RESEARCH ARTICLE

Pharmacological Evaluation of Cytoprotective Potential of Phyllanthus Emblica (PE) and Asparagus Racemosus (AR) in Preventing Gastric Erosions, Ulcerations and Inflammation induced in Rats

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ABSTRACT

Background: Most of the currently available therapies for peptic ulcer disease have their own limitations and an ideal therapeutic agent is not yet available in the market.Hence,we decided to explore the cytoprotective potential of Indian Medicinal Plants namely Phyllanthus emblica and Asparagus racemosus along with ranitidine as a positive control in various animal models.

Aims & Objective: To evaluate the cytoprotective effect of the extracts of Phyllanthus emblica (PE) and Asparagus racemosus (AR) in preventing gastric erosions, ulcerations and inflammation induced in rats.

Materials and Methods: Cold immobilization stress, ethanol (99.9%), aspirin were the experimental models selected for inducing gastric erosions and ulcerations. Ulcer index , erosions, oedema, congestion and inflammation were the parameters studied for assessing gastric damage whereas carrageenin-induced rat paw oedema model was used to induce inflammation. Ranitidine was used as a positive control.

Results: The two drugs chosen for the study namely Phyllanthus emblica and Asparagus racemosus prevented gastric damage significantly in the ethanol group ($p<0.05$) and cold stress group ($p<0.05$). In the aspirin group, erosions, oedema, congestion and inflammation were remarkably reduced by PE cold water extract (PECWE) and PE hot water extract (PEHWE) and in the AR treated groups when compared with the control group. PECWE and PEHWE showed significant anti-inflammatory activity ($p<0.05$) comparable to aspirin thereby proving their role in inflammatory disorders. In our study, AR also showed a significant protection ($p<0.05$) against ethanol, cold stress and aspirin group.

Conclusion: The present study supports that ethanol, cold simmobilization stress and aspirin induce gastritis, gastric erosions and ulcerations and Phyllanthus emblica and Asparagus racemosus have significant potential as prominent cytoprotectants against these toxic effects.

KEY WORDS: Ulcer Index; Inflammation; Oedema; PE; AR

INTRODUCTION

Acute gastritis, erosions and ulcerations have been documented in association with habitual use of NSAIDs like aspirin, ibuprofen, spicy foods, alcohol, severe stress following extensive burns, trauma etc. in the stomach. H pylori positive individuals are also prone to gastric tumours and malignancies.[1]

Unfortunately, medical management of acute gastritis leaves to be desired. The purely symptomatic and supportive management consists of withdrawal of the offending agent, treatment of the associated disease, general supportive measures, and treatment with anti-ulcer agents like H2 blockers, PPI and PG analogues.[1] Most of the currently available therapies have their own limitations and an ideal therapeutic agent is not yet available in the market.

Ayurveda, the Indian traditional system of Medicine offers some strategies that are useful in the treatment of acid-peptic disorders. We chose 2 plants Phyllanthus emblica and Asparagus racemosus and compared them with ranitidine in different models of experimental gastritis.

We first set up experimental models of gastritis. Of the various models of gastritis described, we chose the NSAIDs[2], ethanol[3] and cold immobilization[4] stress-induced gastritis models, as they were cheap and easily available. After standardizing the models, we decided to explore the cytoprotective potential of Indian Medicinal Plants namely Phyllanthus emblica and asparagus racemosus along with ranitidine as a positive control.

The purpose of the study was to evaluate the efficacy of Phyllanthus emblica and Asparagus racemosus in preventing or reducing the severity of gastritis, and its related complications like ulcers, haemorrhage and perforation. However, there is paucity of data regarding the established role of the above mentioned drugs as cytoprotective agents, and hence we set out to investigate the same in rats.

Further, we also selected the carrageenin induced rat paw oedema model to explore the anti-inflammatory effects of Phyllanthus emblica and Asparagus racemosus.

MATERIALS AND METHODS

Animals

246 Wistar rats of either sex weighing between 200-250 gms were used for the study. The rats were kept fasting for a period of 24 hrs,prior to the day of experimentation with free access to drinking water upto the beginning of the experiment. Standard Ethical guidelines were followed during the study.

