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Preliminary Pharmacological, Acute Toxicity Test And Anti-Ulcer Activity Extract Of Plant *Caesalpinia Pulcherrima*

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Abstract
Open sores on the inside of the stomach and upper portion of the small intestine are known as peptic ulcers. Stomach pain is the most typical symptom of a peptic ulcer. Caesalpinia pulcherrima, commonly known as peacock flower or Pride of Barbados, has been studied in scientific studies for its ability to cure ulcers. *Caesalpinia pulcherrima* is a species of flowering plant native to the tropical and subtropical parts of the Americas. It is a member of the pea family Fabaceae. Preliminary studies revealed the presence of alkaloids, flavonoids, tannins, and saponins in the aqueous and methanolic extract of Caesalpinia pulcherrima. *Caesalpinia pulcherrima* extracts have also been shown to protect mucosa by increasing mucin production and inducing prostaglandin synthesis. It has been frequently demonstrated that Caesalpinia pulcherrima extracts considerably lower the incidence and severity of ulcers in experimental mice. These findings suggest that it may be useful as a natural ulcer remedy; nevertheless, additional human clinical trials are needed to confirm these advantages. In summary, *Caesalpinia pulcherrima's* anti-inflammatory, antioxidant, and mucosal-protective properties make it a promising botanical treatment for ulcers. Future clinical trials should be conducted to validate these first findings and explore the treatment's potential for gastrointestinal ulcers.

Keywords: Plants; *Caesalpinia pulcherrima*; Peptic ulcer; Phytochemical investigation; Anti-ulcer activity.

Introduction

We are blessed with an extremely rich botanical diversity, with many different kinds of plants growing throughout the nation. All three dimensions of biodiversity—species diversity, genetic variety, and habitat diversity—are abundant in India(1). Thousands of species are known to have therapeutic potential in India, and using various sections of several medicinal plants to treat particular illnesses has long been popular(2). The Indian subcontinent derives significant economic benefits from the use of medicinal herbs. Because herbal medicine has fewer adverse effects, is more culturally acceptable, and is more compatible with the human body, it continues to be the primary source of primary healthcare for roughly 75–80% of the population, mostly in developing nations. But in the industrialized world, their use has significantly increased during the past few years(3).

One significant source of novel chemicals with possible medical applications is medicinal plants. Numerous illnesses have been treated with them in traditional medicine. Numerous plants produce compounds that are beneficial to maintaining animal and human health(4). They include aromatic compounds, the majority of which are either their oxygen-substituted derivatives, such as tannins, or phenols themselves. At least 12,000 secondary metabolites have been isolated, which is thought to represent less than 10% of the total(5). Alkaloids are among the various compounds that plants use as defensive strategies against insects, herbivores, and microbes(6).

One of the most common digestive problems is peptic ulcer, which is brought on by an imbalance between the defensive (gastric mucosal integrity) and offensive (gastric acid secretion) elements. The usage of non-steroidal anti-inflammatory drugs (NSAIDs) is the cause of bleeding and stomach ulcers(7). The term "peptic ulcer disease" describes gastric secretion-induced breaches in the mucosa of the stomach and small intestine, primarily the proximal duodenum. Peptic ulcer disease primarily affects the proximal duodenum and distal stomach, although it can also arise as high as the Barrett esophagus and as low as the Meckel diverticulum with gastric neterotopia(8).

A well-targeted therapeutic approach is necessary for the treatment of ulcers. The main therapeutic strategies for peptic ulcer disease have involved lowering the generation of stomach acid and increasing the production of gastric mucosa(9). As a result, an increasing number of medications—both synthetic and herbal—are being developed to treat peptic ulcers in a way that is more innovative and effective(10, 11). Numerous herbs have been shown to have strong analgesic

