

ANTI-ULCER ACTIVITY OF METHANOLIC EXTRACT OF *HIBISCUS ROSA SINENSIS* LEAVESSHRUTI SRIVASTAVA^{1*}, JATIN JAISWAL², DR. HEMENDRA GAUTAM³, SURABHI SHARMA⁴, DR. CH. V RAO⁵^{1,2,3,4}Invertis Institute of Pharmacy, Invertis University, Bareilly, ⁵Pharmacognosy and Ethnopharmacology Division, N.B.R.I, Lucknow.
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ABSTRACT

Objective: The antiulcer activity of various extracts of *Hibiscus rosa sinensis* leaves was evaluated in pyloric ligation induced gastric ulcer in albino rats.

Method: The leaves extracts were prepared by cold maceration process with petroleum ether, alcohol and water separately.

Results: Oral administration of aqueous and alcohol extracts (200 and 400 mg/kg) of *Hibiscus rosa sinensis* leaves were evaluated for antiulcer activity and compared with the standard drug, omeprazole (50 mg/kg). From the results, it can be concluded that the aqueous extract of *Hibiscus rosa sinensis* roots (400 mg/kg) showed highly significant ($P < 0.001$) dose-dependent antiulcer activity.

Conclusion: These results lend scientific support for the plant as folk medicine.

Keywords: *Hibiscus rosa sinensis*, Antiulcer activity, Pyloric ligation, Maceration.

INTRODUCTION

Hibiscus rosa sinensis L. (Malvaceae) is an annual or perennial herbaceous bush and has several forms with varying colours of flowers. It is native to China and grown widely as an ornamental plant throughout India. The flowers are considered emollient, and an infusion of the petals is used as a demulcent and refrigerant drink in fevers; [1] its decoction is given in bronchial catarrh [2] in India. Previous studies show that the plant possesses anti-complimentary, anti-diarrhetic and anti-phlogistic [3] activities. The leaves and flowers have been found to be effective in the treatment of heart disorders [4]; used as an anti-spermatogenic and androgenic [5], anti-tumor [6], anticonvulsant, [7] anti-diabetic [8] and antiulcer activities and also as a hair growth promoter [9,10]. The root of *Hibiscus rosa sinensis* is traditionally used for the treatment of ulcer among the Kani tribes in Kanyakumari district, Tamil Nadu, India. No reports are available on the anti-ulcer activity of *Hibiscus rosa sinensis* leaves. Hence, the present study focuses on the scientific investigation of antiulcer activity of *Hibiscus rosa sinensis* leaves.

MATERIALS AND METHODS

Plant Material

The fresh leaves of *Hibiscus rosa sinensis* were collected from Raebareilly district Uttar Pradesh, in the month August 2012. It was further identified and authenticated by Birbal Sahani Institute of Palaeobotany, Lucknow and voucher specimen (13392) was kept at departmental herbarium of Birbal Sahani Institute of Palaeobotany, Lucknow, India. These were dried as quickly as possible in a good air draft or in shade and stored in airtight glass jars until use.

Preparation of extract

The dried, powdered leaves of *H. rosa sinensis* (1000g) were extracted by soxhlet extraction method with methanol for 6 days. The extract was concentrated under vacuum on rotary evaporator and then dried in lyophilizer under reduced pressure and obtained 28.3g. A known volume of the residual extract was suspended in 0.5 % Carboxy methylcellulose (CMC) for animal administration.

Experimental Animals

In bred Wistar rats of either sex obtained from Central Drug Research Institute (Lucknow, India) weighing 200-250g were used for the study. They were kept in the departmental animal house of National Botanical Research Institute at $26 \pm 2^\circ\text{C}$ and relative humidity 45-55%, light and dark cycles of 12 and 12 h, respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (Hindustan Lever) and

water *ad libitum*. All animals were handled according to the guidelines for investigations of experimental pain in conscious animals [11].

Acute toxicity studies

Albino mice weighing 20-25 g selected by random sampling technique were used in the study. Acute oral toxicity was performed 13. The animals were fasted overnight, provided only water after which extract was administered to the groups orally at the dose level of 5mg/kg body weight by gastric intubation and the groups were observed for 14 days. If mortality was observed in 2 or 3 animals (out of 6), then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg / kg body weight. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 h. [12]

Experimental design

Pylorus ligation induced ulcers

Wistar albino rats of either sex weighing 200-220 g were fasted for 36 hrs with water *ad libitum*. In this study the animals were divided into five groups. Group I was considered as control group and received 0.5 ml of vehicle (Sodium CMC, 0.3%), group II was positive control, group III was treated with standard drug omeprazole (80 mg/kg), IV and V were received methanolic extract of *Hibiscus rosa sinensis* at two doses 200 and 400 mg/kg b.w. respectively. The treatments were administered orally. The animals were deprived of food and water during the postoperative period and were sacrificed 6 hrs after pylorus ligation by over dose of ether anaesthesia. The gastric juice was collected and centrifuged at 1000 rpm for 10 minutes. [13,14] The volume of gastric juice (ml) as well as pH of gastric juice was noted. The gastric ulcer score was recorded according to the method described by Aguwa and Ukwe (1997). Gastric content were assayed for total acidity by titration against 0.01N NaOH using phenolphthalein as indicator. The volume of gastric content was measured and the total acidity and free acidity were estimated. The data concerning the pH, acid secretion was analysed by One-Way analysis of variance (ANOVA) showed in Table 1.