Drugs

Phyllanthus emblica
Asparagus racemosus
Ranitidine

Chemicals

Ethanol 100% (haymen)
Aspirin (UDCT laboratory, Mumbai)
Carboxymethylcellulose

Methods of Preparation

A. Phyllanthus emblica: Four different formulations of Phyllanthus emblica, namely dry powder (PEDP), hot water extract (PEHWE), cold water extract (PECWE) and incinerated powder (PEIP) were administered orally in the dose of 270 mg/Kg. These were prepared as mentioned below.

- *Dry powder*: dried fruits were ground to get a fine powder which was then passed through # 100 mesh.
- *Hot water extract*: 25 gms of dried powder fruit were taken in a clean glass stoppered flask to which 250 ml of distilled water was added. The solutionwas boiled for 2 minutes, cooled and filtered to get a clear solution. The filtrate was then autoclaved at 1200° C for 15 minutes, cooled and dispensed in sterile 10 ml vials under aseptic conditions.
- *Cold water extract*: 10 gms of dried powdered fruit were taken in a clean glass powdered flask to which 100 ml of distilled water was added.

The solution was sonicated for 1 hour and kept overnight. On the next day, the solution was filtered to get a clear solution and dispensed in sterile 10 ml vials under aseptic conditions.

- *Incinerated powder*: About 30 gms of commercially available fruits were taken in a clean earthenware pot. The pot was closed with lid and sealed with mud. Sealed pot was kept in a muffled furnace at 200 ± 5 ° C. After exactly 2 hours, pot was removed and cooled to room temperature. Seal was then opened and charred mass was ground to uniformly fine powder which was then passed through # 100.

B. Asparagus racemosus: The dried root was obtained from the market and identified by pharmacognostic methods. It was then powdered and suspended in water. A decoction was prepared by boiling and the unfiltered decoction was administered orally to rats in the dose of 270 mg/kg.

C. Ranitidine: It was obtained form Glaxo India Limited and was administered in the dose of 27 mg/kg orally.

The present study was divided into two phases to evaluate different effects of Phyllanthus emblica (PE) and Asparagus racemosus (AR). Ranitidine (Ran) was used as a positive control throughout the study.

Phase I: Cytoprotective effect (Figure 1)
Phase II: Anti-inflammatory effect (Figure 2)

