

ANTI ULCER ACTIVITY OF THE ETHANOLIC EXTRACT OF LEAVES *PHYSALIS ANGULATA*.I

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## ABSTRACT

**Aim:** The present study was designed to investigate the antiulcer effect of ethanolic extract of *Physalis angulata* leaves (EEPAL) using different models of gastric ulceration in rats.

**Method:** Acute gastric ulceration in rats is induced by oral administration of noxious chemicals like aspirin and ethanol. Anti-secretory studies and total gastric acid output were studied using pylorus ligation technique. EEPAL was administered at the doses of 250 and 500 mg/kg orally to the respective experimental groups. Omeprazole was used as a reference drug. The antiulcer activity was assessed by determining and comparing the ulcer index in the test groups with that of the control group and standard group animals.

**Results:** EEPAL at the dose of 500mg/kg showed a significant reduction in the ulcer index and also significantly inhibited gastric mucosal damage induced by aspirin and ethanol. In pylorus ligated rats, EEPAL significantly reduced the basal gastric acid secretion.

**Conclusion:** The present study suggested that the protective effect of EEPAL might be mediated by anti secretory and cytoprotective mechanisms. Moreover, further insight into the precise mechanism of action is essential to explore the complete potency of EEPAL and increase its usage in contemporary medicine.

**Keywords:** *Physalis angulata*, Anti ulcer activity

## INTRODUCTION

Gastric hyperacidity and gastro duodenal ulcer is a very common human problem now a days<sup>1</sup>. Gastric ulcer is believed to be due to an imbalance between offensive (acid, pepsin and H-pylori) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors). These agents have been implicated in the pathogenesis of gastric ulcer including enhanced gastric acid and pepsin secretion<sup>2</sup>, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility. Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor (PAF), leukotrienes, endothelins, bile or exogenous factors including NSAIDs<sup>3</sup> or stimulating the mucosal defences (mucus, bicarbonate, normal blood flow, prostaglandins, nitric oxide)<sup>4</sup>. The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Hence, efforts are on to find a natural product that suitable to treat the ulcer.

*P. angulata* is an annual herb indigenous to many the tropical parts in the world. It can be found on most continents in the tropics including Africa, Asia and the America. It grows up to 1m with small stem, cream-colored flowers and light yellowish & orange colored fruits.

It is a medicinally important plant used in traditional medicine as analgesic, antirheumatic, to treat sore throat and abdominal pain. It is considered as antipyretic, anti-nociceptive, anti-diuretic, anti-inflammatory for hepatitis and cervicitis<sup>6</sup> and alpha amylase inhibitory activity<sup>7</sup>. Some species have edible fruits and tea of their roots is considered in medicine.

## MATERIALS AND METHODS

## Plant material

The fresh leaves of *Physalis angulata* were collected from local areas of Chengalpattu, Tamil Nadu, India and plant was authenticated by Prof. P.Jayaraman, Botanist, Director of National institute of Herbal science, Chennai. The leaves were dried in shade and were ground to a coarse powder.

## Extraction

Air dried *Physalis angulata* leaves extraction was prepared by maceration technique using ethanol as solvent for 72 hrs at room temperature. The extract was concentrated by simple evaporation at

room temperature. A suspension of EEPAL in 5% (w/v) carboxymethyl cellulose was prepared for oral administration.

## Animals

Albino wistar rats of either sex weighing approximately 180-200 gm were used for the antiulcer study. The animals were housed in cages under standard laboratory conditions (12/12 hour light/dark cycle at 25±5°C) and were fed with a commercial rat diet and water ad libitum. All procedures involved in using the animals were carried out according to Institutional Animals Ethics Committee (IAEC) (XII/VELS/PCOL/23/2000/CPCSEA/IPEC).

## Phytochemical analysis

A portion of residue from each extracts was subjected for phytochemical analysis to check the presence of alkaloids, steroids and flavonoids<sup>8</sup>.

## Acute toxicity studies

Toxicity studies of the ethanolic leaf extract were carried out using Albino mice of either sex weighing between 20 and 25g. The LD<sub>50</sub> of the ethanol extract of leaf was found to be safe up to 5000 mg/kg (p.o.).

## Ethanol induced ulcer model

In this model ulcer was induced by administration of absolute ethanol (90%) (1ml/200g). The animals were divided into four groups each consisting of six rats and were fasted for 36 hours prior to administration of ethanol.

Group I- Positive control group which received ethanol.

Group II & III- Test groups which received Ethanolic extract of *Physalis angulata* leaves in the dose of 250 and 500 mg/kg respectively.

Group IV- Standard group which received Omeprazole in the dose of 20 mg/kg.

