

DETERMINATION OF ANTIULCER ACTIVITY OF *PLUMERIA RUBRA* LEAVES EXTRACTSMisra Vimlesh*¹, Yadav Garima², Uddin Sheikh Mubeen¹, Srivastava Vivek¹¹Institute of Pharmaceutical Research, GLA University, Mathura, Uttar Pradesh, India²Advance Institute of Biotech & Pramedical Science, Kanpur, U.P., India

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ABSTRACT

Gastric ulcer is one of the most prevalent gastrointestinal disorders, which affects approximately 5-10% of people during their life. In recent years, abundant work has been carried out on herbal medicine to clarify their potential efficacy in gastric ulcer prevention or management. Here, present study was carried out to investigate antiulcer activity of ethanol (EEPR) and chloroform (CEPR) extract of *P. rubra* L. (Family: Apocynaceae) leaves in pylorus ligated and ethanol induced ulceration in the albino rats. 250 mg/kg of both ethanol and chloroform extracts were selected for the Pharmacological study. In pylorus ligation induced ulcer model, various parameters were studied viz. gastric volume, pH, total acidity, free acidity, protein assay, mucin assay, antioxidant estimation and ulcer index. Ulcer index and percentage inhibition of ulceration was determined for ethanol induced ulcer model. The results suggest that the ethanol and chloroform extract of *P. rubra* possess the significant antiulcer activity.

Key words: *Plumeria rubra*, Ulcer, Pylorus ligation, Ethanol induced ulcer.

INTRODUCTION

Peptic ulcer disease is a serious gastrointestinal disorder that requires a well targeted therapeutic strategy. A number of drugs including proton pump inhibitors and H₂ receptor antagonists are available for the treatment of peptic ulcer, but clinical evaluation of these drugs has shown incidence of relapses, side effects, and drug interactions. This has been the rationale for the development of new antiulcer drugs and the search for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse. Drugs of plant origin are gaining popularity and are being investigated for a number of disorders, including peptic ulcer. Indian Medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders including peptic ulcer. An indigenous drug possessing fewer side effects is the major thrust area of the present day research, aiming for a management of peptic ulcer.¹

Plumeria rubra (Linn.) (Hindi name: Lal champa; English: True Frangipani) belongs to the Apocynaceae family and have laticiferous trees and shrubs.² They are recognized as excellent ornamental plants and often seen in the graveyards.³ *Plumeria* plants are famous for their attractiveness and fragrant flowers. The essential oils from the flowers are used for perfumery and aromatherapy purposes. The decoction of the bark and roots of *P. rubra* is traditionally used to treat asthma, ease constipation, promote menstruation and reduce fever. The latex is used to soothe irritation.⁴ The fruit is reported to be eaten in West Indies. In India, however, it has been used as an abortifacient. The flowers are aromatic and bechic and widely used in pectoral syrups. The flowers decoction of *P. rubra* was reported to use in Mexico for control of diabetes mellitus. The Leaves of *P. rubra* are used in ulcers, leprosy, inflammations and rubefacient.⁵

According to Ayurveda; root is bitter, carminative, thermogenic, laxative, leprosy etc. Leaves are useful in inflammation, rheumatism, antibacterial, bronchitis, cholera, cold and cough, Antipyretic, antifungal, stimulant etc.⁶ Looking to the scope of herbal drug and increasing demand especially in disease of liver, cancer, diabetes, hypertension, renal disease, inflammation, infectious diseases and skin diseases etc.⁷

MATERIAL AND METHOD**Collection of plant material**

Fresh leaves of *Plumeria rubra* (Linn) was collected from Allahabad (U.P), India and authenticated by experts of Botany at Birbal Sahni institute of Palaeobotany, Lucknow (U.P), India

Extraction of *Plumeria rubra* leaves

The leaves were dried in shade and then powdered and passed through sieve no.40. The powder was packed into a Soxhlet apparatus and extracted with petroleum ether (60-80°) for 18 h. The same marc was then extracted with ethanol and afterwards the ethanol extract was added to distilled water and extracted with chloroform. The water was removed under the reduced pressure and the material we got was ethanol extract. The extracts were dried below 50°C in water bath for 5 h when a solid mass was obtained in case of alcoholic and chloroform extract respectively and were stored in airtight containers in refrigerator. The extracts thus obtained were subjected to phytochemical analysis.

Experimental animal

Albino rats of either sex, weighing 100-150 g were selected for antiulcer activity. Institutional animal ethical committee approved the experimental protocol; animals were housed under standard conditions of temperature (24± 2°C) and relative humidity (30-70%) with a 12:12 light: dark cycle and had free access to standard pellet food and water *ad libitum*.

