interval between reference drugs and aspirin should be 1 hour) and the process described above was followed[13-15].

RESULTS AND DISCUSSION

Acute oral toxicity study of the crude extracts in mice

The acute oral toxicity study was conducted using the limit test procedure according to OECD test guidelines using female mice. One group at the dose of 2000mg/kg consisting of 03 animals were used; animals received a single dose by inragastric intubations started with extract dissolved with CMC and were observed for mortality signs of gross toxicity or behavioral changes (excitability, convulsions, lethargy, sleep), 30 min and one to four hour and 24th hour post dosing at least once daily for 14 days for the immediate and delayed toxicity during the observation period. The acute oral toxicity studies conducted on aqueous extract of crude plants no mortality and showed no toxicity symptoms up dose of 2000mg/kg body weight in mice

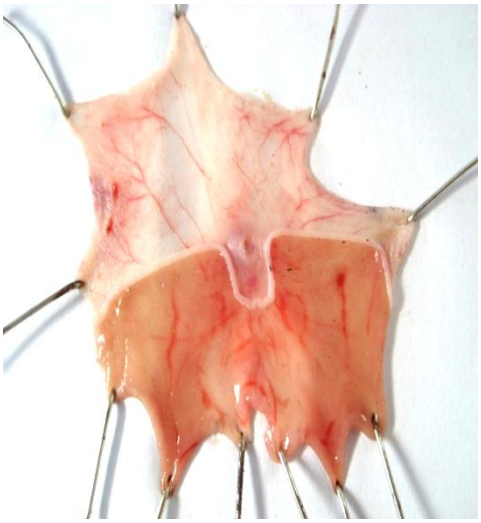
Pylorus ligated (Shay) rats

In the pylorus ligated induced ulcer model, it was observed that the treatment with *Emblica officinalis* aqueous extract (400 mg/kg) and ranitidine (30 mg/kg) significantly reduced the ulcer index in comparison with negative control group ($p < 0.01$). Whereas *Emblica officinalis* aqueous extract (200 mg/kg) treated animals not shown any significance in compare with the control group (Table-1). However, the gastric volume, pH, free acidity and total acidity parameters were significantly reduced in compare with the negative control group ($p < 0.05$). Treatment with *Emblica officinalis* extract (400 mg/kg) and ranitidine (30 mg/kg) reduced significantly all the evaluated parameters in comparison with control group ($p < 0.01$). Hence, the low dose of the extract has not produced any anti-ulcer activity but it produced anti secretary activity; it was evidenced by compare with the high dose of the extract (Figure-1).

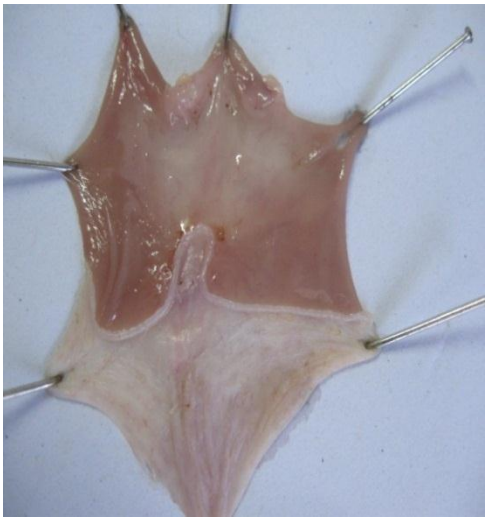
Table -1 Effect of *Emblica officinalis* extract in Pylorus ligated (Shay) rat (n=6)

Group	Dose (mg/kg)	Gastric volume (ml)	Ulcer score	pH	Free acidity(meq/l)	Total acidity(meq/l)
Control		1.38± 0.09	2.58 ±0.30	1.46 ± 0.16	42.8 ±0.74	83.5 ±0.99
Ranitidine	30	0.68± 0.04**	0.83 ±0.16**	3.05 ± 0.16**	29.6 ±0.76**	66.1 ±1.04**
EO –I	200	1.11 ± 0.06*	1.91 ±0.23^{ns}	2.08 ±0.15*	35.0 ±0.51*	72.8 ±1.19*
EO-II	400	0.9 ± 0.03**	1.16 ±0.16**	2.91 ±0.15**	29.83 ±1.07**	67.3 ±1.68**