Figure-1: Phase I: Cytoprotective Effect

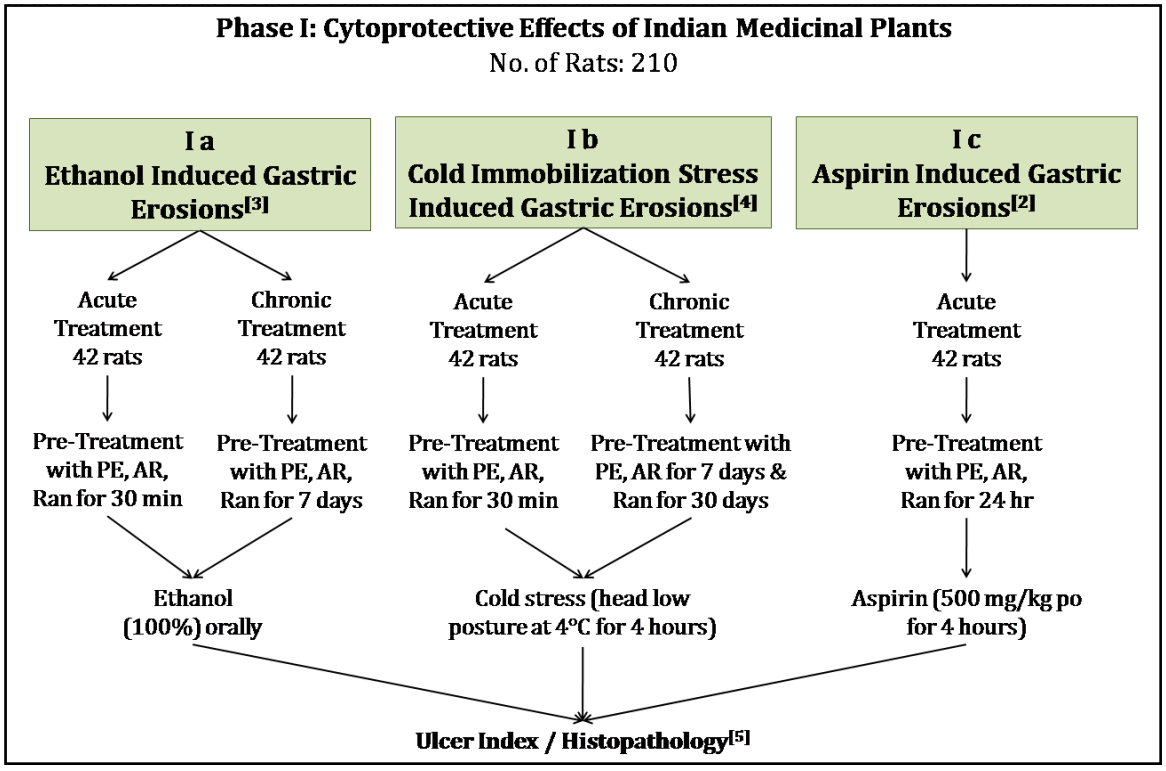
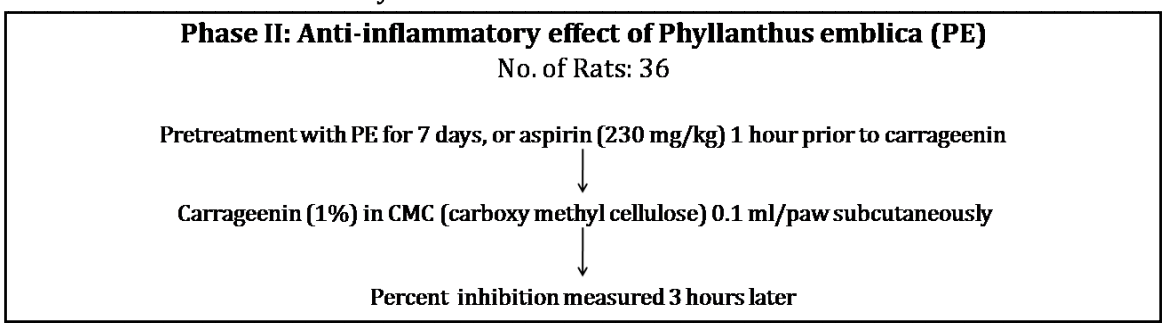


Figure-2: Phase II: Anti-inflammatory Effect



Parameters for Assessment of Damage

1. *Ulcer index (UI)*: it was determined by the following formula[5]

   $UI = \frac{10}{x}$ Where, x = $\frac{\text{Total mucosal area}}{\text{Total ulcerated area}}$

2. *Histopathology* [5]: After calculating the ulcer index, the stomach was stored in formalin for histopathology. Paraffin sections were made and stained with haematoxilin eosin and observed for erosions, oedema and congestion. Microscopically, congestion was measured by calculating the number of dilated submucosal vessels. Erosions and oedema were graded as, 0 = no damage, 1 = mild damage, 2 = moderate damage, 3 = severe damage

Percent inhibition of inflammation was calculated as follows:

1. Control: $\mathbf{X}$ (% **inflammation**) = $\frac{B-A}{A} \times 100$
2. Test Drug: $\mathbf{Y}$ (% **inflammation**) = $\frac{D-C}{C} \times 100$

   A – initial reading (control), B – final reading (control), C – initial reading (test drug), D – final reading (test drug).

   The unit of measurement was mmHg
3. Percent Inhibition = $\frac{Y-X}{X} \times 100$

Statistical Analysis: It was done using the student's t-test for the parametric data and Wilcoxon test for the non-parametric data.

