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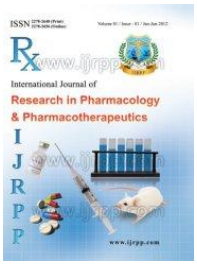

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Anti-ulcer activity on cold stress induced ulcerative rats with column chromatographically isolated *Ceiba pentandra* flavonoids

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ABSTRACT

This study evaluated the anti-ulcer activity of the ethanolic extract of *Ceiba pentandra* leaves on experimentally-induced gastric ulcer in rats. Animals were pre-treated with varied doses of the extract and reference drug pantoprazole. Ulcer was induced in the animals by fasting them for 12 hours and by Cold Stress Induced Ulceration. A total of 20 rats were divided into four groups of five animals, each used for each assay.Histological studies of the gastric wall of the ulcer- induced rats were carried out. Mean ulcer index along with the percentage of ulcer inhibition by the extract and drugs were calculated for each group. *Ceiba pentandra* produced a significant dose dependent inhibition of gastric lesions in Cold Stress Induced ulcers evidenced by the reduced ulcer index of the treated groups. Histological examination of the gastric wall of the control rats revealed severe damage of the gastric mucosa, haemorrhages, along with oedema and leucocyte infiltration of sub-mucosal layer while the *Ceiba pentandra* extract-treated rats showed little damage of the gastric mucosa. These results showed that *Ceiba pentandra* possess ulcer protective properties against experimentally induced ulcers and validates its traditional use in the treatment of stomach pain and ulcer.

Key Words: Ceibapentandra, pantoprazole, gastric ulcer, mucosal damage, rats.

INTRODUCTION

Gastric ulcer, also known as Peptic Ulcer Disease, [PUD] is the most common ulcer of the gastro-intestinal tract. It results from persistent erosions and damage of the stomach wall that might become perforated and develop into peritonitis and massive haemorrhage [1,2]. This occurs as a result of imbalance between some endogenous aggressive factors such as hydrochloric acid, pepsin, refluxed bile, leukotrienes, Reactive Oxygen Species (ROS) and cytoprotective factors, which include the function of the mucus bicarbonate barrier, surface active phospholipids, prostaglandins (PGs), mucosal blood flow, non-enzymatic and enzymatic antioxidants [3-5]. The gastric mucosa protects itself from gastric acid with a layer of mucus, the secretion of which is stimulated by certain prostaglandins. NSAIDs block the function of Cyclooxygenase 1 (COX-1), which is essential for the production of these prostaglandins [6]. Secretion of gastric acid is recognized as a central component of the gastric ulcer. Thus, its main therapeutic target is the control of this secretion using

antacids, H_2 receptor blockers (ranitidine, famotidine, cimetidine) or proton pump blockers (omeprazole and lansoprazole) [7]. The success of these commercially available antiulcer drugs in the treatment of gastric ulcer is usually overshadowed by various side effects; for example, H2- receptor antagonists e.g. cimetidine may cause gynaecomastia in men (enlargement of breast in men, due to harmonic imbalance)[8] and galactorrhoea in women (abnormal copious milk secretion)[9], while protonpump inhibitors can cause nausea, abdominal pain and constipation [10]. Due to these side effects, there is a need to find new anti-ulcerogenic compound(s) with potentially less or no side effects, and natural products derived from plant sources have always been the main sources of new drugs for the treatment of various diseases including gastric ulcer [11].

Ceiba pentandra also known as 'Silk Cotton Tree' and locally as 'Dum' is a tropical tree of the order Malvales and the family "Bombacaceae"[12]. The plant is native to Mexico, Central America and the Caribbean, Northern South America, and to Tropical West Africa and is widely reputed in the African traditional medicine. The tree is also known as the Java cotton, Java kapok, Silk cotton or ceiba. Various morphological parts of this plant have been reported to be useful as effective remedies against diabetes, hypertension, body aches, uterine contractions and rheumatism [12,13]. The plant has also been reported to possess antiulcerogenic activity[14]. The traditional medicine practitioners of the Igbo tribe, Nigeria, use a boiled concoction mix which includes the stem bark and leaves of *Ceiba pentandra* as a remedy for stomach pains. Thus, the present study was initiated to evaluate the anti-ulcer activity of the ethanolic extract of Ceibapentandraleaves.

MATERIALS AND METHODS

The leaves of *Ceiba pentandra* was authenticated by Dr. A. Madhusudhan Reddy, Assitant professor, Yogi Vemana University, Kadapa, Andhra Pradesh, India.