properties and to be beneficial in healing stomach ulcers that have been artificially generated in experiments. Caesalpiniaceae plants are good sources of coarse particles. The dried powder has a variety of therapeutic applications. Caesalpinia steeped for 72 hours in 50% ethanol. The final product, pulcherrima(12). Alcoholic extract, commonly referred to as "Peacock Flower" in colloquial language, was heated, filtered, and concentrated on the leaves, flower, and plate. *Caesalpinia pulcherrima* is used as a stimulant, tonic, and anti-ulcer activity(13). While the leaves are used as a cathartic, the bark is utilized as an abortifacient after being macerated for 24 hours. There is proof that *Caesalpinia pulcherrima* can help treat peptic ulcers, including the discomfort associated with them(14, 15). This study aims to assess the anti-ulcer properties of ethanolic and aqueous extracts of aerial portions of Caesalpinia pulcherrima (Linn.) Sw.

MATERIALS AND METHODOLOGY

Sterilization of Glassware

Glassware were soaked overnight in cleaning solution and washed thoroughly with running tap water. They were then cleaned with detergent solution and rinsed several times with tap water and finally in distilled water and air dried. The glassware and media were sterilized in an autoclave at 15psi for 20 minutes, at 120ºC.

Collection of Plant Material

Plant material were collected from medicinal garden of near kissan college of parola, and it was verified by Dr. S.R. Kshirsagar, an assistant professor and the department head of botany in Dhule. First, tap water was used to wash the verified plant materials, and then distilled water. The stems and leaves of the plant were separated, allowed to dry entirely in the shade, and then ground into a coarse powder. For later use, the powder was stored in an airtight container.Fresh Caesalpinia pulcherrima, weighing approximately 1 kg, rinsed under running tap water and allowed to air dry at room temperature until it reached a consistent weight. Using a mortar and pestle, the dried plant samples were ground into a powder, which was then kept in sterile polythene bags until needed.

Phytochemical Studies

Preparation of extract

1kg of finely ground plant material were put into a Soxhlet apparatus in a little muslin fabric bag. To stop any leaks, glycerin was sprayed at the intersection of the extraction chamber and the condenser mouth. To make sure the powder was completely submerged, 150 ml of an appropriate solvent was poured into the extraction chamber through the open end. Using the Soxhlet equipment, the extraction procedure was carried out in steps using various solvents, including methanol, until the extract lost its color. Next, Whatman filter paper number one was used to filter the extracts. For additional analysis, the filtrate was dried at 40°C and kept in storage at 4°C(16, 17).

Preliminary Phytochemical Investigation

Caesalpinia pulcherrima was used in the initial phytochemical experiments to identify the elements of phytochemicals qualitatively(18, 19).

To test for alkaloids

An exact drop of Mayer's reagent was carefully poured down the edge of a test tube and combined with one milliliter of the filtrate. Next, the solution was watched to see if a white or yellowish precipitate formed.

Test for tannin

One milliliter of the material was mixed with one milliliter of ferric chloride solution. Next, the resultant test solution was examined to see if a green or black precipitate had formed(20).

Test for saponin

To create a stable, long-lasting froth, two milliliters of the sample and five milliliters of distilled water were combined and agitated hard. After adding three drops of olive oil to the foam, it was forcefully shook once more, and the creation of an emulsion was watched for.

Test for carbohydrate

Two milliliters of concentrated sulfuric acid and two drops of 1% alcoholic alpha-naphthol were gently dropped down the test tube's sides to every milliliter of the extract. This caused a violet ring to appear at the intersection of the two layers(21).

Test for terpenoids

To create separate layers, 5 milliliters of the sample were combined with 2 milliliters of chloroform and concentrated sulfuric acid. After that, the mixture was checked to see if the interface started to turn reddish-brown.

Test for flavonoids

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A part of each sample's aqueous filtrate was added to five milliliters of diluted ammonia solution, and then concentrated sulfuric acid was added. The combination was examined to see if a yellow coloring emerged(22).

Test for steroids
Carefully pouring down the test tube's sides were one milliliter of the aqueous extract, ten milliliters of chloroform, and an equal volume of pure sulfuric acid. The sulfuric acid layer displayed a yellow tint with green fluorescence, whereas the upper layer turned red. This observation suggests that steroids are present.