RESULTS

Ethanol Induced Damage: Acute Study

42 rats divided into 7 groups (6 rats each) were used

Table-1: Ethanol Induced Damage: Acute Study

Groups	Drugs	Ulcer Index
1	D/W	0.80 ± 0.09
2	PEDP	0.62 ± 0.16
3	PEHWE	0.45 ± 0.13 *
4	PECWE	0.45 ± 0.15 *
5	PEIP	0.31 ± 0.06 **
6	Ran	0.35 ± 0.07 **
7	AR	0.11 ± 0.05 **

* p < 0.01; ** p < 0.001 (D/W : Distilled water, Ran : Ranitidine, PEDP : Phyllanthus emblica dry powder, PEHWE : Phyllanthus emblica hot water extract, PECWE : Phyllanthus emblica cold water extract, PEIP : Phyllanthus emblica incinerated powder, AR : Asparagus racemosus)

Ethanol Induced Damage: Chronic Study

42 rats divided into 7 groups (6 rats each) were used

Table-2: Ethanol Induced Damage: Chronic Study

Groups	Drugs	Ulcer index	Erosions	Oedema	Congestion
1	D/W	1.03 ± 0.17	2.33 ± 0.57	2.00 ± 0.00	5.16 ± 4.32
2	PEDP	0.43 ± 0.29 *	2.33 ± 0.57	1.00 ± 1.00	3.00 ± 0.58
3	PEHWE	0.31 ± 0.13 **	1.33 ± 0.57	1.33 ± 0.57	4.28 ± 1.82
4	PECWE	0.41 ± 0.12 **	1.33 ± 0.57	1.33 ± 0.57	4.65 ± 2.42
5	PEIP	0.27 ± 0.12 **	0.66 ± 0.57	0.66 ± 0.57	6.73 ± 3.59
6	RAN	0.24 ± 0.07 ***	0.66 ± 0.57	0.66 ± 0.57	4.32 ± 2.01
7	AR	0.32 ± 0.16 **	1.00 ± 0.00	0.33 ± 0.57	3.71 ± 0.77

* p < 0.001; ** p < 0.0001; *** p < 0.00001 (D/W : Distilled water, Ran : Ranitidine, PEDP : Phyllanthus emblica dry powder, PEHWE : Phyllanthus emblica hot water extract, PECWE : Phyllanthus emblica cold water extract, PEIP : Phyllanthus emblica incinerated powder, AR : Asparagus racemosus)

Cold Immobilization stress: Acute Study

42 rats divided into 7 groups (6 rats each) were used in this part of the study (Table 3)

Table-3: Cold Immobilization Stress: Acute Study

Groups	Drugs	Ulcer index	Erosions	Oedema	Congestion
1	D/W	1.28 ± 0.06	2.33 ± 0.57	2.33 ± 0.57	3.90 ± 0.65
2	PEDP	0.15 ± 0.04 *	0.66 ± 0.57	0.33 ± 0.57	4.00 ± 2.28
3	PEHWE	0.09 ± 0.05 **	1.00 ± 0.00	0.33 ± 0.57	4.58 ± 2.37
4	PECWE	0.09 ± 0.08 **	0.33 ± 0.57	1.00 ± 1.00	3.18 ± 2.48
5	PEIP	0.19 ± 0.13	1.00 ± 1.00	1.00 ± 0.00	4.10 ± 1.52
6	RAN	0.07 ± 0.01 ***	1.00 ± 0.00	0.66 ± 0.57	3.59 ± 1.42
7	AR	0.30 ± 0.09	---	---	---

* p < 0.01; ** p < 0.001; *** p < 0.001 (D/W : Distilled water, Ran : Ranitidine, PEDP : Phyllanthus emblica dry powder, PEHWE : Phyllanthus emblica hot water extract, PECWE : Phyllanthus emblica cold water extract, PEIP : Phyllanthus emblica incinerated powder, AR : Asparagus racemosus)

