



E: ISSN 2278-4136
P: ISSN 2349-8234
JPP 2014; 3 (3): 137-145
Received: 29-07-2014
Accepted: 15-08-2014

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Gastroprotective effect of flower extracts of *Hibiscus rosa sinensis* against acute gastric lesion models in rodents

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Abstract

Hibiscus rosa sinensis L. (malvaceae) flower extracts were selected and evaluated for gastroprotective activity by using Pylorus ligation, aspirin induced and ethanol induced ulceritis in rats. Phytochemical and pharmacological screening of extracts revealed the presence of flavonoids, alkaloids and tannins and significant ($p < 0.05$) gastro protective action in all the gastric ulcer induced models compared to reference standard. Aqueous extract 250 mg/kg offered more percentage of protection, 84.17%, 77.12% and 76.8% in pylorus ligation, aspirin induced and ethanol induced ulcer models, respectively. The protective effect observed is attributed to its effect on mucus production and free radical scavenging effect of flavonoids and tannins present in crude extract. Our study report the gastroprotective activity of flower extracts of *Hibiscus rosa sinensis* against experimentally induced gastric erosions in rats.

Keywords: Gastroprotective, Flavonoids, *Hibiscus rosa sinensis*, Lansoprazole, Sucralfate

1. Introduction

Peptic ulcer disease encircles gastric and duodenal ulcers are one of the major ailments affecting about 60% human adults and nearly 80% child population in tropical countries [1]. Peptic ulcer is one of the common problems in gastrointestinal tract. This is due to the imbalance between the protective factors and aggressive factors, which are affected by factors, include gastric mucosal layer thickness, bicarbonate, mucus secretion, mucosal blood flow, cell regeneration, prostaglandins and acid – pepsin secretion. Smoking, alcohol, corticosteroids, administration of NSAIDs, stress and *Helicobacter pylori* are the major contributing factors; develop the incidence of peptic ulcers in the world [2]. Amongst the most prevalent causes are NSAIDs, alcohol and reactive oxygen metabolites [3-7].

Currently the drugs used in the treatment of peptic ulcers which include H₂ receptor inhibitors, proton pump inhibitors, mucoprotectives, antacids and anticholinergics. They offer only symptomatic relief with menacing adverse effects [8]. For this reason, the development of natural medicines with antiulcerogenic properties is still a thrust area to researchers.

Hibiscus rosa sinensis L. (Malvaceae) is an ornamental shrub in tropical regions, with varying colors of flowers. The plant has been evaluated for various diseases like tumors, diarrhea, inflammation heart diseases [9], anti-diabetic, hypolipidemic and post-coital antifertility activity [10]. *Hibiscus rosa sinensis* flowers consists of cyanidin, quercetin, calcium oxalate, thiamine, riboflavin, niacin and ascorbic acid as chemical constituents [11-13]. *Hibiscus rosa sinensis* flower extracts showed Cardio protective activity [14], Anti-Fertility Activity [15] The present study was designed to investigate the effect of the aqueous and ethanolic flower extracts of *Hibiscus rosa sinensis* in ulcer induced models.

2. Materials and Methods

2.1 Plant Material and extraction

The fresh flowers of *Hibiscus rosa sinensis* were collected from the garden of the AU College of pharmaceutical sciences, Visakhapatnam. The authenticated drug *Hibiscus rosa sinensis* was shade dried and powdered coarsely. The coarse powder of the *Hibiscus rosa sinensis* was extracted with the solvents ethanol [64.5-65.5 °C] and distilled water. The extracts were concentrated to a residue.

2.1.1 Phytochemical screening

Preliminary Phytochemical screenings were conducted according to Gupta *et al* [16]. Phytochemical analysis was performed for the identification of flavonoid, alkaloids, glycosides and Tannins.