Experimental Procedures**Pylorus Ligation Induced Gastric Mucosal Ulcers**

Healthy wistar albino rats of either sex weighting between 100-150g were taken for the studies. The animals were divided in five groups (each contain 6 animals). The animals in all groups were kept for 24 h fasting with free access to water and avoidance of coprophagy, after that group I serve as Normal control. Group II received distilled water orally. Group III received omeprazole (20mg/kg, p.o.). Group IV received EEPR (250mg/kg, p.o.) and group V received CEPR (250 mg/kg, p.o.).

After 30 m of the treatment, the pylorus ligation was done under the diethyl ether anesthesia. For carrying out pylorus ligation firstly the incision was made on the peritoneal region of the rat, then stomach was taken out of the peritoneal cavity and the pyloric end of the stomach was ligated with the help

of thread. Then the stomach was replaced in the cavity and the incision was sutured and animals were allowed to recover in their cages for four hours. After 4 h the rats were sacrificed. The stomach was then taken out by opening the sutures and stomach is isolated. During the isolation proper care was taken so that the gastric juices do not flow out from the cardiac end of the stomach. The stomach was then opened from the greater curvature with the help of scissor and gastric juice was collected in a appropriate measuring vessel for the further gastric secretions studies, and ulcer index was measured in the stomach after cleaning it under low pressure water by the simple stage microscope under 10x magnification.⁸ The result of the ulcer index is given in table 1.

Measurement of Various Parameters

1. Ulcer Index:

The following arbitrary scoring system was used to grade the incidence and severity of lesion

0 = Normal, 1 = Red colouration, 2 = Spot ulcers,
3 = Haemorrhagic streaks, 4 = Ulcers > 3 but < 5 and
5 = Ulcers > 5

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

$$\% \text{ protection} = \frac{\text{Control mean ulcer index} - \text{test mean ulcer index}}{\text{Control mean ulcer index}} * 100$$

2. Volume and pH of Gastric Juice:

The collected gastric juice is subjected to centrifugation in centrifugation tube. The tubes were centrifuged at 1000 rpm for 10 min and the gastric volume is directly read from the graduation on the tubes. The supernatant was then collected and pH was determined by using a digital pH meter.⁹ The result is given in table 1.

3. Biochemical Parameters

Preparation of Tissue Homogenate:

Stomach was dissected out from the sacrificed animal and rinsed with distilled water. Stomach was then homogenized into ice cold 0.15 M Tris-HCl (pH 7.4) to give 10%w/v homogenate. The homogenate was then centrifuged at 15000 rpm for 15 min at 4°C in cooling centrifuge. Supernatant was used for determination of antioxidant activity.

a) Reduced Glutathione:

The supernatant (40µl) was mixed with 400µl tris and 360µl water. Then 0.2mL (200µl) DTNB solution was added and absorbance was measured at 412 nm. Standard curve for GSH was prepared using glutathione.¹⁰ The result is given in table 3.

b) Catalase (CAT):

100µl of supernatant was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0 ml of freshly prepared 30 mM H₂O₂. Decrease in absorbance was read at 240 nm for 3min at interval of 30 sec. The activity was calculated using extinction coefficient of H₂O₂ 0.041micromole/cm². Results were expressed as micromole of H₂O₂ utilized/min/gm tissue. The rate of Decomposition of H₂O₂ was measured spectrophotometrically from changes in absorbance at 240 nm.¹¹ The result is given in table 3

c) Superoxide Dismutase Assay:

Take 0.1 ml Supernatant as sample and add 1.2 mL sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml phenazine methosulphate (186 µM), 0.3 ml of 300 µM

Nitroblutetrazolium and 0.2 ml NADH (750 µM) and incubate at 30°C for 90 s. Add 0.1 ml glacial acetic acid stir with 4.0 ml n-butanol, allow to stand for 10 min, centrifuge and separate butanol layer and take OD at 560 nm (take butanol as blank). The result is given in table 3

d) Protein assay:

Take 50 µl sample, 50 µl 1M NaOH and 5ml Bradford Reagent, incubate for 5 min at room temp. Take OD at 595nm.¹² The result is given in table 2

e) Gastric wall mucin:

Gastric wall mucus was determined according to the modified procedure of Corne et al. (1974). The glandular segments from stomachs which had been opened along their greater curvature were removed and weighed. Each segment was transferred immediately to 10 ml of 0.1% w/v Alcian blue solution (in 0.16 M sucrose solution, buffered with 0.05 M sodium acetate pH 5). After immersion for 2 h, excess dye was removed by two successive rinses with 10 ml of 0.25 M sucrose, first for 15 and then for 45 min. Dye complexed with the gastric wall mucus was extracted with 10 ml of 0.5 M magnesium chloride by shaking intermittently for 1 min at 30 min intervals for 2 h. Four milliliters of blue extract were then shaken vigorously with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3600 rev./min for 10 min and the absorbance of the aqueous layer was recorded at 580 nm. The quantity of Alcian blue extracted per gram of net glandular tissue was then calculated. The result is given in table 2