The plant material was shade-dried after collection and powdered. The powdered plant material was extracted with ethanol and filtered with the help of No. 1 Whatmann paper filter paper. The extraction was done with soxhlet apparatus. The temperature was set at 80^0c as the boiling point of ethanol is 78.4^0c. The dried crude extract obtained was stored in a sterile container.

ANIMALS

Male wistar rats of 150-200 g body weight were obtained from Raghavendra enterprises, Bangalore, and they were housed under standard husbandry conditions, 25 ± 5^0C temperature, 12 h light / dark cycle with standard rat feed (Pravan agro Ltd. India) with water adlibitum.

Isolation of flavonoids by Column Chromatography

The ethanolic extract of plant was developed with column chromatography by taking Ethyl acetate, Formic acid, Acetic acid, Water in the proportions of 0.6, 3.2, 6.2, 6.5, *% V/V*, respectively, which is found to have maximum elution of flavonoids with TLC technique. The components present in extract were separated as different colored bands, and each separated component is collected as fractions from the column as given in fig. 3.

Fig. 1.Isolation by Column Chromatography

Screening of Isolated Compound

The isolated compound is screened for its identification.

Phytochemical screening

The isolated component is screened phytochemically by conducting qualitative chemical tests, and it responded positively for alkaline reagent test.

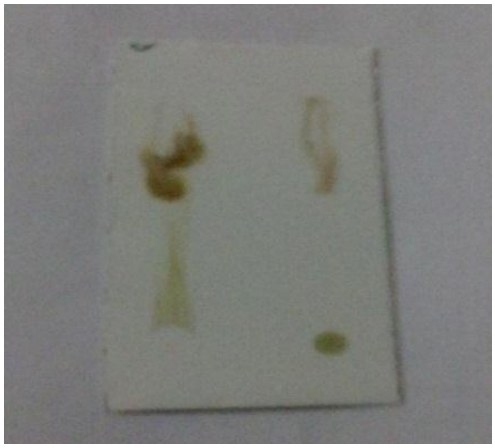
Alkaline Reagent Test

To the isolated component, few drops of sodium hydroxide solution is added, formation of intense yellow colour that disappears up on addition of few drops of dil. acid indicated presence of flavonoids. Thus isolated component is identified as flavonoids.

Confirmation of Flavonoid Isolation

Thus eluted component was spotted on TLC plate along with standard flavonoid drug, 'Quercetin' The distance of eluted spots is identified under UV, and the R_f value is calculated for plant drug in comparision with the Quercetin as given in fig.4. From the resulted R_f value of 0.5 it was identified as flavonoid.

Fig. 2. TLC Plate with Elution of Isolated Flavonoid and Quercetin



PHYTOCHEMICAL ANALYSIS

The phytochemical analysis of ethanolic extract of plant was developed with column chromatography by taking Ethyl acetate, Formic acid, Acetic acid, Water in the proportions of 0.6, 3.2, 6.2, 6.5, % *V/V*, respectively, which is found to have maximum elution of flavonoids with TLC technique. Thus isolated flavonoid was spotted on TLC plate along with standard flavonoid drug Quercetin. The distance moved by the spots is identified under UV, and the R_f value was obtained for plant extract in comparison with the Quercetin.

COLORIMETRIC ESTIMATION

The isolated flavonoids were estimated by colorimetry at the wave length of 405 nm to 516 nm and its absorbance was measured as 0.625 nm.

ANTI-ULCER ACTIVITY (COLD STRESS INDUCED ULCERATION)

The gastric ulcers were induced in rats of either sex weighing between 150 g to 200 g due to psychological stress by means of cold restrain. The rats were divided into four groups, each containing five animals. Fasted for 12 hours, and allowed free access to water. The first group received control vehicle only (SCMC), and the second group received standard pantaprazole in the dose of 40 mg / kg, third group received isolated flavonoids of ethanolic extract of *Ceiba pentandra* leaves 200 mg/kg, fourth group received isolated flavonoids of ethanolic extract of leaves of *Ceiba pentandra* 400 mg/kg orally as per body weight respectively. After administration of drugs the animals were kept under the stress in cages for 3 hrs in cold restrain environment. After 3 hrs the animals were anaesthetized with anesthetic ether, stomach was incised along the greater curvature, and ulceration was scored. The ulcer index and the length of each

ulcer were determined and % of ulcer protection was measured from the formula:

$$\% \text{ Ulcer protection} = \frac{\text{control mean ulcer index - test mean ulcer index}}{\text{control mean ulcer index}} \times 100$$