2.1.2 Acute toxicity studies

Acute toxicity study conducted as per OECD guidelines 420 using albino Swiss mice. The extracts were found to be safe up to 2000 mg/kg body weight. According to LD50 dose, 1/8 and 1/4 dose i.e. 250 mg/kg and 500 mg/kg were selected for vivo studies [17].

2.2 Experimental procedure

2.2.1 Animals

Albino Wistar rats of either sex weighing between 150 to 200 gm. The animals were housed polypropylene cages and maintained at (20-25) °C and relative humidity [30-70%] with a 12/12 light/dark cycle. They were fed with standard pellet diet and have water *ad libitum*. The pharmacological and acute toxicity protocols were approved by the institutional animal ethical committee (Regd. No. 516/01/A/CPCSEA) The wistar albino rats weighing 150-180g were divided into six groups (n=6). The groups were treated according to the protocol. These groups were as follows: Group-I- Control treated with 2% gum acacia, Group-II – Standard received lansoprazole 8 mg/kg in 2% gum acacia, Group-III & IV are treated with ethanolic extract of *Hibiscus rosa sinensis* 250 & 500mg/kg and Group-V& VI are treated with aqueous extract of *Hibiscus rosa sinensis* 250 & 500 mg/kg, respectively.

2.2.2. Pylorus ligation induced model

In the pyloric ligation induced gastric ulcer model rats were fasted in individual cages for 24 hrs, care was taken to avoid coprophagy. Pylorus ligation was made 1 h after the treatment schedule and after 4hrs of ligation the animals were sacrificed and the stomach was removed and gastric juice volume, pH, ulcer index & % protection were determined and compared with control and standard [18].

% protection = $[(C-T)/C] \times 100$.

C=Ulcer index in control, T= Ulcer index in Test

2.2.3. Aspirin Induced Ulcers

After one hour of the administration of the extract/Lansoprazole, all the groups received Aspirin at dose of 200 mg/Kg once daily for five days. On day 5, Four hours after the administration of Aspirin, the animals were sacrificed and the stomach was excised, opened along the greater curvature and ulcer index & % protection were identified [18, 19].

2.2.4 Ethanol Induced Ulcers

The study medications were administered 1 hour prior administration of Absolute alcohol at the dose of 1 ml/100 g. The rats were sacrificed 1hr after the administration of ethanol. The abdomen was dissected to retrieve the stomach and analyzed for ulcer index & % protection [19].

2. 3 Statistical analysis

Results were analyzed by using one-way ANOVA followed by post hoc Tukey's test using Graph pad Prism-5 software. The results were expressed as Mean \pm SEM. P<0.001 was considered as significant.

3. Results

3.1 Preparation of extract and properties

Successive Soxhlet extract process as yielded 3.8% dark brown colored ethanolic extract, 4.7 % dark brown colored aqueous extract.

Table 1: Successive Soxhlet extraction of *Hibiscus rosa sinensis* flowers

S. No	Solvent	Colour & Consistency	%Yield
1	Ethanol	Dark brown and sticky	4.8%
2	Water	Dark brown and powder	6%

3.1.1 Preliminary phytochemical screening

Flavonoids, tannins and alkaloids are present in ethanolic and aqueous extract [Table 2].

Table 2: Preliminary phytochemical screening

S. No	Type of phyto chemical constituents	Ethanolic Extract	Aqueous Extract
1	Carbohydrates	+++	+++
2	Proteins	—	+
3	Flavonoids	+++	+++
4	Steroids	+	+
5	Tannins and Phenolic compounds	—	—
6	Saponin glycosides	+	+
7	Cardiac glycosides	—	—
8	Starch	—	++
9	Alkaloids	++	++