Test for amino acids
Amino acids can be detected by adding a few drops of Ninhydrin reagent to one milliliter of the extract and watching for the formation of a purple color.

Test for phenols
A few milliliters of a neutral ferric chloride solution were added individually to one milliliter of each extract that had been dissolved in either alcohol or water. Any observable alteration in hue suggested the presence of phenolic chemicals.

Test for protein
One milliliter of diluted extract was mixed with one milliliter of 1% NaOH and five milliliters of 5% CuSO4. The formation of a deep blue color indicated the presence of proteins(19, 23, 24).

Acute Oral Toxicity Test
The Organization for Economic Co-operation and Development (OECD) – 425 guideline(25, 26) was followed in order to test the acute oral toxicity of *Caesalpinia pulcherrima* in female, nulliparous, non-pregnant rats weighing 180–220 g. Animals were given a three-hour fast before the experiment. *Ceasalpinia pulcherrima* was given to the animals as a single dose, and over the 48-hour trial period, their death was monitored (short term toxicity). The extract was given to the rats in varying quantities. A dosage escalation of 2000 mg/kg was used. A toxic dose was defined as the one at which two of the three rats showed signs of death. Acute oral toxicity (AOT) 425 was utilized to compute the LD50 of *Ceasalpinia Pulcherrima*(27, 28).

Animals
For testing, 180–200 g of Swiss and Wistar albino rats were obtained from Bioneeds, DCS's Annasaheb Ramesh Ajmera College of Pharmacy in Nagaon, Dhule. Over the course of seven days, all animals were acclimated to typical husbandry settings, which included a 12:12 h light/dark cycle, room temperature of 24 ± 1 o C, and relative humidity of 45–55 percent. A distinct set of animals was utilized for each experimental group, and precautions were taken to guarantee that the animals used for one response were not used for another. To minimize nonspecific stimuli, animals were accustomed to laboratory circumstances 48 hours before the trial began. Committee for the Purpose of Control and Supervision of Experiments with Animals (CPCSEA) rules and ethical principles were strictly adhered to in all experiments(29, 30).

Aspirin induce gastric ulcer
Aspirin used orally caused gastric ulcers. Male wistar rats were split into six experimental groups at random: control, aspirin-induced, ranitidine (175 mg/kg), and *Caesalpinia pulcherrima* (Cp) therapy (100, 200, and 400 mg/kg) (**Table 1**). For seven days, rats were gavaged once a day at the same time and continually pretreated with medicines. Rats were given 200 mg/kg body weight of aspirin orally on the seventh day following an overnight fast in order to produce an acute gastric ulcer(31). Four hours later, the animals were slaughtered, and the stomach was removed, sliced along the grater curvature, and carefully cleaned with 5.0 milliliters of 0.9% NaCl. (32).

Table 1. Grouping of animals for aspirin induce antiulcer activity

Groups	**Treatment**
1	Vehicle control
2	Aspirin induce 200mg/kg oral
3	Standard (ranitidine 175mg/kg oral)
4	*Caesalpinia pulcherrima* 100mg/kg oral
5	*Caesalpinia pulcherrima* 200mg/kg oral
6	*Caesalpinia pulcherrima* 400mg/kg oral

Ethanol induce ulcer
The previously described procedure involved the oral administration of ethanol to create gastric ulcers. Six experimental groups were created by randomly assigning Swiss albino mice to different groups: normal, ethanol-induced, ranitidine (20 mg/kg), and *Caesalpinia pulcherrima* therapy (Cp, 100, 200, and 400 mg/kg) (**Table 2**). For seven days, mice were

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gavaged once a day at the same time and continually pretreated with medicines(33). After an overnight fast, mice were given ethanol (0.2 mL/20 g body weight) orally on the seventh day to cause an acute stomach ulcer(34). After four hours, the animals were sacrificed, and the stomach was removed and cut along the grater curvature. The stomach was then meticulously cleaned with five milliliters of 0.9% NaCl.(35, 36).