Parameters Evaluated from Gastric Juice:

Total Acidity and free Acidity:

Gastric juice (1ml) was pipette into a 100ml conical flask and diluted with 9ml distilled water. Two or three drops of Topfer's reagent was then added and titrated with 0.01 N sodium hydroxide until all traces of red color disappeared and the color of the solution was yellowish-orange. The volume of alkali added was noted. This volume corresponds to free acidity. Two or three drops of phenolphthalein were then added and the titration was continued until a definite red tinge appeared; the volume of alkali added was noted. The volume corresponds to total acidity; Acidity was expressed in terms of mEq/L. The result is given in table 1.

Ethanol Induced Gastric Mucosal Ulcer

Healthy wistar albino rats of either sex weighting between 100-150g were taken for the studies. The animals were divided in five groups (each contain 6 animals). The animals in all groups were kept for 24 h fasting with free access to water and avoidance of coprophagy, after that group I serve as Normal control. Group II received distilled water orally. Group III received Sucralfate (50mg/kg, p.o.). Group IV received EEPR (250mg/kg,p.o.) and group V received CEPR (250 mg/kg,p.o.).

One hour after treatment, all the rats received 1ml of 99.5% ethanol to induce gastric ulcer. One hour later, the animals were sacrificed by excess dose of diethyl ether, and the stomachs were removed and opened along the greater curvature. The stomachs were gently rinsed with water to remove the gastric contents and blood clots, for subsequent scanning.¹³ The result of the ulcer index is given in table 4.

Statistical analysis All values are expressed as mean ±S.E.M. statistical significance was determined by using student's t-test values with p<0.5* were considered significant.

Table: 1 Effect of EEPR and CEPR on Pylorus Ligation Induced Model

Group	Ulcer index	% protection	Gastric pH	Gastric volume	Free acidity mEq/L	Total acidity mEq/L
Normal control	0.00±0.00		2.11±0.04	2.25±0.06	31.62±0.64	52.48±0.49
Ulcer control	4.53±0.12		2.31±0.07	5.11±0.06	95.55±0.57	117.41±0.59**
Standard omeprazole (20mg/kg)	0.39±0.07**	91.39	4.76±0.10**	2.40±0.08**	33.62±0.50**	57.10±0.52**
EEPR (250mg/kg)	1.13±0.04**	75.05	4.13±0.08**	3.23±0.07**	38.38±0.25**	61.9±0.46**
CEPR (250mg/kg)	1.67±0.10**	63.13	3.30±0.12**	4.26±0.06**	45.95±0.27**	67.26±0.28**

Values are expressed as Mean±SEM, n=6 animal in each group *** P<0.001, **P<0.01, * P<0.5, All values are compared to control group.

Table: 2 Effect of EEPR and CEPR on Total protein and Mucin

Group	Total protein gm/dL	Mucin mcg/mL
Normal control	38.35±0.41	201.2±13.15
Ulcer control	13.27±0.41	102.60±6.90
Standard omeprazole (20mg/kg)	35.91±0.35**	197.20±15.75**
EEPR (250mg/kg)	31.85±0.67**	171.22±22.03**
CEPR (250mg/kg)	30.23±0.51**	169.32±21.01**

Values are expressed as Mean±SEM, n=6 animal in each group *** P<0.001, **P<0.01, * P<0.5, All values are compared to control group.

Table: 3 Effect of Ethanol and Chloroform Extract of *Plumeria rubra* on CAT, SOD, and GSH

Group	CAT (µ/mg protein)	SOD (µ/mg protein)	GSH (µ/mg protein)
Normal Control	98.37±0.37	120.32±0.98	5.67±0.15
Ulcer control	66.12±0.42	85.97±0.46	2.82±0.54
Standard (Omeprazole 20mg/kg)	93.80±0.73***	112.32±0.89***	4.55±0.15**
EEPR 250mg/kg	75.74±0.89***	97.94±0.72***	4.03±0.03*
CEPR 250mg/kg	71.59±0.45***	94.34±0.33***	3.95±0.01*

Values are expressed as Mean±SEM, n=6 animal in each group *** P<0.001, **P<0.01, * P<0.5, All values are compared to control group.

Table: 4 Effect of EEPR and CEPR on Ethanol induced Ulcer Model

Group	Ulcer Index	% protection
Normal control	0.00±0.00	-
Ulcer control	4.85±0.12	-
Standard(Sucralfate 50mg/kg)	0.72±0.06**	85.15
EEPR 250mg/kg	1.34±0.05**	72.37
CEPR 250mg/kg	2.13±0.12**	56.08

Values are expressed as Mean±SEM, n=6 animal in each group *** P<0.001, **P<0.01, * P<0.5, All values are compared to control group.