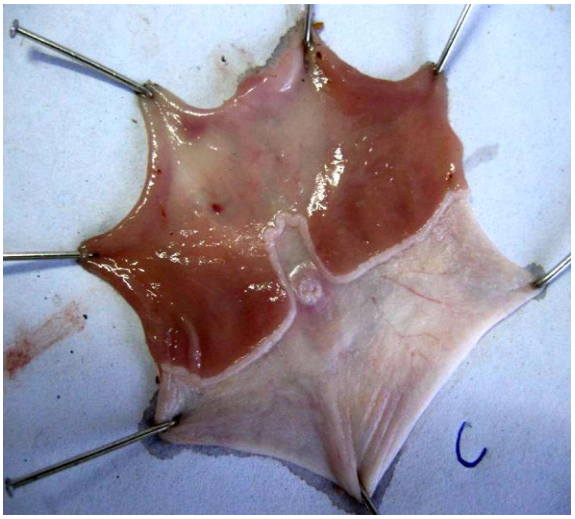
Values are expressed in terms of mean ± S.E.M. statistical analysis was carried by GraphPad InStat DTCG through one way ANOVA. *, P < 0.05; **, P < 0.01, ***, P < 0.001.



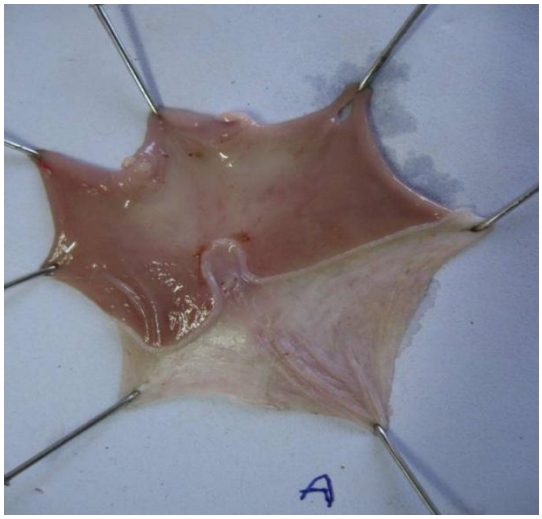
CONTROL
(Ulcer score 2.58±0.30)



RANITIDINE (30mg/kg)
(Ulcer score 0.83±0.16**)



LOW DOSE (200mg)
(Ulcer score1.19± 0.23*ns)



HIGH DOSE (400mg)
(Ulcer score1.16 ± 0.16**)

Figure-1 PYLORUS LIGATED RATS (SHAY RAT METHOD)

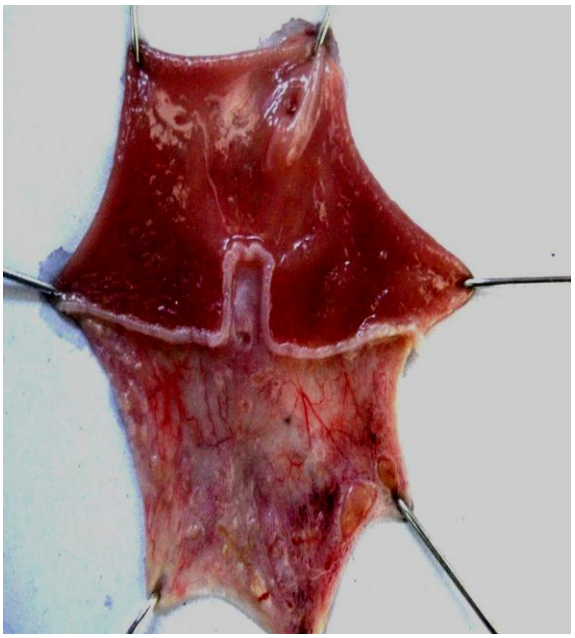
Aspirin-induced ulcerogenesis in pylorus ligated rats

Regarding the aspirin-induced ulcer model, it was observed a significant reduction in ulcer index in animals treated with *Emblica officinalis* aqueous extract (400 mg/kg) and ranitidine (30 mg/kg), in comparison with negative control group ($p < 0.01$). Furthermore, reduced the volume of gastric juice, total, free acidity and raised gastric pH significantly ($p < 0.01$) in comparison with the control group (Table-2). However, *Emblica officinalis* aqueous extract (200 mg/kg) treated animals not shown any significance in compare with the control group. The gastric volume and pH parameters were not significantly reduced in compare with the negative control group. The parameters of free acidity and total acidity were significantly reduced in compare with the negative control group ($p < 0.05$). Hence, the low dose of the extract neither produced anti-ulcer nor produced anti secretary activity; it was evidenced by compare with the high dose of the extract (Figure-2).