Table 2. Grouping of animals for Ethanol induce antiulcer activity

Groups	**Treatment**
1	Vehicle control(Saline water)
2	Ethanol induce 0.2 ml/per b.w
3	Standard (ranitidine 20mg/kg oral)
4	*Caesalpinia pulcherrima* 100mg/kg oral
5	*Caesalpinia pulcherrima* 200mg/kg oral
6	*Caesalpinia pulcherrima* 400mg/kg oral

RESULTS AND DISCUSSIONS

Phytochemical Investigations

Table 3 presents the preliminary phytochemical analysis results. It demonstrates that the plant of *Caesalpinia pulcherrima* leaves has an aqueous and methanolic extract. *Caesalpinia pulcherrima* aqueous extract showed the presence of tannins, saponins, alkaloids, and flavanoids. Similar to the aqueous extract, the methanolic extract of *Caesalpinia pulcherrima* contains tannins, saponins, alkaloids, and flavanoids. The outcome of the *Caesalpinia pulcherrima* methanolic extract's phytochemical screening.

Figure 1. Preliminary Phytochemical Investigation

Caesalpinia pulcherrima's aqueous extract showed the presence of tannins, saponins, alkaloids, and flavanoids. Similar to the aqueous extract, the methanolic extract of *Caesalpinia pulcherrima* contains tannins, saponins, alkaloids, and flavonoids.

Table 3. Showing phytochemical chemical screening
(-- = Negative (absent); + = Positive (present))

Category	**Name of the test**	**Methanolic Extract**
Alkaloids	a) Mayer's test	+
	b) Wagner's test	+
	c) Dragendorff's test	+
Flavonoids	a) Sulphuric acid test	+
	b) Zinc +HCL+T.S	+
Phenolics andTannins	a) Ferric chloride test	+
	b) Dil.iodine solution	+
Carbohydrates	a) Molish's test	--
	b) Fehling's test	--
	c) Benedict's test	--
Fixed oil and Fats	a) Stain test	--
Saponins	a) Froth test	+
Steroids	a) Salkowski test	--
	b)Liebermann Burchard test	--

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Chromatographic Analysis by Thin-Layer Chromatography

The presence of a spot with an Rf value of 0.70-0.72 was detected in the thin layer chromatogram of the methanolic extract of *Caesalpinia pulcherrima* when a solvent system containing acetic acid, ethanol, hexane, ethylacetate, and chloroform in the ratio of 10:2:5:1:1 was used. The 12 cm solvent run in the TLC analysis took 25 minutes to finish, whereas the *Caesalpinia pulcherrima* solute ran about 7 cm. *Caesalpinia pulcherrima* patches showed up. The dots, with Rf in the range of 0.70-0.72, were dark bluish-violet in color on a yellow-light brown backdrop (**Figure 2**).

$$\text{RF value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Figure 2. Thin layer Chromatography chromatogram of methanolic extract of *Caesalpinia pulcherrima*

Acute Toxicity Studies

Caesalpinia pulcherrima's acute oral toxicity was assessed using the 2011 Organization for Economic Co-operation and Development (OECD) – 425 guideline. During the initial stage of the acute toxicity investigations, no significant toxicity symptoms were noted at a dosage of 100 mg/kg. However, at dosages of 2000 and 1000 mg, there were symptoms such as salivation, rubbing at the application site, mouth and nose on the cage floor, and restlessness. The toxicity indicators seen during the study's second phase were identical to and more severe than those seen during the first. The dosage of 2000 mg/kg, which is the geometric mean of the doses for which there is zero mortality (0/3) and total mortality (0/3), was determined to be the dosage at which all animals survived.

Pharmacological Studies

Ethanol induce ulcer

This study examined the anti-ulcer properties of *Caesalpinia pulcherrima* L. leaves. The findings showed that the lowest ulcer index was seen while using ranitidine in combination with 100 mg/kg, 200 mg/kg, and 400 mg/kg of *Caesalpinia pulcherrima*. According to the methanol induces study, the gastric juice of the group (CP) that received 100, 200, and 400 mg/kg b.wt. of *Caesalpinia pulcherrima* L. methanolic extracts, it showed a substantial ($P<0.01$) fall in pH, which lowers the ethanol induce group (**Table 4**).