RESULT AND DISCUSSION

Peptic ulcers are due to imbalances between offensive (NSAIDs, alcohol, acid and pepsin) and defensive (bicarbonate, mucosal blood supply) factors. The mucosal barrier is normally impermeable, to back diffusion of hydrogen ions, but it is weakened by either decreased mucus secretion or by disturbance in the turnover of epithelial cells, caused by stress, the adrenocorticosteroid hormone, cortisone, aspirin, phenylbutazone, smoking and so on.¹⁴ The leaves extracts of *Plumeria rubra* showed ulcer-protective effects against all five animal models.

Most widely used method for producing experimental gastric ulcers is pylorus ligation, as it is suitable for first line antiulcer screening, because the agents are retained in the stomach and may act by a variety of mechanisms. Reproducibility and high incidence of ulceration has been reported by this method. A major advantage of this method is that one can measure gastric secretory rate, percentage ulceration and ulcer severity in the same animal. It is reported that pyloric ligation induced ulcer model on rats is probably the single model which can give idea regarding clinical usefulness of an antiulcer drug.

Gastric acid and pepsin are important factors for the formation of ulcers in pylorus-ligated rats. In the stomach, digestive effect of accumulated gastric juice in the induction of gastric ulcers is well documented in the pylorus ligation model. Biochemical constituents of the gastric secretion have a considerable role to play in the pathophysiological state of gastric mucosa.¹⁵ In addition to gastric acid secretion, reflex or neurogenic effects have also been suggested as playing an

important role in the formation of gastric ulcers in the pylorus ligation model. In a recent study role of free radical in gastric ulcer induce by pylorus ligation is also reported.

In an attempt to clarify the mechanism of action of EEPR and CEPR, its influence on gastric secretion by using pylorus-ligation induced ulcer in rats was conducted. In the group of animals treated with EEPR and CEPR decrease in Ulcer index and volume of gastric juice was observed. Increase in pH and decrease in total acidity were significant as compared to disease group when extract dose was administered.

Preventive anti-oxidants, such as catalase (CAT) enzymes and reduced glutathione (GSH) are the first line of defense against reactive oxygen species. CAT reduces peroxy radical H₂O₂.¹⁶ Reduced glutathione (GSH) is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation.

The level of CAT SOD and GSH was significantly decreased in Ulcer control group as compared to normal group. Administration of EEPR and CEPR (250 mg/kg, p.o.) increased CAT, SOD and GSH levels as compared to the ulcer control animals, which suggests its efficacy in preventing free radical-induced damage.

EEPR and CEPR significantly inhibited lesion formation in the glandular stomach and reduced acid secretory parameter and volume of gastric secretions.

Antacids such as aluminium hydroxide gel mainly act by decreasing pH of gastric juice and hence reduce total acidity of gastric juice. Anticholinergics such as pirenzepine inhibit effect of vagal stimulation i.e. they compete with

acetylcholine for M₁ receptor on gastric parietal cell and hence decreases acid output. Proton pump inhibitors also acts by decreasing the gastric acid output.

Various causes are responsible for the development of ulcers due to pylorus ligation model such as increased metabolism of carbohydrates, increased synthesis of nucleic acids and other compensatory mechanisms. Moreover, the status of mucus secretion is important to determine the status of mucosal barrier.

This mucus consists of mucin type of glycoprotein. This high molecular weight glycoprotein are mainly responsible for viscous and gel forming capacity of mucus. Increased mucus secretion by gastric mucosal cell prevent gastric ulceration by several mechanism such as losing of stomach wall friction during peristalsis and gastric contraction, improving the buffering of acid gastric juice, and by acting as an effective barrier to back diffusion of H⁺ ions.¹⁷ The EEPR and CEPR is significantly capable of increasing the Mucous secretion.

In the ethanol induced ulcer model, ulcers are caused due to perturbations of superficial epithelial cells, notably the mucosal mast cells, leading to the release of vasoactive mediators including histamine and reactive oxygen species¹⁸ resulting in the damage of rat gastric mucosa. Mucosal blood flow has been considered to be an important factor in the damage caused by alcohol and is modulated by prostaglandin.¹⁹ The significant effectiveness of extracts in protecting against mucosal damage caused by alcohol is an indication of its effect on prostaglandins synthesis and on the free radical scavenging activity.²⁰ Thus, it could be assumed that the existence of the cytoprotective effect of compound is present in the ethanol and chloroform extract of the *Plumeria rubra* because it showed the significantly less ulcer index in the observation when the results were compared with the control group.

CONCLUSION

Present study indicates that ethanolic extract of *Buchanania lanzan* spreg. roots have good antiulcer mactivity.

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