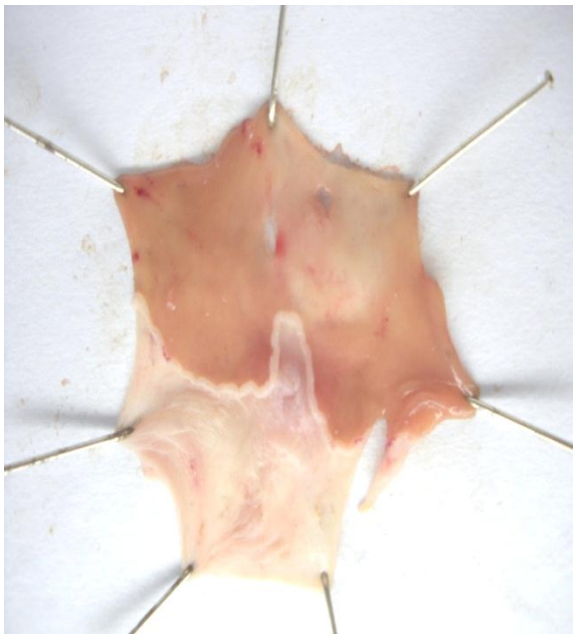
Table-2 Effect of *Emblica officinalis* extract in *a*spirin-induced ulcerogenesis in pylorus ligated rat (n=6)

Group	Dose (mg/kg)	Gastric volume (ml)	Ulcer score	pH	Free acidity(meq/l)	Total acidity(meq/l)
Control		1.76± 0.08	2.91 ±0.23	1.13 ± 0.06	55.0 ±1.34	88.3 ±1.43
Ranitidine	30	0.96± 0.09**	1.08 ±0.15**	2.83 ± 0.14**	34.3 ±0.84**	64.6 ±1.17**
EO –I	200	1.76 ± 0.07^{ns}	2.41 ±0.15^{ns}	1.50 ±0.11^{ns}	51.0 ±1.21*	80.3 ±2.14*
EO-II	**400**	0.93± 0.07**	1.16 ±0.24**	2.80 ±0.10**	38.3 ±0.98**	72.0 ±0.85**

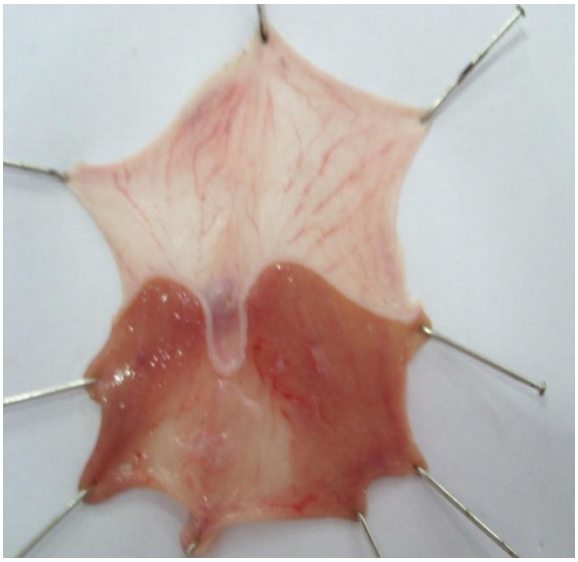
Values are expressed in terms of mean ± S.E.M. statistical analysis was carried by GraphPad InStat DTCG through one way ANOVA. *, P < 0.05; **, P < 0.01, ***, P < 0.001.



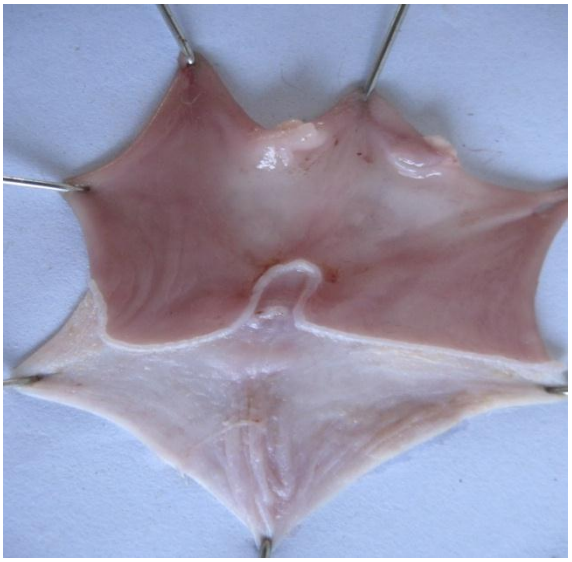
CONTROL
Ulcer score2.91± 0.23



RANITIDINE (30mg/kg)
ulcer score 1.08±o.15**



LOW DOSE(200mg)
Ulcerscore 2.41±0.15ns



HIGH DOSE(400mg)
ulcer score1.16±0.24**

Figure-2 ASPIRIN INDUCED ULCEROGENISIS IN PYLORUS LIGATED RATS.

