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# Anti-ulcer activity of ethanolic extract of *Caesalpinia pulcherrima* flowers on *ethanol induced gastric ulcers in rats*

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**ABSTRACT**

To evaluate the anti-ulcer activity of *Caesalpinia pulcherrima on* ethanol induced gastric lesion in albino rat. Gastric lesions were induced in rats by oral administration of ethanol 80% Ethanol (1 ml, p.o).The anti-ulcer activity of ethanolic extract of flowers of C*aesalpinia pulcherrima* (CPFE) was compared with standard drug (Famotidine). The parameter studied were ulcer index, pH, gastric juice volume, total acidity, tissue glutathione, and total protein against ethanol induced gastric ulcer in rats. The results obtained from the present study have shown that flower of *Caesalpinia pulcherrima* possesses antiulcer effect on ethanol induced ulcer where, a decrease in ulcer index, total acidity, total volume of gastric secretion and increase in total protein, glutathione content and pH of gastric secretion when compared with toxic was observed. The present study suggests the anti-ulcerative activity of ethanolic extract of flower of *Caesalpinia pulcherrima*.

**Key words:** *Caesalpinia pulcherrima*, ethanol induced ulcer.

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## INTRODUCTION

An estimated 15,000 deaths occur each year as a consequence of peptic ulcer disease (PUD). Ulcer is defined as the erosion in the lining of the stomach or duodenum and is caused by the disruptions of the gastric mucosal defense and repair systems.[1]Gastric ulcer is one of the most widespread disease is believed to be due to an imbalance between aggressive and protective factors. The defensive factors (mucus, mucosal blood flow, formation of $HCO^-_3$ and $PGE_2$) are impaired or overpowered by the aggressive factors (acid, pepsin, NSAID'S and *Helicobacter pylori*), in gastric ulcer patients there is loss of pyloric sphincter tone which precipitates duodenal-gastric reflux of bile.[2] Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid (antacid) and the second with re-enforcing gastric mucosal protection (antiulcer).[3] In Indian pharmaceutical industry, antacids and antiulcer drugs share 6.2 billion and occupy 4.3% of the market share.[4]

The newer research in pathophysiology of ulcer remarkably increases the interest in developing newer and better drug. The clinical evaluation of existing anti-ulcer drug therapy showed the development of tolerance and incidence of relapse and side effects that make their use objectionable. The indigenous drug of herbal origin possessing fewer side effects. Using medicine of plant origin is based upon the premise that plants contain natural substances that can promote health and alleviate illness. An indigenous drug possessing fewer side effects is major thrust area of present research. Several plants like *Allophytus serratus*[5], *Ocimum sanctum*[6], *Embelisca officianalis*[7], *Biodes pilosa*[8] have been investigated for anti-ulcer activity.

*Caesalpinia pulcherrima* flowers are used for intestinal worms, cough. The leaf and flower extract are used as antibacterial against gram positive bacteria. The juice from the leaves is said to cure fever, the juice from the flower is said to cure sores, and the seeds are said to cure cough, breathing difficulty, and chest pain. The root is also said to induce abortion in the first trimester of pregnancy. [9,10]

Previously, no scientific work has been reported on the anti-ulcerogenic activity of this plant. The present study was therefore undertaken to evaluate the anti-ulcerogenic activity of *Caesalpinia pulcherrima* flowers extract on albino rats. In this study, various parameters like ulcer index, gastric juice volume, pH, total acidity, tissue glutathione, and tissue protein were determined.

## MATERIALS AND METHODS

### Collection of plant

The fresh plant of *Caesalpinia Pulcherrima* belonging to the family Fabaceae was collected from the local area of Aurangabad, Maharashtra. The plant was identified and authenticated by Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad.

### Extraction of plant material

The flowers of *Caesalpinia Pulcherrima* plant were dried under the shade condition, powdered with the help of grinder and stored in an airtight container. The powder of flower was weighed (300 g). A 95% w/v ethanolic extract was prepared by soxhlet extraction method. The dried powdered flowers of *Caesalpinia Pulcherrima* were extracted with 95% v/v ethanol for 36 hr using soxhlet extractor. The combined extracts were concentrated at 40°C to obtain dark brownish residue. The yield obtained from the above process was found to be 1.3% w/w. The extracts were preserved in a refrigerator for further use.