After 45 min of oral administration of Ethanolic extracts and Omeprazole all the groups were treated with Ethanol. After 1 hr all the animals were anaesthetized with anesthetic ether and stomach was incised along the greater curvature and ulceration was scored. A score for the ulcer was studied similar to pyloric ligation induced ulcer model<sup>9,10</sup>.

**Aspirin induced ulcer model**

In this model ulcer was induced by administration of Aspirin (200mg/kg p.o). The animals were divided into four groups each consisting of six rats and were fasted for 36 hours prior to administration of aspirin.

Group I- Positive control group which received Aspirin.

Group II & III- Test groups which received Ethanolic extract of *Physalis angulata* leaves in the dose of 250 and 500 mg/kg respectively.

Group IV- Standard group which received Omeprazole in the dose of 20 mg/kg.

After 45 min of oral administration of Ethanolic extracts and Omeprazole all the groups were treated with Aspirin. After 4 hours all the animals were anaesthetized with anesthetic ether and stomach was incised along the greater curvature and ulceration was scored. A score for the ulcer was studied similar to pyloric ligation induced ulcer model<sup>9, 10</sup>.

**Pyloric ligation in rats**

The animals were divided into four groups each consisting of six rats and were fasted for 48 hours prior to experimentation.

Group I- Positive control group which received Aspirin.

Group II & III- Test groups which received Ethanolic extract of *Physalis angulata* leaves in the dose of 250 and 500 mg/kg respectively.

Group IV- Standard group which received Omeprazole in the dose of 20 mg/kg.

After 45 min of oral administration of Ethanolic extracts and Omeprazole all the groups were subjected to pyloric ligation under ether anesthesia. Ligation was done without causing any damage to the blood supply of the stomach. After 4 hours of surgery, rats were sacrificed and ulcer scoring was done<sup>9, 10</sup>. Gastric juice was collected and gastric secretion studies were performed<sup>11, 12</sup>.

**Scoring of ulcer will be made as follows**

Normal stomach..... (0)

Red coloration..... (0.5)

Spot ulcer..... (1)

Hemorrhagic streak... (1.5)

Ulcers..... (2)

Perforation..... (3)

Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer protection was determined as follows

% Protective = Control mean ulcer index - Test mean ulcer index / Control mean ulcer index X 100

**Statistical Analysis**

The values are represented as mean  $\pm$  S.E.M and statistical significance between treated and control groups was analyzed using of one way ANOVA, followed by Dunnett's test where P<0.01 was considered statistically significant.

**RESULTS****Table 1: Phytochemical Analysis of leaf extract of *Physalis angulata***

Extract	Alkaloids	Glycosides	Saponins	Carbohydrates	Tannins	Flavonoids	Steroids	Triterpenoids	Lignins	Proteins	Amino acids
Aqueous	+	-	-	-	-	-	+	-	-	-	-
Hexane	+	-	-	-	-	-	+	-	-	-	-
Trichloro methane	-	-	-	-	-	-	+	-	-	-	-
Methanol	+	-	-	-	-	+	+	-	-	-	-
Ethanol	+	-	-	-	-	+	+	-	-	-	-

The qualitative phytochemical study reveals the presence of alkaloids, phytosterols and flavanoids.

**Table 2: Effect of *Physalis angulata* leave extracts on various parameters in Ethanol induced gastric ulcers**

Group	Treatment	Ulcer index	% Protection	pH of gastric content
I	Control ( 1ml/ animal)	10.7 $\pm$ 0.27	-	3.2 $\pm$ 0.58
II	Omeprazole (20mg/ kg)	2.45 $\pm$ 0.37**	77%	5.18 $\pm$ 0.37**
III	EEPAL 250mg/ kg	4.25 $\pm$ 0.41*	60%	3.43 $\pm$ 0.20*
IV	EEPAL 500mg/ kg	3.66 $\pm$ 0.25**	65%	4.65 $\pm$ 0.15*

\*\*P<0.01 Vs control group; P<0.05 Vs control group. All values are represented as mean  $\pm$  S.E.M, and statically significance using of one way ANOVA followed by Dennett's test where P<0.05 was moderately significant and P<0.01 was considered as statically significant.

EEPAL showed the ability to significantly reduce the ulceration of stomach induced by absolute ethanol. EEPAL at dose of 250mg/kg, 500 mg/kg and omeprazole at 20 mg/kg produced a significant

(p<0.01) reduction in the ulcer index 4.25, 3.66 and 2.45 and has protection index of 60%, 66% and 77% and pH of gastric content 3.48, 4.65 and 5.18 respectively as shown in table 2.

