GASTRO-PROTECTIVE POTENTIAL OF FLOWERS OF *HIBISCUS ROSA-SINENSIS* (L.) IN MUCOSAL LESION ON RATS

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ABSTRACT

Study was conducted to evaluate the gastro-protective activity of aqueous (HAE) and ethanolic (HEE) extracts of *Hibiscus rosa-sinensis* Linn. (Malvaceae) flowers in experimental animals. Aqueous and ethanolic extracts were investigated for pylorus ligation and indomethacin induced ulcer models in albino Wistar rats. The pH, gastric volume, ulcer index, ulcer score and free and total acidity were determined by pylorus ligation induced ulcer model and ulcer score, ulcer index and percentage protection were determined by Indomethacin induced ulcer model. Phytochemical screening of aqueous and ethanolic extracts were conducted for and it showed that extracts have alkaloids, glycosides, tannins, flavonoids and mucilage. Both the extracts at dose of 200 and 400mg/kg showed significant ($P<0.05$) reduction in gastric volume (1.20±0.17, 0.83±0.20, 0.90±0.12, 0.96±0.17) and ulcer index (1.50±0.19, 1.33±0.11, 2.16±0.03, 0.75±0.17, 0.92±0.22, 0.31±0.04, 0.42±0.06, 0.19±0.04) as compared to the control group (1.47±0.15, 3.00±0.21, 1.64±0.13) in both of the models. It can be concluded that both the extracts possess gastro-protective activity that might be due the presence of flavanoids, tannins and/or mucilage.

Key Words: *Hibiscus rosa-sinensis*, gastro-protective, pylorus ligation, indomethacin, ulcer index

INTRODUCTION

Hibiscus is a large genus that contains herbs, shrubs and trees widely distributed in the tropical and sub-tropical region of world. *Hibiscus rosa-sinensis* Linn. (Malvaceae) is commonly known as jasut in Hindi and China rose in English. It is a native of China and is grown throughout in India as an ornamental plant. Plant can be propagated by cutting from mature wood of current growth. It is an evergreen woody, glabrous, showy shrub of 5-8 ft in height. Leaves are bright green, ovate, coarsely toothed above; flower are solitary, axillary, bell shaped, large 4-6 inch in diameter with pistil and stamens projecting from the centre[1].

Folklorically the flowers are used as demulcent, emollient, refrigerant, aphrodisiac emmenagogue and as anthelmintic[2]. A decoction of flower is used in bronchial catarrh. The dark red petals in the form of mucilaginous infusion are used in ardor-urinae, strangury, cystitis and other irritable conditions of the genito-urinary tract[3]. The flower of *H.rosa-sinensis* have been reported to possess various activities such as analgesic[4], anticonvulsant[5], anti-diabetic[6], antipyretic[7], wound healing[8], antibacterial[9], immunomodulatory[10], anti-estrous[11], antioxidant[12] and hair growth[13].

Peptic ulcer is a serious gastrointestinal disorder which occurs in that part of the gastrointestinal tract which is exposed to gastric acid and pepsin i.e. the stomach and duodenum. It occurs due to the imbalance between the aggressive (acid, pepsin and *H. pylori*) and defensive (gastric mucus, prostaglandins, nitric oxide and bicarbonate secretion) factors[14].

MATERIAL AND METHODS

The flower of *H. rosa-sinensis* Linn were collected from the gardens of Mathura district (27.6056N, 77.5930E), Uttar Pradesh and were authenticated by Birbal Sahni Institute of Palaeobotany, Lucknow, Uttar Pradesh, India and a herbarium specimen was deposited in the Department of Pharmacognosy with Ref. No. 13374.

Preparation of Extracts[27]

The flowers of *H.rosa-sinensis* were collected and dried in shade and coarsely powdered. It was then passed through the sieve no. 20. A weighted quantity (360g) of the powder drug was successively extracted with petroleum ether (60-80^{0}C) using Soxhlet extractor. Defatted drug was subjected to ethanolic extraction and extract was dried by

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distilling off the solvent and then drying in desiccators. The marc collected after ethanolic extraction was subjected to aqueous extraction by maceration process for seven days consecutively. The extract was then dried by evaporating the water and stored for further activity. All extracts were subjected to phytochemical screening to find out the presence of various phytochemical constituents. The polarity of different solvents affect the efficiency of the extraction and each solvent has extracted out different phyto-chemicals.