Note: - Absent, + Indicates presence, ++ More clarity, +++ Better response

3.2. Pylorus Ligation Ulcer Model, Aspirin induced ulcer model and Ethanol Induced Ulcers

Extracts at 250 & 500 mg/kg p.o significantly (P<0.001) decreased and increased the gastric P^H & volume, respectively when compared to control & reference standard (Table 3 & figure 1). Both the extracts showed tendency to reduce the ulcer index and increase the % protection in all the induced models. Maximum % protection was observed by aqueous extract at 500 mg/kg i.e. 81.85% (pylorus ligation model), 73.7% (Aspirin induced model), 76.8% (Ethanol induced model). Significant gastro protective activity was identified with both the extracts at 500 mg/kg p.o. (Table 4&5 and Figure 2, 3)

Table 3: Effect of Aqueous and Ethanolic extracts of *Hibiscus rosa sinensis* flowers on pH, volume of gastric secretion by pylorus ligation induced ulcer in rats

Groups	Volume of gastric juice	pH
Control (2% acacia)	6.35 ± 0.14	1.3±0.10
Lansoprazole (8 mg/kg)	1.03±0.10 ^a	5.73±0.04
Eth. extract (250 mg/kg)	6.5±0.10 ^b	1.71±0.04 ^{a,b}
Eth. extract (500 mg/kg)	4.45±0.11 ^{a,b}	1.98±0.04 ^{a,b}
Aq. extract (250 mg/kg)	6.26±0.11 ^b	1.11±0.10 ^b
Aq. extract (500 mg/kg)	5.03±0.11 ^{a,b}	1.23±0.08 ^b

Data expressed as Mean± S.E.M, $P<0.001$, a indicates significant difference when compared with control, b indicates significant difference when compared with lansoprazole.

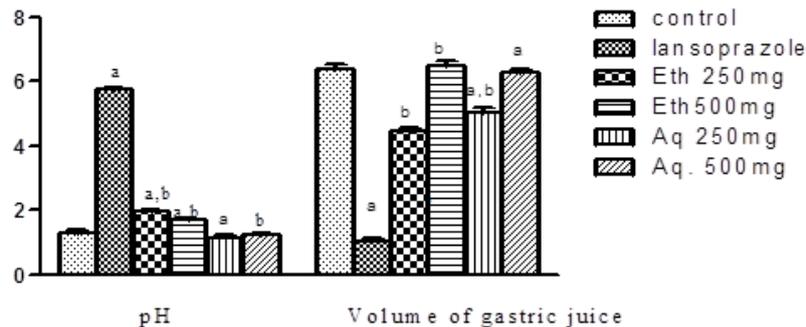


Fig 1: Effect of *Hibiscus rosa sinensis* on pH & Gastric juice volume in pylorus ligation model. Data are expressed as Mean ± S.E.M (n=6), $P<0.001$. a, indicates significant difference when compared with control, b, indicates significant difference when compared with lansoprazole.

Table 4: Effect of Aqueous and Ethanolic extracts of *Hibiscus rosa sinensis* flowers on ulcer index in pylorus ligation, Aspirin induced and Ethanol induced ulcer in rats

Groups	Ulcer index		
	Pylorus ligation model	Aspirin Induced Model	Ethanol induced model
Control (2% acacia)	7.33±0.31	5.83±0.24	6.08±0.15
Lansoprazole (8 mg/kg)	1.1±0.21 ^a	1.16±0.21 ^a	1.33±0.16 ^a
Eth. Extract (250 mg/kg)	2.16±0.45 ^a	2.58±0.39 ^a	1.66±0.27 ^a
Eth. Extract (500 mg/kg)	1.5±0.46 ^a	1.91±0.61 ^a	1.58±0.30 ^a
Aq. extract (250 mg/kg)	2.41±0.51 ^a	2.33±0.33 ^a	1.91±0.2 ^a
Aq. extract (500 mg/kg)	1.33±0.31 ^{a,b}	1.53±0.45 ^a	1.41±0.2

Data are expressed as Mean±S.E.M (n=6), $P<0.001$. a, indicates significant difference when compared with control, b, indicates significant difference when compared with lansoprazole