**Table 3: Effect of *Physalis angulata* leaf extracts on various parameters in Aspirin induced gastric ulcers.**

Group	Treatment	Ulcer index	% Protection	pH of gastric content
I	Control	6.16 $\pm$ 0.25	-	3.4 $\pm$ 0.22
II	Omeprazole (20mg/ kg)	1.3 $\pm$ 0.25**	78%	5.38 $\pm$ 0.27**
III	EEPAL 250mg/ kg	3.3 $\pm$ 0.40*	45%	3.6 $\pm$ 0.15*
IV	EEPAL 500mg/ kg	2.18 $\pm$ 0.24**	66%	4.8 $\pm$ 0.08*

\*\*P<0.01 Vs control group; P<0.05 Vs control group. All values are represented as mean  $\pm$  S.E.M, and statically significance using of one way ANOVA followed by Dennett's test where P<0.05 was moderately significant and P<0.01 was considered as statically significant.

EEPAL at dose of 250 mg/kg, 500 mg/kg and omeprazole at 20 mg/kg produced a significant reduction in the ulcer index 3.3, 2.1 and 1.3 and protection index 45%, 60% and 78% respectively as shown in table 3.

After 1 hour of treatment with EEPAL, pyloric ligated rats for 4h resulted in accumulation of gastric secretory volume, ulceration and

increase in free and total acidity. EEPAL at dose of 250mg/kg, 500 mg/kg and omeprazole at 20 mg/kg produced a significant ( $p < 0.01$ ) reduction in the ulcer index 3.5, 2.5 and 2.1 and has protection index of 66%, 76% and 79% and  $p^H$  of gastric content 3.56, 4.41 and 4.71 and free acidity as 44.7 meq/ltr, 34.6 meq/ltr, 32.5 meq/ltr and total acidity as 69.4 meq/ltr, 62.3 meq/ltr, 55.8 meq/ltr respectively as shown in table 4.

**Table 4: Effect of *Physalis angulata* leaf extracts on various parameters in pyloric ligation induced gastric ulcers**

Group	Treatment	Ulcer index	%Protection	$p^H$ of gastric content	Free acidity (meq/lit)	Total acidity (meq/lit)
I	Control	10.58±0.37	-	2.54±0.12	95.8±1.8	109.8±5.26
II	Omeprazole (20mg/ kg)	2.16±0.25**	79%	4.71±0.22**	32.5±1.45**	55.8±1.45*
III	EEPAL 250mg/ kg	3.58±0.37*	66%	3.56±0.16*	44.7±2.83*	69.4±1.79*
IV	EEPAL 500mg/ kg	2.5±0.44**	76%	4.41±0.14**	34.6±2.50*	62.3±0.81*

\*\* $P < 0.01$  Vs control group; \* $P < 0.05$  Vs control group. All values are represented as mean  $\pm$  S.E.M, and statically significance using of one way ANOVA followed by Dunnett's test where  $P < 0.05$  was moderately significant and  $P < 0.01$  was considered as stastically significant.

## DISCUSSION

Ulcer index parameter was used for the evaluation of anti ulcer activity since ulcer formation is directly related to factors such as reduction in gastric  $p^H$ , decrease in free and total acidity. The antiulcer effect of EEPAL may be due to the presence of alkaloids and flavanoids. Previous studies on flavanoids<sup>13</sup> and some alkaloids<sup>14</sup> have been shown to possess antiulcer effect that suppresses the gastric secretion having a local action on protection of the gastric mucosa.

In ethanol induced ulcer model, ulcers are caused due to perturbations of superficial epithelial cells. Notably the mucosal mast cells leading to release of the vasoactive mediators including histamine thus causing damage to gastric mucosa. Mucosal damage caused by alcohol is modulated by prostaglandin. The effectiveness of EEPAL protection against mucosal damage caused by ethanol is indication of its effect on prostaglandins.

Aspirin has been reported to produce ulcers by both local and systemic effect<sup>15</sup>. Aspirin causes direct irritant effect and mucosal damage by interfering with prostaglandin synthesis increasing acid secretion by increase the  $H^+$  ion transport /back diffusion of  $H^+$  ions resulting over production of leukotrienes and other products of 5-lipoxygenase pathway<sup>16</sup>.

Pyloric ligation induces gastric ulcers because of an increase in acid-pepsin accumulation due to pyloric obstruction and subsequent mucosal digestion and breakdown of the gastric mucosal barrier<sup>17</sup>. Hence estimation of acid secretion, pepsin secretion and mucus secretion is a valuable part of the study to clarify the mechanism of action of the drug under trial.

Overall EEPAL has shown a substantial and significant protection against gastric ulcers in the models. This protective effect might have been mediated by anti-secretory and cytoprotective mechanisms. Moreover, further insight into the precise mechanism of action is essential to exploit the complete potency of EEPAL and increase its usage in contemporary medicine.

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