### Animals

Albino Wistar rats weighing 200–250 g rats weighing of either sex were obtained from Wockhardt Ltd. Aurangabad, for this study. The animals were housed in separate groups (six rats in each cage) in clean sanitized polypropylene cages. They had free access to standard pellet diet and water *ad libitum*. The animals were maintained under day and night 12:12 hr cycles and with maintenance of room temperature at 25 $\pm$ $2^0$C. All procedures were performed in accordance with the Institutional Animal Ethics Committee (IAEC) constituted as per the direction of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

### Toxicity study

Chronic oral toxicity study was done according to OECD guidelines 423. In this experiment two groups of Wistar rats (n=3) were used. Animals were fasted overnight with water *ad libitum* and food was withheld for 3-4 hrs after oral administration of the extracts. One group of animals were treated with starting dose of 2000mg/kg body wt orally and the maximum dose of 5000 mg/kg body weight was administered to rats. Another group was treated with normal saline. Observation includes mortality and clinical signs, which includes changes in skin fur, eyes and mucous membranes. The gross behaviors like body positions, locomotion, rearing, tremors, gait was observed.

### Ethanol Induced Anti-ulcer Activity

### Grouping of animals

**Group I:** Animals served as control and received0.1% Tween-80 (10 ml/kg, p.o).
**Group II:** Animals received80% Ethanol (1 ml, p.o).
**Group III:** Animals received standard drug Famotidine (30 mg/Kg, p.o) + Ethanol.
**Group IV:** Animals received ethanol extract of *C.Pulcherrima* flower suspended in 0.1% tween 80 (250 mg/Kg, p.o) + Ethanol.
**Group V:** Animals received ethanol extract of *C.Pulcherrima* flower suspended in 0.1% tween 80(500 mg/Kg, p.o) + Ethanol.

### Procedure

Albino Wistar rats of either sex weighing between 200–250 g were deprived food for 24 hr prior to the experiment but were allowed free access to water *ad libitum*. During this time they were kept in restraining cages to prevent coprophagy. The rats were administered either the vehicle, standard drug or the EECP orally in 0.1% Tween 80 suspension 1 hr prior to administration of 1 ml 80% ethanol. Untreated animals were served as control. The animals were sacrified after 2 hr and stomach was opened. The stomachs were carefully excised keeping oesophagus closed and open along greater curvature, and luminal contents were removed. The ulcer index was determined. [11, 12] The gastric content was collected in test tube and centrifuged at 3000 rpm for 10 min. The pH of the supernatant was

measured using digital pH meter. The volume of supernatant was measured and expressed as ml/100 g body weight. The pH of the supernatant was measured using digital pH meter. [13, 14]

An aliquot of 1.0 ml of gastric juice was pipetted out to a 50 ml conical flask and 2/3 drops of Topfer′s reagent were added to it and titrated with 0.01 N Sodium Hydroxide (NaOH) until all traces of the red color disappeared, and the color of the solution turned yellowish orange. The volume of 0.01 N NaOH was noted, which corresponded to the free acidity. Then 2-3 drops of phenolphthalein were added and titration was continued until a permanent pink color was developed. The volume of total alkali consumed was noted which corresponded to the total acidity. The mucosa was flushed with saline and stomach was pinned on board.

The lesion in the glandular portion was examined under a 10 x magnifying glass and length was measured using a divider and scale and gastric lesion was scored as follows:

0 - Normal colored stomach,
0.5 - Red coloration,
1- Spot ulceration,
1.5 - Hemorrhagic streak,
2 - ulcers
3- Perforations

Ulcer index of each animal was calculated by adding the values and their mean values were determined and percentage inhibition was calculated. [15] After the examination of ulcer index the stomachs were homogenized in 0.15 M KCl to make a 10 % homogenate for the estimation of tissue Glutathione [16] total protein. [17]

## RESULTS

The present study has been undertaken to evaluate the antiulcer effect of ethanolic extracts of flowers of *Caesalpinia pulcherrima* on ethanol induced ulcer. The results obtained from the present study have shown that ethanolic extract of *Caesalpinia pulcherrima* possess antiulcer effect on ethanol induced ulcer.

**Table-1: Effect of Ethanolic extract of *C.pulcherrima* of flower on ulcer index, gastric volume, pH, and against ethanol induced gastric ulcer in rats.**

| Group | Treatment (mg/kg p.o) | Ulcer index | Gastric volume (ml) | pH |
|---|---|---|---|---|
| I | Control | 2.90 ± 0.16* | 3.63 ± 0.08* | 6.83 ± 0.44* |
| II | Toxic (Ethanol, 1ml) | 20.11 ± 0.32 | 2.20 ± 0.22 | 4.83 ± 0.33 |
| III | Standard (Famotidine, 3) | 3.78 ± 0.17* | 1.48 ± 0.04* | 7.91 ± 0.23* |
| IV | CPFE 250 + EtOH 1ml | 10.01 ± 0.12* | 1.35 ± 0.14* | 6.83 ± 0.33* |
| V | CPFE 500 + EtOH 1ml | 5.70 ± 0.25* | 2.93 ± 0.15* | 7.75 ± 0.21* |

