

Screening of Antiulcer Activity of *Caesalpinia pulcherrima* L. Bark. Against Aspirin Induced Ulcer in Rats

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Abstract: The study aimed to investigate the antiulcer effects of the hydroalcoholic and aqueous extracts of bark of *Caesalpinia pulcherrima* Linn. for antiulcer activities. 200 (CP1) and 400 (CP2) mg/kg b.wt & (CPA) 300 mg/kg b.wt. of the hydroalcoholic and aqueous extracts of *Caesalpinia pulcherrima* L respectively were evaluated by pylorus ligation models for protection against Aspirin induced ulcer method. Volume of the gastric content, pH were investigated & After centrifugation, acidity is determined by titration with 0.01 N NaOH. Ulcer Index & % of protection were calculated. The CP and CPA significantly controlled ($P < 0.001$) the Aspirin induced ulcer development. At 200 (CP1) and (CPA) 300 mg/kg both the doses by decreasing the ulcer score in both the ulcer models, It is concluded that the hydroalcoholic and aqueous extracts of bark of *Caesalpinia pulcherrima* L. can be used as antiulcer herbal medicine.

Key words:

INTRODUCTION

Gastric ulcer, one of the most widespread disorder, [1]. When the gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, bacterial products (*Helicobacter pylori*) and drugs, the gastric ulcer prevalence increases [2].

These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility [3].

Plants belonging to the family Caesalpinaceae have wide range of medicinal uses. *Caesalpinia pulcherrima*. Vernacularly known as Peacock Flower is widely distributed in India and its leaves, flower, bark and seeds are used in Indian medicine [4]. The plant is considered as a tonic, stimulant and emmenagogue. The bark is used as abortifacient while leaves are used as cathartic [5]. An attempt has been made in the present study to evaluate the antiulcer actions of Hydroalcoholic and aqueous extracts of *Caesalpinia pulcherrima* L.

MATERIALS AND METHODS

Collection of Plant & Authentication: The bark of the *Caesalpinia pulcherrima*. (L.) was collected from the Pune District of Maharashtra, India. The plant was authenticated from Botanical survey of India, Pune (Ministry of environment and forest) –A voucher specimen No- ATNCAEPU2.

Preparation of Extract

Hydro Alcoholic Extract: The dried barks of *Caesalpinia pulcherrima*. (L.) Sw. was collected, dried and powdered to get coarse particles. The dried powder (1300g) were soaked in 50% ethanol for 72 hours. The obtained alcoholic extract was filtered and concentrated on hot plate.

Aqueous Extract: The aqueous extract of the bark was prepared by macerating coarse powder for 24 hours by soaking in cold water then filtered and concentrated.

The extracts obtained were concentrated under reduced pressure to yield hydroalcoholic (18.70%) and aqueous (12.51%) extracts.

Phytochemical Test: Phytoconstituents were identified by qualitative chemical tests like test for acidic compounds, carbohydrates, mucilage, flavonoids, tannins, steroids & Triterpenoides on hydro alcoholic and aqueous extracts of aerial parts of *Caesalpinia pulcherrima* L.

Animals: Wistar albino rats of both sexes weighing between 150-200 gm were used. animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA). Albino rats were obtained from the National Institute Of Biosciences. The animals were housed in Poly propylene cages and maintained at 24°C±2°C under 12h light/ dark cycle and were feed *ad libitum* with standard pellet diet and had free access to water. Institutional Animal Ethics Committee approved the experimental protocol (IAEC NO.1249/ac/09/CPCSEA).

Acute Toxicity Study: The acute oral toxicity study was carried out for hydro alcoholic and aqueous extract of *Caesalpinia pulcherrima*. (L.) using fixed dose method according to OECD guideline no.420 [8] Healthy adult female Swiss albino mice weighing between 25 to 35 g were used for study. Animals were divided into four groups, three animals each, fasted overnight. The different doses like 5, 50, 300 and 2000 mg/kg b. w. were administered to the Group 1, II, III, IV respectively. After administering the hydro alcoholic extract to different groups the behavioral changes like body temperature, CNS activity, urination, defecation etc were observed for 24 hrs for any signs of toxicity.