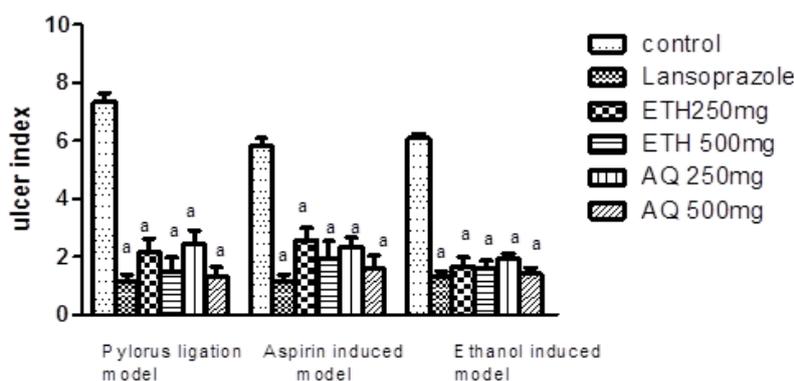


Fig 2: Effect of Aqueous and Ethanolic extracts of *Hibiscus rosa sinensis* flowers on ulcer index in pylorus ligation, Aspirin induced and Ethanol induced ulcer in rats. Data are expressed as Mean ± S.E.M (n=6), $P<0.001$. a, indicates significant difference when compared with control, b, indicates significant difference when compared with lansoprazole

Table 5: Effect of Aqueous and Ethanolic extracts of *Hibiscus rosa sinensis* flowers on % protection in pylorus ligation, Aspirin induced and Ethanol induced ulcer in rats ethanol induced ulcer in rats

Groups	% Protection		
	Pylorus ligation model	Aspirin Induced Model	Ethanol induced model
Lansoprazole (8 mg/kg)	84.99%	80.10%	78.12%
Eth.Extract (250 mg/kg)	70.53%	55.7%	72.6%
Eth.Extract (500 mg/kg)	77.54%	67.2%	74%
Aq.extract (250 mg/kg)	67.12%	60.03%	68.4%
Aq.extract (500 mg/kg)	81.85%	73.7%	76.8%

Data are expressed as Mean \pm S.E.M (n=6), $P < 0.001$. a, indicates significant difference when compared with control, b, indicates significant difference when compared with lansoprazole

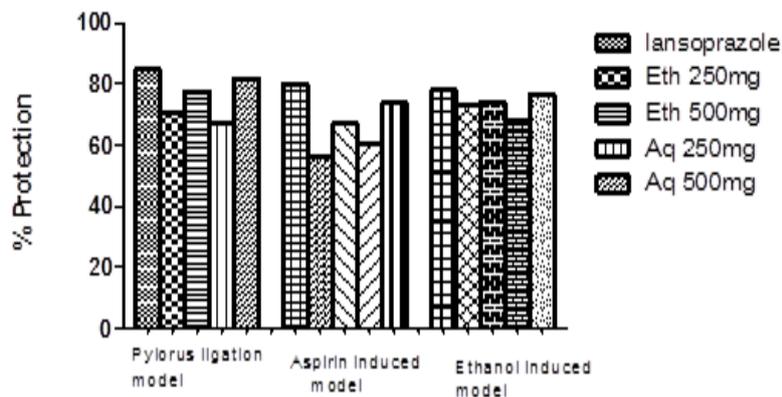


Fig 3: Effect of Aqueous and Ethanolic extracts of *Hibiscus rosa sinensis* flowers on % protection in pylorus ligation, Aspirin induced and Ethanol induced ulcer in rats. Data are expressed as Mean \pm S.E.M (n=6), $P < 0.001$. a indicates significant difference when compared with control, b, indicates significant difference when compared with lansoprazole

4. Discussion

Peptic ulcer presents a major global health problem in terms of both morbidity and mortality because the treatment regimens offer symptomatic relief with toxicity [20]. However, the use of herbal medicine and plant secondary metabolites gain importance worldwide for the augmentation of different ailments. Literature reports on medicinal plants revealed the gastroprotective effect in different ulcer induced models in rodents [21]. They showed promising antiulcer activity, when compared to conventional agents like H_2 -blockers, proton pump inhibitors and cytoprotective agents [22].