Cold Immobilization Stress: Chronic Study

42 rats divided into 7 groups (6 rats each) were used in this part of the study

Table-4: Cold Immobilization Stress: Chronic Study

Groups	Drugs	Ulcer index	Erosions	Oedema	Congestion
1	D/W	0.47 ± 0.11	2.66 ± 0.57	2.33 ± 0.57	4.96 ± 1.80
2	PEDP	0.10 ± 0.04 ***	0.66 ± 0.57	0.66 ± 0.57	3.52 ± 0.62
3	PEHWE	0.05 ± 0.04 ****	0.66 ± 0.57	0.66 ± 0.57	5.06 ± 1.64
4	PECWE	0.05 ± 0.02 ****	0.66 ± 1.15	0.66 ± 0.57	3.62 ± 0.77
5	PEIP	0.14 ± 0.08 **	0.33 ± 0.57	0.66 ± 0.57	6.35 ± 2.34
6	RAN	0.29 ± 0.05 *	1.00 ± 0.00	0.66 ± 0.57	4.35 ± 0.13
7	AR	0.15 ± 0.04 **	0.33 ± 0.57	0.33 ± 0.57	6.37 ± 2.72

* p < 0.01; ** p < 0.001; *** p < 0.0001; **** p < 0.0001
(D/W : Distilled water, Ran : Ranitidine, PEDP : Phyllanthus emblica dry powder, PEHWE : Phyllanthus emblica hot water extract, PECWE : Phyllanthus emblica cold water extract, PEIP : Phyllanthus emblica incinerated powder, AR : Asparagus racemosus)

Aspirin Induced Damage

42 rats divided into 7 groups (6 rats each) were used in this part of the study

Table-5: Aspirin Induced Damage

Groups	Drugs	Erosions	Oedema	Congestion	Inflammation
1	D/W	1.66 ± 0.75	1.66 ± 0.98	2.00 ± 1.26	1.66 ± 1.16
2	PEDP	0.00 ± 0.00	2.00 ± 0.00	0.50 ± 0.70	0.00 ± 0.00
3	PEHWE	0.50 ± 0.70	2.00 ± 0.41	1.50 ± 0.70	0.50 ± 0.70
4	PECWE	1.00 ± 1.41	1.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.70
5	PEIP	2.00 ± 1.41	2.00 ± 0.00	1.50 ± 0.70	1.00 ± 0.00
6	RAN	1.00 ± 0.00	1.50 ± 0.70	1.50 ± 0.70	0.00 ± 0.00
7	AR	0.50 ± 0.70	2.00 ± 0.00	0.50 ± 0.70	0.50 ± 0.70

(D/W : Distilled water, Ran : Ranitidine, PEDP : Phyllanthus emblica dry powder, PEHWE : Phyllanthus emblica hot water extract, PECWE : Phyllanthus emblica cold water extract, PEIP : Phyllanthus emblica incinerated powder, AR : Asparagus racemosus)

Anti-inflammatory Effect of Phyllanthus Emblica

36 rats divided into 6 groups (6 rats each) were used in this part of the study

Table-6: Anti-inflammatory Effect of Phyllanthus Emblica (Mean ± SD)

Group No.	Drugs	% Increase (Paw Volume)	% inhibition
1	D/W	40.24 ± 12.75	---
2	PEDP	49.13 ± 20.70	22.00
3	PEHWE	13.89 ± 10.50	65.48
4	PECWE	16.35 ± 8.31	59.36
5	PEIP	97.47 ± 24.15	-142.00
6	ASP	13.70 ± 10.20	65.96

(D/W : Distilled water, Ran : Ranitidine, PEDP : Phyllanthus emblica dry powder, PEHWE : Phyllanthus emblica hot water extract, PECWE : Phyllanthus emblica cold water extract, PEIP : Phyllanthus emblica incinerated powder, AR : Asparagus racemosus)

DISCUSSION

This study confirms the potential of different formulations of phyllanthus emblica (amalaki) and Asparagus racemosus (Shatavari) in preventing gastritis and its related complications like erosions, ulcerations, haemorrhage and perforation.