Animals

Male albino Wistar rats weighing between 150-230 g was used for the experimental work. Institutional Animal Ethics Committee (Ref. no.- GLAIPR/IAEC/2011-12/134) approved the experimental protocol. Animals were maintained under standard conditions in an animal house approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Gastro-protective Activity by Indomethacin-Induced Ulcer Model[15-17]

Male albino rats weighing between 150-230 g were selected and divided into six groups of six animals each.

Group I Control (Indomethacin 20 mg/kg p.o. in 1.0 % w/V CMC)
Group II Standard (Ranitidine 50 mg/kg p.o. in 1.0 % w/V CMC)
Group III HAE 200 mg/kg p.o. in 1.0 % w/V CMC
Group IV HAE 400 mg/kg p.o. in 1.0 % w/V CMC
Group V HEE 200 mg/kg p.o. in 1.0 % w/V CMC
Group VI HEE 400 mg/kg p.o. in 1.0 % w/V CMC

Food was withdrawn for 24 h before the experiment and water was allowed *ad libitum.* Indomethacin 20 mg/kg was administered to produce ulcers. The test drugs were administered orally 30 min prior to indomethacin challenge. After 4 h animals were sacrificed and stomach was removed and opened along the greater curvature and the stomach was excised and inflated for scoring ulcer and ulcer index.

Gastro-protective Activity by Pylorus Ligation-Induced Ulcer Model [18, 19, 20]

Male albino Wistar rats weighing between 150 – 230 g were divided into six groups of six animals each.

Group I Control 1.0 % w/V CMC p.o.
Group II Standard (omeprazole 30 mg/kg p.o. in 1.0 % w/V CMC)
Group III HAE 200 mg/kg p.o. in 1.0 % w/V CMC
Group IV HAE 400 mg/kg p.o. in 1.0 % w/V CMC
Group V HEE 200 mg/kg p.o. in 1.0 % w/V CMC
Group VI HEE 400 mg/kg p.o. in 1.0 % w/V CMC

In this method, male albino rats were fasted in individual cages for 24 h. Care was taken to avoid coprophagy. Aqueous and ethanolic extract of flowers and standard drug were administered 30 min prior to pyloric ligation to the respective groups. The animal was anesthetized with pentobarbitone (45mg/kg b.w.)[21]. Then the abdomen was sutured. At the end of 4 h after ligation the animals were sacrificed and the stomachs were opened and another ligature was placed around the esophagus to prevent the drainage the gastric juice. Gastric juice was collected and drained into test tubes and then centrifuged at 3000 rpm for 30 min. and the volume was noted. The pH of the gastric juice was recorded by digital pH meter. Then the contents were subjected to the analysis of free and total acid strength. The stomachs were then washed with running water to observe for ulcers in the glandular portion of the stomach. The number of ulcers per stomach was noted and severity of the ulcers scored microscopically with the help of hand lens (10x).

The ulcer scoring done as below[22]

Normal stomach..... 0
Red coloration..... 0.5
Spot ulcer..... 1.0
Hemorrhagic streak..... 1.5
Ulcers..... 2.0
Perforation 3.0

Mean ulcer score for each animal are expressed as ulcer index.

Calculation of Ulcer Index[22]

$UI = (UN+US+UP) \times 10^{-1}$

UI = Ulcer index

UN = Average of number of ulcer per animal

US = Average of severity score

UP = Percentage of animal with ulcer

The percentage protection was calculated using the formula[23]

$$\text{Percentage protection} = \left\{1 - \frac{Ut}{Uc}\right\} \times 100$$

where Ut = Ulcer index of treated group

Uc = Ulcer index of control group.