Induction of Gastric Ulcer: Animal were divided into five groups, six rats each. The control group (C), received distilled water orally. The second group(CO) administered commercial medicine Omeprazole (20mg/kg) b.w. were administered orally for group 2 as reference drug. the third and fourth group (CP1 and CP2) received hydro alcoholic extract (50%) of *Caesalpinia pulcherrima* L. 200 & 400mg/kg b.wt respectively and the fifth group (CPA) received the water extract of *Caesalpinia pulcherrima* L. 300mg/kg b.wt animals were given the plant extract once aspirin was administered to the animals in dose of 200mg/kg 45min after giving the extracts & omeprazole treatment. Animal were sacrificed 4 hours later & the stomach was then excised & cut along the greater curvature, washed carefully with 5.0 ml of 0.9%NaCl then after the ulcers were scored by a person unaware of the

experimental protocol in the glandular portion of the stomach. ulcer index was then calculated by adding the total number of ulcer per stomach as well as the total severity of ulcer per stomach [9-10].

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

$$U.I. = \frac{\text{No. of ulcer positive animals}}{\text{Total no. of animals}} \times 2$$

$$\text{Protective(\%)} = \frac{\text{Control mean ulcerindex} - \text{Test mean ulcerindex}}{\text{Control mean ulcer index}} \times 100$$

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1 \text{ N}} \times 100 \text{ mEq/L/100g}$$

The above equation is use to calculate the acidity of the stomach content.

Free and Total Acidity: Free and total acidity were determined by titrating with 0.01 N NaOH using Topfer's reagent and phenolphthalein as indicator. The free and total acidity were expressed as μequiv/100 g.

Statistical Analysis: Data were statistically computed using one way ANOVA followed by Dunnett's test [REF.15].

RESULT

Acute Toxicity Study: Animals did not show any sign of toxicity during the observation period (24 h). The extracts either the alcohloic or aquous were administered safely up to a maximum dose 2000 mg/kg b.w. of *Caesalpinia pulcherrima* L.

Screening the Chemical Constituents of the Plant Extract: The phytochemical screening revealed that hydroalcoholic and aqueous extract contained flavonoids, alkaloids, steroids, tannins and phenolic compounds, glycosides, saponins, cardiac glycoside like cardenolids and carbohydrates.

Aspirin Induced Ulcers in Rats: In the present study the anti ulcer activity of bark of *Caesalpinia pulcherrima* L. Revealed that the minimum ulcer index was observed with Omeprazole in CO, followed by CP1 and CPA. (Table1 and Fig. 1).

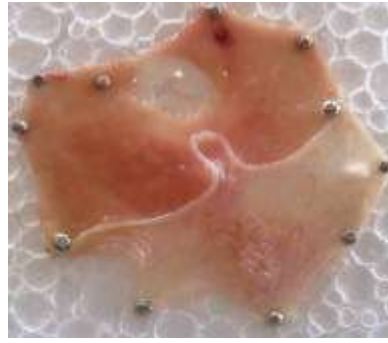
Table 1: Effect of hydroalcoholic(50%) extract of *Caesalpinia pulcherrima* L. on Aspirin induced gastric ulcer model

Parameters	C	CO	CP1	CP2	CPA
Ulcer index	13.3±0.7	5±0.22***	6±0.51***	8.66±0.33*	7±0.42***
Protection(%)	-	62.40	54.88	34.88	47.36
Gastric juice vol.(ml)	7.5±0.06	3±0.00***	7±0.00***	8.2±0.08***	7.4±0.07
pH of gastric juice	3±0.77	4.8±0.74	2.8±0.47	3.6±0.98	3±0.77
Total acidity	62±1.14	40±0.00***	45±1.00***	3.6±0.98***	3±0.77***

Values are expressed as mean ±SEM of 5 observation, statistical comparisons as follows: significant at ***P<0.001 as compared to control



a) Control (P.L.) shows severe damage



1 b) Omeprazole (20 mg/kg) shows of mucosal layer protected mucosal layer



c) hydroalcoholic extract of *Cp2* (400 mg/kg) Shows protected mucosal layer



d) hydroalcoholic extract of *Cp1* (200 mg/kg) Shows protected mucosal layer



e) Aqueous extract of *CpA* (300 mg/kg) Shows protected mucosal layer

Fig. 1: Macroscopical view of Aspirin induced Ulcer

DISCUSSION

Ulcers develop when the normal defense and repair mechanisms of the lining of the stomach or duodenum are weakened, making the lining more likely to be damaged by gastric acid. A peptic ulcer is a score on the lining of the stomach, small intestine or esophagus [11].