Till today, management of gastritis and gastric erosions has been mainly supportive and included treatment of associated disease, maintenance of oxygen, blood volume, fluid and electrolyte requirements, antacids and H2 receptor blockers. No specific pharmacological agent has been shown to be effective in preventing either gastritis or its related complications.

The primary aim of the study was to evaluate the ability of Phyllanthus emblica and Asparagus racemosus in preventing acute gastritis and its related complications.

We began this study by setting up an experimental model of gastric erosions by ethanol, cold immobilization stress and aspirin. To confirm the reproducibility of the models, gastric erosions were induced by ethanol (100%), cold stress at 4-6º C, and aspirin in the dose of 500 mg/kg. Reddish black streaks or patches were seen on the mucosal surface of the stomach when cut open along the greater curvature. Ulcer index was very high especially with ethanol and histopathology showed congestion and dilatation of the submucosal vessels with areas of focal ulceration.

Ulcer index was reduced from 0.80 ± 0.09 to 0.45 ± 0.13 in the PEHWE and PECWE groups which was statistically significant.

Grading of erosions and oedema by histopathology in all the three models clearly showed that gastric damage was significantly reduced and on certain occasions completely prevented. The ease of induction of gastric erosions, the relatively low cost and the reproducibility proved to be the salient advantages while carrying out the study.

The two drugs chosen for the study namely PE and AR could prevent gastric damage significantly in ethanol and the cold stress group macroscopically.

PE proved highly effective in preventing gastritis and erosions. In the D/W treated group, when ethanol was administered in the dose of 1 ml/ 100 gm to rats, the stomach showed the presence of red streaks, patches and occasional petechiae. When ethanol was administered to the drug treated groups, i.e. PE and AR, the ulcer index fell significantly with minimal streaks, the congestion was reduced significantly and histopathology showed only minimal areas of focal ulcerations.

In the aspirin group, erosions, oedema, congestion and inflammation were significantly reduced in the PEHWE and PECWE and in the AR treated groups when compared with the control group which explains the gastric cytoprotective role of PE and AR. PEHWE and PECWE showed significant anti-inflammatory activity comparable to aspirin thereby proving their role in inflammatory disorders.

Ayurvedic literature revealed that PE had anti-inflammatory effects.[6] Further, if a drug could combine cytoprotective with anti-inflammatory effects,it would be a novel addition to the therapeutic armamentarium. Hence we took up the study to evaluate the anti-inflammatory effects of different effects of PE.

PE has been found to be effective in the treatment of amalapitta (peptic ulcer) and in dyspepsia.[7]

PE is a plant extensively used in Ayurveda as a Rasayana.[8] This term has been used by Sushruta for agents which, among other things, strengthens life.

The fact that PE successfully prevented gastritis and its associated complications like erosions and ulcerations suggests that there is some truth in shloka. The question arises whether we can interpret the term 'strengthening life as 'strengthening tissues', i.e. can we attribute this effect to cytoprotective actions of PE? Further, its anti-inflammatory – cytoprotective effects suggests a mechanism different from prostaglandins (PGs). PE is a richest source of vitamin C.As such, it may be exerting both cytoprotective and anti-inflammatory effects by scavenging free radicals.

Asparagus Racemosus

In our study, the aqueous extract of AR showed a significant protection ($p<0.05$) against ethanol damage, cold stress and aspirin as it was evident from ulcer index and histopathology. This effect could be due to its direct cytoprotective effects. In fact, AR, is a common ingredient of formulations prescribed for the treatment of peptic ulcers.

The anti-oxytoxic action of AR may prevent this increased ACTH secretion during stress thus contributing to its anti-ulcer effects.

CONCLUSION

Induction of gastric erosions by ethanol, cold immobilization stress and Aspirin were found to be easy, cheap and reproducible models.

PE and AR could be novel and revolutionary therapeutic strategies to prevent development of gastric erosions. This was confirmd by assessing cytoprotection by ulcer index macroscopically and grading erosions, edema, congestion and and inflammation microscopically.

Our study confirms the cytoprotective effects of different effects extracts of PE as well as AR and shows them to be comparable to ranitidine.

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