Determination of free acid and total acid strength[24, 25]

One mL of gastric juice was pipetted out into 100 mL conical flask, 2 –3 drops of Topfer's reagent (dimethylaminoazobenzene) added and titrated with 0.01 N sodium hydroxide until all traces of red colour disappeared and the colour of the solution turned to yellowish orange. The volume of the alkali added was noted. This volume corresponds to free acid. Then, 2 – 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge reappeared. Again, the total volume of alkali added was noted. The volume corresponds to total acid.

Strength of acid was calculated by using the formula

$$\text{Strength of Acid} = \left[\frac{\text{Volume of NaOH x Normality of NaOH x 100}}{0.1}\right] \text{mEq/L}$$

Determination of gastric volume and pH[25]

The gastric juice obtained after pyloric ligation was subjected to centrifugation for 1 h at 3000 rpm and volume was measured by pipette. An aliquot of 1 mL gastric juice was diluted with 1 mL of distilled water and pH of the solution was measured by using digital pH meter.

RESULTS AND DISCUSSION

The extracts of flower of *H.rosa-sinensis* were investigated phyto-chemically and pharmacologically. Phytochemical evaluation includes various qualitative tests such as test for alkaloids, glycosides, flavonoids, vitamins, tannins, sterol and mucilage. The results of qualitative phytochemical screening revealed that ethanolic extract and aqueous extract have alkaloids, tannins, glycosides, mucilage, reducing sugar and flavonoids (Table I).

As shown in Fig. 2, the results obtained when ulceration of the gastric mucosa was provoked by pylorus ligation are shown in Table III and IV. Pyloric ligation has caused the accumulation of gastric secretions of 1.47±0.15 mL with pH 2.17±0.50 in a control group. The free acid and total acid of the gastric secretion were found to be 92.33±7.26 and 169.33±17.47 mEq/L, respectively. Pretreatment with the aqueous and ethanolic extract of *H. rosa-sinensis* flower at doses of 200 and 400 mg/kg, significantly ($^{*}P<0.05$) reduced the volume of gastric secretion and increaseed the pH of the gastric juice. In addition, total acid and free acid were also reduced significantly ($^{**}P<0.01$) in dose dependent manner. Further it was observed that ulcer index and ulcer score of extracts treated and standard were also reduced significantly ($^{**}P<0.01$) as compared to control group (3.00±0.21, 18.00±1.28).

As shown in Table II, animals treated with aqueous extract at dose of 200 and 400 mg/kg exhibited respective inhibition percentage of 43.90 and 81.09. The ethanolic extract at the same above doses, significantly inhibited the ulcerogenic effect of indomethacin by 74.39 and 88.41 percentage while ranitidine (50 mg/kg) induced an inhibition of 93.29 percentages. The ulcer score (9.83 ± 0.76) of control was compared to extract (5.5±1.32*, 1.83±0.21**, 2.5±0.37**, 1.17±0.25**) and ranitidine (0.67±0.17**) treated animals and the ulcer index (1.64 ± 0.13) of control was compared to extract (0.92±0.22*, 0.31±0.04**, 0.42±0.06**, 0.19±0.04**) and ranitidine

Table I: Results of Phytochemical Screening of *H. rosa-sinensis* Extracts

Sr. No.	Chemical Constituents	Pet. Ether extract	Ethanolic extract	Aqueous extract
1.	Alkaloids	Negative	Positive	Positive
2.	Sterols	Positive	Negative	Negative
3.	Proteins	Negative	Negative	Negative
4.	Tannins	Negative	Positive	Positive
5.	Amino acids	Negative	Negative	Negative
6.	Glycosides	Negative	Positive	Positive
7.	Mucilage	Negative	Positive	Positive
8.	Flavonoids	Negative	Positive	Positive
9.	Reducing sugar	Negative	Positive	Positive
10.	Saponin	Negative	Negative	Negative

(0.11±0.03**) showed significant (**P<0.01, *P<0.05) reduction in dose dependent manner (Fig. 1).

Peptic ulcers are caused due to increase in gastric acid and/or decrease in gastric mucosal protection mechanisms. Potent anti ulcerogenic and ulcer healing drugs act via decreasing offensive factors or increasing the defensive factors. In this work, we have studied gastroprotective activity of aqueous and ethanolic of *H. rosa-sinensis* flower in two different models including indomethacin and pylorus ligation induced ulcers[15].