The current study showed that gastric juice in the group (CP1) and (CPA) received 200 & 300 mg/kg b.wt. of the hydroalcoholic and aqueous extracts of *Caesalpinia pulcherrima* L respectively Showed a significant ($P < 0.01$) increase in gastric juice pH, reduces the gastric volume, total acidity when compared to control.

Different therapeutic agents including plant extracts are used to inhibit the gastric acid secretion, or to stimulate the mucosal defence mechanism by increasing the mucus production that protect surface epithelial cells, or interfering with PG synthesis [11-12].

Gastrointestinal injury is induced by various chemical agents.

Aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of H^+ ions [13] and is certified in the current study as elaborated in table 1 where the anti ulcer activity of bark of *Caesalpinia pulcherrima* L.

Revealed that the minimum ulcer index was observed with Omeprazole in CO, followed by CP1 and CPA.

In stomach, prostaglandins play a vital protective role by stimulating secretion of HCO_3^- and mucous, maintaining mucosal blood flow and regulating mucosal cell turnover and repair. Thus the suppression of prostaglandin synthesis by NSAIDs results in increased susceptibility to mucosal injury and gastro duodenal ulceration[14]. This is in agreement with the obtained results where the total acidity was decrease in CP1, CPA & Omeprazole compare to normal control which is responsible for decreasing severity of ulcer or treating ulcer by maintaining mucosal membrane. It is also shown that ROS (reactive oxygen species) plays an important role in pathogenesis of mucosal damage caused by aspirin besides inhibition of COX enzymes. The present study The bark is rich in active ingredients like caesalpin-type diterpenoids, sitosterol, pulcherrimin, lupeol, lupeol acetate, myricetin, quercetin and rutin, flavonoids, carotenoids, glycosides, peltogynoids, phenols and steroids [6-7]. Observed result showed that Hydroalcoholic and aqueous extracts reduced aspirin induced ulcers suggesting possible involvement of prostaglandin and mucus. CP is also reported to possess antioxidant activity that might have also contributed in

antiulcer activity exhibited by extracts. The (CP1) and (CPA) and Omeprazole significantly decreased the total acidity; this suggests that it having an antisecretory effect.

The current study showed that gastric juice in the group (CP1) and (CPA) received 200 & 300 mg/kg b.wt. of the hydroalcoholic and aqueous extracts of *Caesalpinia pulcherrima* L respectively Showed a significant ($P < 0.01$) increase in gastric juice pH, reduces the gastric volume, total acidity when compared to control.

Bark extract of *Caesalpinia pulcherrima* L. showed a significant reduction in ulcer index when compared to normal control ($p < 0.001$). both doses of bark CP1 & CP2. (200 & 400 mg /kg p.o.) showed a significant reduction in total acidity ($p < 0.001$) when compared to control. Result are shown in Table 1 show the result obtained with experimental model of aspirin induced acute gastric ulceration in rats. CP1 & CP2 at dose 200 mg/ kg & 400 mg/ kg body weight demonstrated reduction mean ulcer score when compared to the animal not treated with extract (control).

The result of experimentally induced ulceration with aspirin showed that CP1 & CP2 cause decrease ulcer score when compared to the control. Antiulcer effect of CP1 at 200 Mg/kg of body wt. is likely mediated which might be similar to that of Omeprazole which equally reduced the severity of gastric lesion developed by aspirin in this study. Omeprazole is a known stable analogue of PGE_2 . This drug inhibit the gastric acid secretion, both basal & that occurring in response to food & also increase the secretion of mucus & bicarbonate.

Overall, CP1 at 200mg/kg body wt. has shown a substantial and significant protection against gastric ulcers in all the models.

CONCLUSION

Different concentration of aqueous and hydroalcoholic extract of *Caesalpinia pulcherrima* L. were used. among all hydroalcoholic extract *Caesalpinia pulcherrima* L. of 200mg/kg of body wt showed significant antiulcer activity.

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