DISCUSSION

Topical injury by ion trapping and reduction of mucus gel hydrophobicity was once thought to be an important mechanism of NSAID-induced gastric damage. Later, NSAIDs were shown to damage the stomach mainly by suppression of gastric prostaglandin synthesis [16]. The discovery of two isoforms of cyclooxygenases (COX), COX-1 and COX-2, sparked an enormous drive by the pharmaceutical industry to develop COX-2-selective NSAIDs as gastric-sparing anti-inflammatory analgesics. Now, good evidence exists that selective inhibition of COX-2 reduces but does not eliminate risk of gastro duodenal ulcers and their complications. Work in animals has shown that neutrophil adherence to gastric microcirculation plays a crucial part in initiation of NSAID injury. Neutrophil adherence damages the mucosa by liberating oxygen free radicals, releasing proteases, and obstructing capillary blood flow. Inhibition of neutrophil adherence alleviates NSAID induced damage in animal models [17]. Attention has focused on the role of nitric oxide (NO) and hydrogen sulphide (H2S), in maintenance of gastric mucosal integrity. NO and H2S increase mucosal blood flow, stimulate mucus secretion, and inhibit neutrophil adherence. NO-releasing and H2S-releasing derivatives of NSAIDs induce much less gastric damage than do their parent drugs [18]. Unlike animal ulcer models, however, NSAIDs gastropathy in man is characterized by an absence of inflammatory cells unless *H pylori* infection is present, whether neutrophil initiate NSAIDs injury in man remains unknown. Acid suppression has been the mainstay of management of NSAIDs-associated ulcer disease. Gastric acid probably exacerbates NSAIDs injury by converting superficial mucosal lesions to produce deeper injury, interfering with platelet aggregation and impairing ulcer healing [19]. Patients taking NSAIDs have about a four-fold increase in risk of ulcer complications such as bleeding compared with non-users. Several risk factors have been identified in these patients, such as history of ulcer or ulcer complications, old age, co morbidities, use of high-dose NSAIDs, concomitant use of corticosteroids, aspirin, or anticoagulants, and *H pylori* infection. A history of ulcer complications is the most important predictor of future ulcer complications associated with NSAIDs use [20]. There were various causes of potentiation of the offensive factor and reduction of the defensive factor. Extracts of *Emblica officinalis* significantly reduced the formation of gastric lesions in rats induced by Pylorus ligated and aspirin-induced ulcerogenic in pylorus rats ulcerogenic procedures [21-23]. A dose dependent response on the intensity of gastric ulceration was noted. Two hundred milligrams was not statistically proper dose in pyloric (Shay) rat and

aspirin-induced ulcerogenic in pylorus ligated rat model. Where as in pyloric ligated (Shay) rat and NSADs induced ulcerogenic in pylorus ligated rats an increase in dose (400 mg) was found to be effective[24-25]. The significant reduction in basal gastric secretion and complete inhibition of ulcers by *Emblica officinalis* ethanolic extract after pylorus ligation suggest that the gastroprotective mechanism of action of the extract on gastric mucosa may involve direct reduction of gastric acid, pepsin and increase mucus secretion though one or more of the possible mechanisms discussed [26-28]. Moreover, gastric acid is an important factor for the genesis of ulceration of pylorus ligation ulcer in rats [29-30]. *Emblica officinalis* aqueous extract was also able to produce a significant reduction of the gastric mucosal damage induced by aspirin (Table 2), indicating the probable defensive implementation in the ulcer pathogenesis. In the results of anti-secretary parameter investigation, the gastric mucosa of rats revealed that the pretreatment of *Emblica officinalis* aqueous extract absolutely inhibited the aspirin induced necrosis of rat stomach. Our results are in corroboration with the anti-ulcer activity of the aqueous crude extract observed under the studies on pharmacological [31-32].

CONCLUSION

In conclusion, the oral administration of an *Emblica officinalis* aqueous extract produced a significant anti ulcerogenic activity without any apparent toxicological effects, which supports the use of *Emblica officinalis* aqueous crude extract for non-steroidal anti-inflammatory drugs (NSADs) induced ulcer condition. Further experiments and detailed phytochemical analyses are underway to determine the phytoconstituent(s) responsible for as well as the anti-ulcer mechanisms involved.

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