Table 4. Results of Ethanol induce Mice

Groups treatment	Ulcer index	Protection	Total acidity
Ethanol induce 0.2ml/b.w oral	0	0	0
Std ranitidine 20 mg/kg oral	1.8±0.1***	47.6	1.60±0.60***
Caesalpinia pulcherrima 100 mg/kg oral	1.1±0.5**	49.20	1.57±0.65**
Caesalpinia pulcherrima 200 mg/kg oral	1.3±0.8*	51.50	1.67±0.54**
Caesalpinia pulcherrima 400 mg/kg oral	1.0±0.4*	53.70	1.70±0.40**

Values are expressed as mean r SEM from 6 rats. Significant at **P < 0.01 and ***P < 0.001as compared to control group using one way ANOVA.

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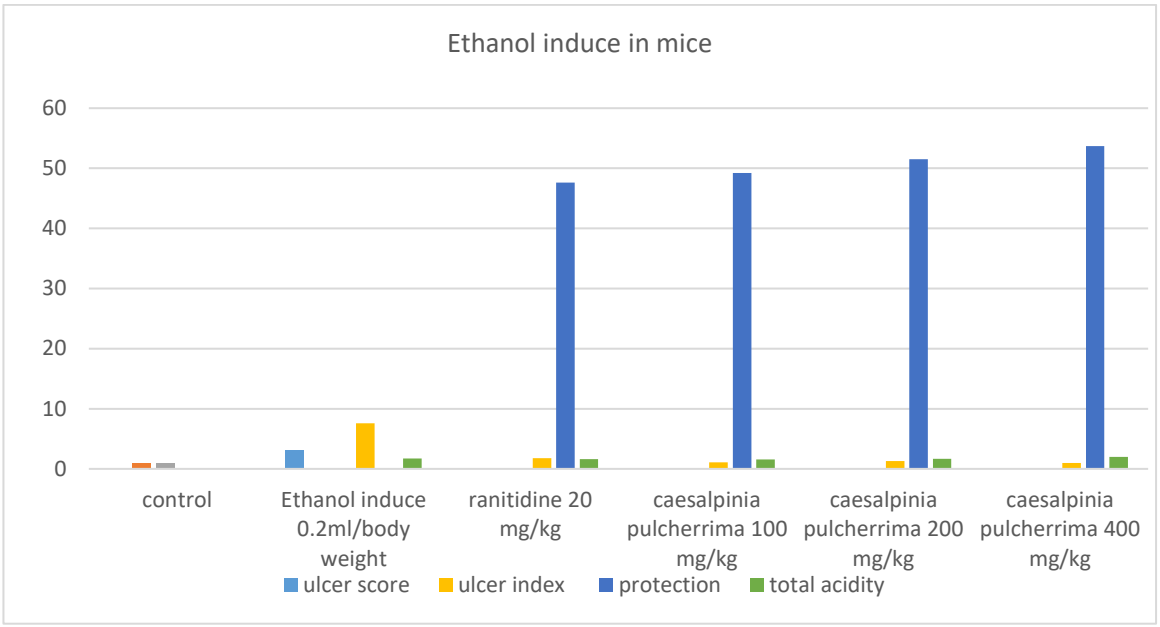

Figure 3. Effect of *Caesalpinia pulcherrima* on antiulcer activity in ethanol induce ulcer

Aspirin induce ulcer

The aspirin induces study showed that gastric juice in the group *Caesalpinia pulcherrima* received 100, 200 and 400 mg/kg b.wt. of the Methanolic extracts of *Caesalpinia pulcherrima* L. It showed a significant (P<0.01) decrease in gastric juice pH, reduces the aspirin induce group (**Table 5 and Figure 4**).