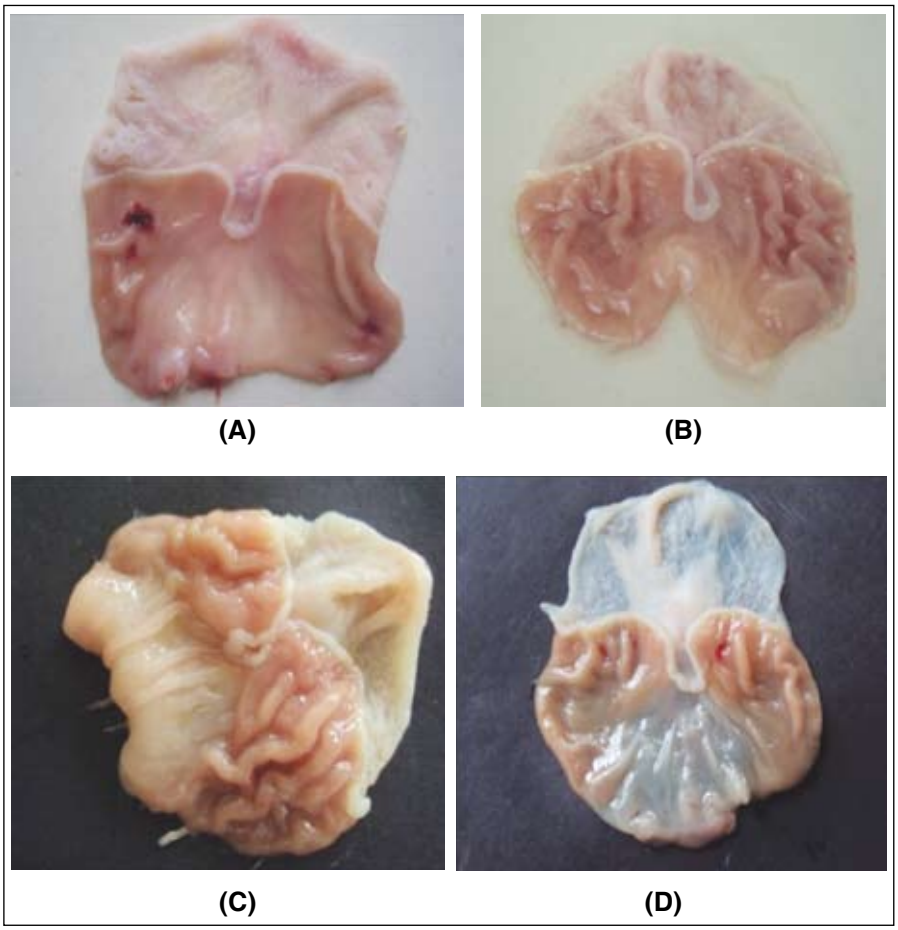

Fig. 1 : Macroscopical view of stomach of indomethacin-induced ulcer model (A) Control (vehicle+indomethacin); (B) Standard (ranitidine+indomethacin); (C) HEE (400mg/kg+indomethacin); (D) HAE (400mg/kg+indomethacin)

Ulcer healing is a genetically programmed repair process, including inflammation, cell proliferation, re-epithelization, formation of granulation tissue, angiogenesis, interactions between various cells and the matrix and tissue remodeling, all resulting in scar formation[14,24]. Various studies and phytochemical analysis showed that flower of *H. rosa-sinensis* is rich in flavonoids and it also has alkaloids. Flavonoids possess anti-oxidant activity and as it is known that peptic ulcer is an inflammatory condition in which the level of various endogenous molecules become depleted. Further it was observed from the preliminary phytochemical screening that flower also possessed mucilage that may form a protective layer over the gastric mucosa that might help in prevention of gastric lesion. It might be possible that pre-treatment with extracts (aqueous and ethanolic) increases the prostaglandins level of gastric mucosa and thus may be helpful in healing of ulcer[26].