*Results are expressed as Mean ±SEM, Data was analyzed by one way ANOVA followed by Dunnett's test. Comparisons were made with toxic group vs all treated groups, * represents statistical significance at P < 0.05.*
*CPFE: C.pulcherrima flower extract*

**Table-2: Effect of EECP of flower on total acidity, tissue glutathione, and total protein against ethanol induced gastric ulcer in rats**

| Group | Treatment (mg/kg p.o) | Total acidity (mEq/Lit) | Tissue glutathione (µmol/gm tissue) | Total protein (mg) |
|---|---|---|---|---|
| I | Control | 66.00 ± 1.03* | 43.89 ± 0.44* | 3.16 ± 0.01* |
| II | Toxic (Ethanol, 1ml) | 56.83 ± 1.07 | 24.22 ± 0.85 | 4.16 ± 0.01 |
| III | Standard (Famotidine, 3) | 33.33 ± 0.98* | 39.12 ± 0.37* | 6.76 ± 0.02* |
| IV | CPFE 250 + EtOH 1ml | 37.50 ± 0.61* | 33.33 ± 0.22** | 6.12 ± 0.03** |
| V | CPFE 500 + EtOH 1ml | 39.16 ± 0.70* | 37.34 ± 0.16* | 6.56 ± 0.03* |

*Results are expressed as Mean ±SEM, Data was analyzed by one way ANOVA followed by Dunnett's test. Comparisons were made with toxic group vs all treated groups, * and ** represents statistical significance at P < 0.05, P < 0.01 respectively.*
*CPFE: C.pulcherrima flower extract.*

**Table- 3: Ulcer Healing effect of Ethanolic extract of *C.pulcherrima* flower on ulcer index, gastric volume and pH**

| Group | Treatment (mg/kg p.o) | Ulcer index | Gastric volume (ml) | pH |
|---|---|---|---|---|
| I | Control | 3.18 ± 0.24** | 3.38 ± 0.18** | 6.66 ± 0.27** |
| II | Toxic (Ethanol 1 ml ) | 19.62 ± 0.37 | 2.32 ± 0.22 | 5.46 ± 0.40 |
| III | Standard (Famotidine, 3) | 4.77 ± 0.08** | 0.66 ± 0.03** | 8.33 ± 0.21** |
| IV | CPFE 250 + Ethanol 1ml | 8.40 ± 0.16** | 1.31 ± 0.04* | 6.91 ± 0.20* |
| V | CPFE 500 + Ethanol 1ml | 5.86 ± 0.22* | 0.81 ± 0.03** | 7.96 ± 0.04** |

*Results are expressed as Mean ±SEM, Data was analyzed by one way ANOVA followed by Dunnett's test. Comparisons were made with toxic group vs. all treated groups, * and ** represents statistical significance at P < 0.05 and P < 0.01.*
*CPFE: C.pulcherrima flower extract.*

**Table-4: Ulcer Healing effect of EECP flower on total acidity, tissue glutathione, and total protein against ethanol induced gastric ulcer in rats**

| Group | Treatment (mg/kg p.o) | Total acidity (mEq/Lit) | Tissue glutathione (µmol/gm tissue) | Total protein (mg) |
|---|---|---|---|---|
| I | Control | 35.5 ± 1.80** | 42.68 ± 0.37* | 5.88 ± 0.19** |
| II | Toxic(Ethanol 1 ml ) | 50.16 ± 2.41 | 31.38 ± 0.50 | 4.32 ± 0.02 |
| III | Standard (Famotidine, 3) | 20.33 ± 0.84** | 40.09 ± 0.16** | 6.86 ± 0.03** |
| IV | CPFE 250 + Ethanol 1 ml | 27.33 ± 0.49** | 33.54 ± 0.28* | 6.79 ± 0.01* |
| V | CPFE 500 + Ethanol 1 ml | 22.66 ± 0.33** | 36.18 ± 0.19** | 6.52 ± 0.02** |

*Results are expressed as Mean ±SEM, Data was analyzed by one way ANOVA followed by Dunnett's test. Comparisons were made with toxic group vs. all treated groups,* and ** represents statistical significance at P < 0.05 and P < 0.01.*
*CPFE: C.pulcherrima flower extract.*

## DISCUSSION

Various studies suggested that changes in gastric motility may play a role in the development and prevention of experimental gastric lesions. Relaxation of circular muscle may protect the gastric mucosa through flattening of the folds. This will increase the mucosal surface area exposed to necrotizing agents and reduce the volume of the irritant on the rugal crests. Such an action has been postulated to play a role in the cytoprotective effect of prostaglandins.