Table 5. Results of Aspirin induce Rat

Drug	**Ulcer index**	**Protection**	**Total acidity**
Normal	0	0	0
Aspirine induce	22.3±0.2	0	4.30±0.40
Std ranitidine 175mg/kg	15.7±0.30***	75	3.8±0.20***
Caesalpinia pulcherrima 100mg/kg	9.1±0.10**	67	3.1**
Caesalpinia pulcherrima 200mg/kg	8.7±0.50***	36.50	2.8±0.15***
Caesalpinia pulcherrima 400 mg/kg	7.3±0.70	78	2±0.45

Values are expressed as mean r SEM from 6 rats. Significant at **P < 0.01 and ***P < 0.001as compared to control group using one way ANOVA followed by Tukey – Kramer's post hoc test.

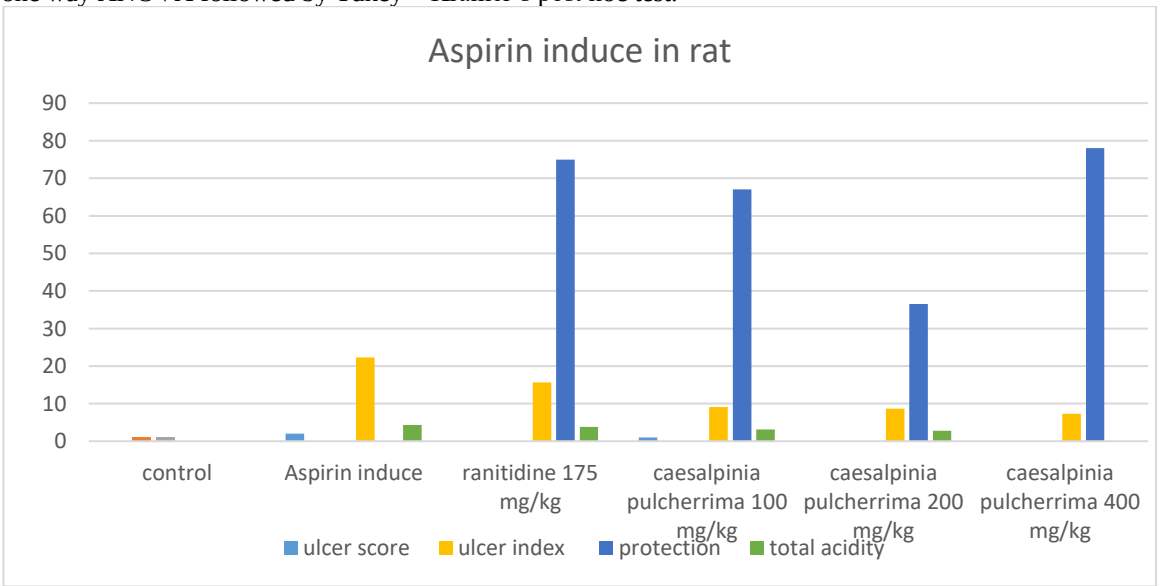

Figure 4. Effect of *Caesalpinia pulcherrima* on Aspirin induce ulcer

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Conclusion

In scientific studies, *Caesalpinia pulcherrima*, also referred to as peacock flower or Pride of Barbados, has been investigated for its ulcer-healing properties. Alkaloids, flavonoids, tannins, and saponins were found in the aqueous and methanolic extract of *Caesalpinia pulcherrima*, according to preliminary investigations. The extracts from *Caesalpinia pulcherrima* have also exhibited protective benefits on the mucosa by boosting mucin secretion and stimulating prostaglandin synthesis. These actions reinforce the mucosal barrier and stimulate natural healing processes in the stomach lining, vital for preventing and treating ulcers. *Caesalpinia pulcherrima* extracts were repeatedly shown to significantly reduce ulcer occurrence and severity in experimental mice. These results point to its promise as a natural ulcer treatment; nevertheless, further human clinical trials are required to validate these benefits. To sum up, *Caesalpinia pulcherrima* has potential as a botanical remedy for ulcers because of its anti-inflammatory, antioxidant, and mucosal-protective qualities. Clinical trials should be carried out in the future to confirm these preliminary results and investigate the therapeutic potential of this treatment for gastrointestinal ulcers.

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