RESULTS AND DISCUSSIONS

RESULTS OF ANTI-ULCER STUDIES IN STRESS INDUCED ULCERATIVE RATS

There are several methods to induce ulceration in rats. In present study, the gastric ulcers were induced in rats of either sex weighing between 150 g to 200 g by psychological stress by means of cold restrain. The rats were divided in to four groups, each containing five animals. Fasted for 12 hours, and allowed free access to water. The first group received control vehicle only (SCMC), and the second group received standard pantaprazole in the dose of 40 mg/kg, third group received isolated flavonoids of ethanolic extract of *Ceiba pentandra* leaves 200 mg/kg, fourth group received isolated flavonoids of ethanolic extract of leaves of *Ceiba pentandra* 400 mg/kg orally as per body weight respectively. After administration of drugs keep the animals in the stress cages for 3 hrs in cold restrain environment. After 3 hrs the animals were anaesthetized with anesthetic ether, stomach was incised along the greater curvature, and ulceration was visualized as shown in fig. 3, fig. 4, fig. 5; and scored as mentioned in table no.1. The number of ulcers and the length of each ulcer were measured from the formula:

$$\% \text{ Ulcer protection} = \frac{\text{control mean ulcer index - test mean ulcer index}}{\text{control mean ulcer index}} \times 100$$

Fig.3 & 4 Incised Stomach of Rats Administered with Isolated Flavonoids

Fig.5 Incised Stomach of Rats Administered with Standard and Control

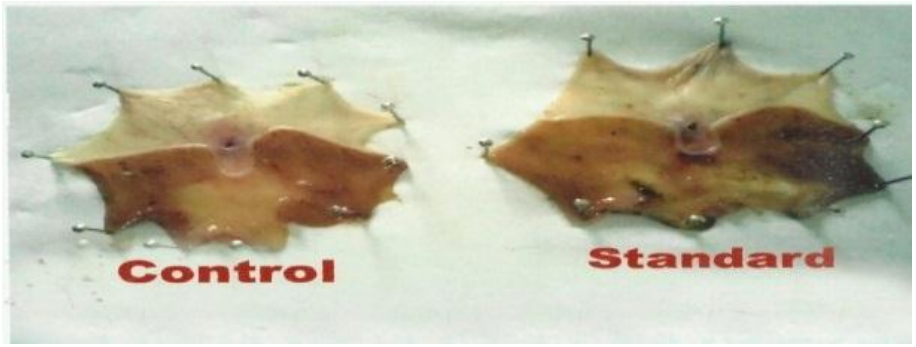


Table.1. Length and Number of Ulcers Visualized

S. No.	TREATMENT	DOSE (mg/kg)	VOLUME (ml)	PH	ACIDITY (mEq/1/100g)	ULCER INDEX
1	Control	-	1.1	2.5	3.00	7
2	Pantaprazole	10	0.9	6.7	6.80	0.5
3	FEECP (200mg/kg)	10	0.9	6.2	6.10	1.5
4	FEECP(400mg/kg)	10	0.8	5.7	5.20	2.5

FEECP – Flavonoids of ethanolic extract of *Ceiba pentandra*

The present study revealed that the flavonoids of ethanolic extracts of Ceibapentandra have significant anti-ulcer activity.

SUMMARY

In this present study an attempt has been made to separate the flavonoids from the ethanolic extract of *Ceiba pentandra* leaves by using the Ethylacetate, Formic acid, Acetic acid, Water in the proportions of 0.6, 3.2, 6.2, 6.5, % V/V respectively as mobile phase and to evaluate the anti-ulcer activity of flavonoids present in *Ceiba pentandra* leaves. There are many marketed anti-ulcer drugs that cause many adverse effects, most commonly gynacomastia is reported with anti- histaminics. To overcome these side effects, an attempt was made to discover anti-ulcer activity in naturally occurring leaves of *Ceiba pentandra*.

The leaves of *Ceiba pentandra* were collected, shade dried and powdered. The powder was extracted with ethanol in soxhlet apparatus. Then a suitable solvent system for the best elution of phenols and flavonoids was selected by performing Thin Layer Chromatography with different solvents.

The flavonoids were isolated by column chromatography by the selected solvent system, and its absorbance was measured using colorimetry. Anti-ulcer activity of isolated flavonoids was studied on cold stress induced ulceration in wistar rats.

CONCLUSION

Finally, we conclude that from the results of pharmacological screening that the isolated phytoconstituents showed significant action on cold stress ulcer induced rats. The present study supports the traditional folk and reveals that flavonoids present in ethanolic extract of *Ceiba pentandra* have significant antiulcer activity. Hence this study can be considered in further researches on *Ceiba pentandra* and also to enable formulation of a new anti-ulcer drug which may have lesser side effects than currently marketed drugs.

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