The present study has been undertaken to evaluate the antiulcer effect of ethanolic extracts of flower of *Caesalpinia pulcherrima* with ethanol induced ulcer. The results obtained from the present study have shown that flower of *Caesalpinia pulcherrima* possesses antiulcer effect on ethanol induced ulcer healing effect. In ethanol induced model, a decrease in ulcer index, total acidity, total volume of gastric secretion and increase in total protein, glutathione content and pH of gastric secretion when compared with toxic was observed.

Ethanol has been shown to increase the risk of ulcer in humans [18] but produces potent ulceration in rats. [19] It is believed to produce reactive species responsible for mucosal injury [20] and lipid peroxidation, a free radical mediated process that ultimately destroys lipids membrane. [21] Ethanol serves as a most common ulcerogenic agent and when given intragastrically to rats it produces severe gastric hemorrhagic erosions. The genesis of ethanol induced gastric lesions is multifactorial with the depletion of gastric wall mucus content as one of the involved factors and this damage induced by ethanol may be due to mucosal leukotriene release. Ethanol-induced damage to the gastric mucosa is associated with a significant production of free radicals leading to an increased lipid peroxidation and damage to the cell and cell membranes. [22] In the present study, famotidine was used as a standard drug. Famotidine is a $H_2$ receptor blocker, is capable of reducing over 90% of basal, food stimulated and nocturnal secretion of gastric acid, stimulated by histamine, gastrin, cholinomimetic drugs and vagal stimulation. Famotidine exerts its antisecretory effect by inhibiting the histamine induced c-AMP dependent pathway. Famotidine has shown to be capable of preventing the development of experimentally-induced gastric ulcers. [23] Also, healing of duodenal ulcer in the rat was accelerated by famotidine. [24]

Total acidity indicates that how much acid is present in the gastric secretion. It is a major aggressive factor which produce ulcer. Gastric secretion is under vagal control and over activity of vagus also contributes to ulcer formation. The vagus nerve stimulates stomach acid secretion via interaction of its chemical mediator (acetylcholine) with the muscarinic receptor. The activation of the muscarinic receptor gives rise to sequential events that result in increased gastric acid secretion. [25]

On ethanol treatment, the mucosal mast cells lead to release of vasoactive mediators including histamine. $H_2$-receptor stimulation increases cyclic AMP and leads to feedback inhibition of histamine release from mast cells and basophils. [26] Histamine is thought to stimulate the production of cyclic AMP by activating the enzyme adenyl

cyclase which causes activation of gastric proton pump and release of hydrogen ions. CPFE treatment showed decrease in the total acidity of the gastric secretion. Serum protein consists of albumin and globulin. In the peptic ulcer the total protein content of serum or gastric juice is increased. This could be due to leakage of plasma protein in to the gastric juice or serum with weakening of the mucosal resistance/barrier of the gastric mucosa. After treatment with CPFE there was a significant increase in protein content of gastric juice which suggest that it might primarily increase the leakage of plasma protein in to the gastric juice with strengthening of the mucosal barrier and decrease in its resistance to the damaging effect of aggressive factors.

Acid volume is a volume (ml) of acid present in the gastric secretion which contains HCl, pepsinogen, mucus, bicarbonates, intrinsic factor and proteins. Volume of acid secretion is also an important factor in the production of ulcer due to exposure of unprotected lumen of the stomach to the accumulating acid. CPFE treatment showed decrease in the acid volume of the gastric secretion.

Higher pH indicates a lower concentration of the hydrogen ion. The hydrogen ion is a major aggressive factor involved in the genesis of ulcer and gastric damage. CPFE treatment showed a increase in pH of the gastric contents. This directly indicates that the CPFE lowers the chances of ulcer and has a protective action upon the gastric mucosa.

Ethanol-induced generation of free radicals reduces the cysteine which is required for GSH synthesis, which is, therefore, decreased. [22] In gastric ulcer tissues, glutathione (g-glutamylcysteinylglycine, GSH) levels were found to be decreased. Data from this study indicates that depletion of gastric GSH is associated with generation of gastric lesion in the rats. GSH is a tripeptide and a superoxide radical scavenger and it protects thiol protein groups required for maintaining the integrity of cell against oxidation. In the present study, CPFE treatment showed increase in the glutathione content.

The ethanolonic extract of *C.pulcherrima* flower extract, showed the presence of flavonoids, alkaloids glycosides, tannins, saponin and triterpenoids. These phytoconstituents present in the ethanolic extract could be the possible chemicals involved in the prevention of gastric ulcers in rats. Further, studies are under process to isolate the possible phytoconstituents responsible for the anti ulcer activity. Many similar active phytoconstituents have shown similar activities.

## CONCLUSION

The present study showed that antiulcer and ulcer healing effect with ethanolic extract of *Caesalpinia pulcherrima* flower caused a beneficial effect on ethanol induced induced gastric ulcers in rats, as evidenced by the reduction in the ulcer scores.

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