Table II: Evaluation of gastro-protective potential of *H.rosa-sinensis* extracts by indomethacin-Induced ulcer model

Sr. No.	Groups	Treatment	Dose (mg/kg)	N Ulcerated	Ulcer Score	Ulcer Index	% Protection
1.	I	Control	1 mL/animal	6/6	9.83±0.76	1.64±0.13	-
2.	II	Ranitidine	50	5/6	0.67±0.17**	0.11±0.03**	93.29
3.	III	HAE	200	6/6	5.50±1.32*	0.92±0.22*	43.90
4.	IV	HAE	400	6/6	1.83±0.21**	0.31±0.04**	81.09
5.	V	HEE	200	6/6	2.50±0.37**	0.42±0.06**	74.39
6.	VI	HEE	400	5/6	1.17±0.25**	0.19±0.04**	88.41

All values are Mean±SEM for 6 rats, Statistical comparison was performed by Graph pad prism software using ANOVA followed by Dunnett's test, **P<0.01, *P<0.05 when all compared with the control group

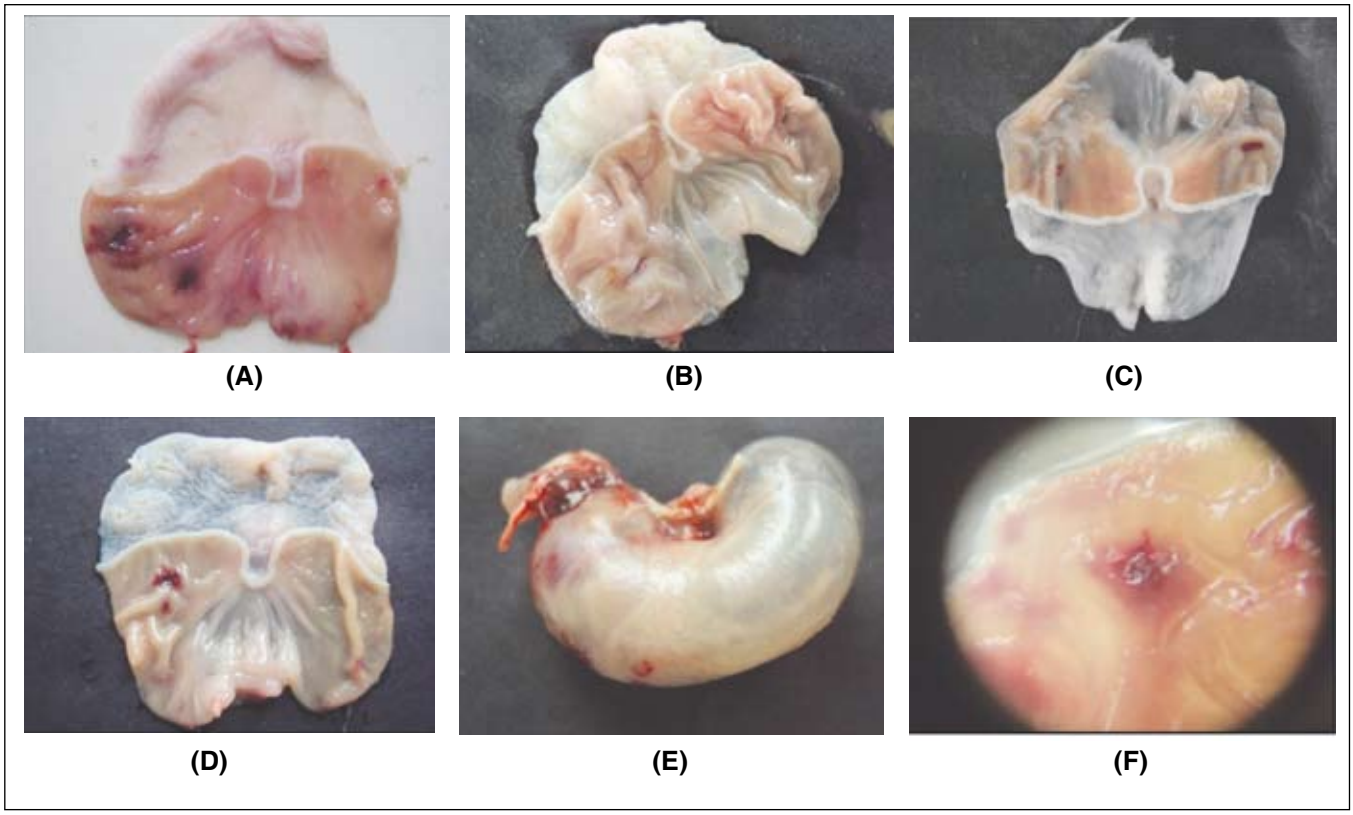

Fig. 2: Macroscopical view of stomach of Pylorus Ligation-Induced ulcer (A) Control (Vehicle+Pylorus ligation); (B) Standard (Omeprazole + Pylorus ligation); (C) HEE (400 mg/kg+Pylorus ligation); (D) HAE (400 mg/kg+ Pylorus ligation); (E) Dissected Stomach (after pylorus ligation); (F) Microscopic view of deep ulcer.

Table III: Evaluation of gastro-protective potential of *Hibiscus rosa-sinensis* extracts by pylorus ligation-Induced ulcer model

Sr. No.	Groups	Treatment	Dose (mg/kg)	pH	Volume (mL)	Free Acid (mEq/L)	Total acid (mEq/L)
1.	I	Control	1mL	2.17±0.50	1.47±0.15	92.33±7.26	169.33±17.47
2.	II	Omeprazole	30	4.41±0.29**	0.48±0.07**	58.00±7.81**	90.33±1.67**
3.	III	HAE	200	2.97±0.49	1.20±0.17	93.00±1.00	128.67±2.19*
4.	IV	HAE	400	3.23±0.26	0.83±0.20	48.33±2.40**	95.00±6.35**
5.	V	HEE	200	3.40±0.29*	0.90±0.12	82.67±4.70	130.33±11.79
6.	VI	HEE	400	3.73±0.17*	0.96±0.17	64.67±7.26*	96.33±9.82**

*All values are Mean±SEM for 6 rats, Statistical comparison was performed by Graph pad prism software using ANOVA followed by Dunnett's test, **P<0.01, *P<0.05 when all compared with the control group.*