RESULTS

In performing preliminary test for pharmacological activity in rats, methanolic did not produce any significant changes in the

behavioural or neurological responses up to 2500 mg/kg b. wt. acute toxicity studies revealed the non-toxic nature of the methanol and chloroform extracts of the leaves of *H. rosa sinensis*. The results of

oral administration of methanolic extracts of *H. rosa sinensis* roots at a dose of 200 and 400 mg/kg b.w. on different biochemical parameters in rats were represented [Table 1].

Table 1: Effect of methanolic extract of *Hibiscus rosa sinensis* leaves on pylorus ligated induced gastric ulcers

Group	Treatment And dose (mg/kg)	Dose mg/kg	Gastric volume (ml/4h)	pH	Free acidity (mEq/l)	Total acidity (mEq/l)	Ulcer score %	Inhibition of ulcer
I	Control (Sodium CMC, 0.3%)	0.5 ml	2.81±0.025	2.87±0.024	226±0.36656	563.5±0.9954	-	-
II	Normal control	-	2.76±0.023	2.08±0.003	223.5±0.673429	550.83±1.30	2.815±0.015498	-
III	Standard (Omeprazole)	50	0.891±0.002	3.76±0.02	127.83±0.30851	482.5±0.5649	0.604167±0.06404	83.05±0.04
IV	MEHRSL	200	2.33±0.010	2.91±0.10	164.5±0.4298	534±0.5795	1.67±0.0066925	26.2±0.2
V	MEHRSH	400	1.55±0.01	3.27±0.02	156±0.366	509.167±2.250	1.0267±0.0084655	64.08±0.4

Values are given as in mean±SEM; (n=6) **P<0.001, *P<0.01 considered for significance (ANOVA followed by Newman-Keuls test).

Figure 1 depicts gastric volume in pylorus ligated induced ulcers in rats. Values are mean ± SEM for 6 rats, *p<0.05, compared to Pylorus ligated control group, **P< 0.001 compared to Pylorus ligated control group. Figure 2 depicts about pH in pylorus ligated induced ulcers in rats. Values are mean ±SEM for 6 rats, ***P< 0.001, compared to pylorus ligation control group. Figure 3 and 4 suggest about free acidity and total acidity in pylorus ligation in rats, values are mean ±SEM for 6 rats, ***P< 0.001, compared to pylorus ligation control group.

Figure 5 suggests about ulcer index in pylorus ligation in rats and values are mean ± SEM for 6 rats, **P< 0.01. Compared to pylorus ligation control, ***P< 0.001, compared to pylorus ligation control. Therefore, the different extracts used for this study showed the tendency to reduce the parameters like volume of acid, free acidity, total acidity and ulcer score. The methanolic extract (400 mg/kg) showed highly significant (p<0.001) reduction whereas the methanolic extract (200 mg/kg) showed significant (p<0.01) reduction in all the parameters when compared with control.

DISCUSSION

Most of the studies demonstrate the importance of natural products in drug discovery. In this study antiulcer activity of methanolic extract of *Hibiscus rosa sinensis* leaves has been studied. The antiulcer activity was evaluated using pylorus ligation model. Most of the studies demonstrate the importance of natural products in drug discovery. The use of phyto-constituents as drug therapy to treat major ailments has proved to be clinically effective and less relatively toxic than the existing drugs. The acute oral toxicity study result showed that the plant leaf is safe.

Peptic ulcer describes a condition in which there is a discontinuity in the entire thickness of the gastric and duodenal mucosa that persists as a result of acid and pepsin in gastric juice.

Pylorus ligation induced model is usually employed to observe the potential of anti-ulcer drugs for their anti-secretory activity by checking the gastric volume and its effect on gastric pH, total acidity and free acidity.

CONCLUSION

The study concludes that the extracts of *H. rosa sinensis* leaves possessed significant antiulcer activity in pylorus ligated rats at the dose of 200 and 400 mg/kg/p.o., which was well compared with omeprazole (50 mg/kg/p.o.). The methanolic extract (400 mg/kg)

showed highly significant (p<0.001) reduction whereas the methanolic extract (200 mg/kg) showed significant (p<0.01) reduction in all the parameters when compared with control.

Thus it has been scientifically proven that these extracts possess enough potential as an anti ulcerogenic agent.

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