In order to probe the effectiveness of *H. rosa-sinensis* extracts in preventing gastric mucosa and also assess their anti-secretory activity, they was tested against pylorus-ligation induced ulcer. Pylorus ligation induced ulcers are a results of auto-digestion of the gastric mucosal barrier, probably due to excess production and accumulation of HCl in the stomach[8, 12]. The aqueous and ethanolic extract of *H.rosa-sinensis* significantly reduced gastric lesion as well as the total acid secretion, while significantly increasing pH. Extracts could significantly reduced the gastric acidity and the volume of gastric acid.

Independently to the experimental ulcer model, there was not any significant difference between the effects of the ethanol and the aqueous extracts from the flower of *H. rosa-sinensis*. Preliminary phytochemical studies of the

Table IV: Evaluation of gastro-protective potential of *H. rosa-sinensis* extracts by Pylorus Ligation-Induced ulcer model

Sr. No.	Groups	Treatment	Dose (mg/kg)	Ulcer Index	Ulcer Score	% Protection
1.	I	Control	1 mL/animal	3.00±0.21	18.00±1.28	-
2.	II	Omeprazole	30	0.61±0.08**	3.67±0.45**	79.67
3.	III	HAE	200	1.50±0.19**	9.00±1.19**	50.00
4.	IV	HAE	400	1.33±0.11**	8.00±0.66**	55.67
5.	V	HEE	200	2.16±0.03	13.00±0.18	28.00
6.	VI	HEE	400	0.75±0.17**	4.50±1.02**	75.00

All values are Mean±SEM for 6 rats, Statistical comparison was performed by Graph pad software using ANOVA followed by Dunnett's test, **P<0.01, when all compared with the control group.

ethanolic and aqueous extract of *H.rosa-sinensis* revealed the presence of flavonoids and mucilage, respectively, which may be responsible for gastro-protective activity by different mechanism.

It can be concluded that aqueous and ethanolic extracts possesses gastroprotective activity which might be due to the presence of flavonoids, tannins and/or mucilage.

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