Ligation of pyloric antrum causes auto digestion and breakdown of defensive mucosal layer. This is due to the accumulation of acid, protein digestive enzyme and reactive oxygen species [23], whereas in Aspirin induced model the damage is attributed to the inhibition of cyclooxygenase enzyme, which is a constitutive enzyme that protects the gastric epithelial cells from acid and pepsin by secreting mucus and bicarbonate but the inhibition of this enzyme makes gastric wall more susceptible towards aggressive factors [24]. In absolute ethanol induced gastritis, it is corrosive and releases histamine and leukotriene C4, which is detrimental to gastric mucosal layer and produces destruction of mucosa and submucosa of gastric layer [25].

Hibiscus rosa sinensis has been reported the presence of alkaloids, glycosides, flavonoids and tannins. Many chemical components were isolated from this plant viz. cyanidin, quercetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin and ascorbic acids [26-28]. Flavonoids and tannins, in addition to antioxidant activity, are also known to produce anti-inflammatory activity and mucus secretion. *Hibiscus rosa sinensis* flower extracts doesn't showed significant effect on the volume and pH of the gastric juice when compared with the control & lansoprazole in pylorus ligation model but they showed significant change in ulcer index and % protection in all the induced models which is comparable to standard lansoprazole and sucralfate. The above results demonstrated that extracts showed potent gastroprotective effect by without affecting pH and gastric volume. This indicates that extract showed protection by increasing mucus secretion. Both the extracts showed maximum protection (Group IV, VI) at 500 mg/kg p.o dose. Among all the groups aqueous extract at 500 mg/kg showed maximum % protection (Group VI). As per the phytochemical analysis revealed that the extracts contain flavonoids and tannins, may be this activity was because of flavonoids and tannins. As flavonoids (Quercetin) and tannins are reported as a good gastroprotective and antioxidants [29].

CONTROL (Saline treated Group)



Note: Severe injuries are seen in the gastric mucosa

Standard (Lansoprazole treated Group)



Note: No ulcerations are seen in lansoprazole



Aqueous extract 250 mg/kg



Aqueous extract 500 mg/kg



Ethanolic extract 250 mg/kg



Ethanolic extract 500 mg/kg

Fig 4: Gross appearance of gastric mucosa in pylorus ligation induced model

CONTROL (Saline treated Group)



Note: Severe injuries are seen in the gastric mucosa



Standard (Lansoprazole treated Group)



Note: No ulcerations are seen in lansoprazole



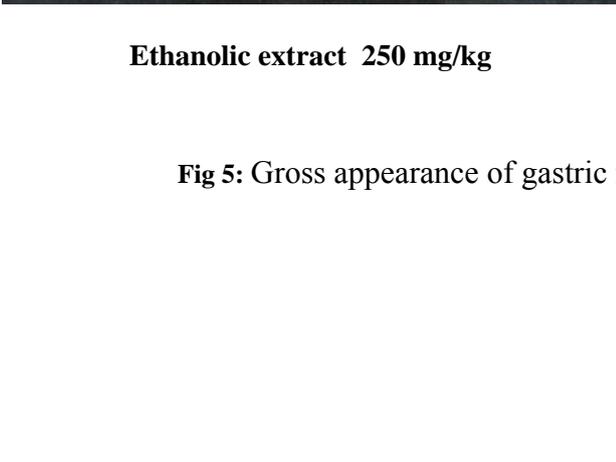
Aqueous Extract 250 mg /kg



Aqueous extract 500 mg /kg



Ethanollic extract 250 mg/kg



Ethanollic extract 500 mg/kg

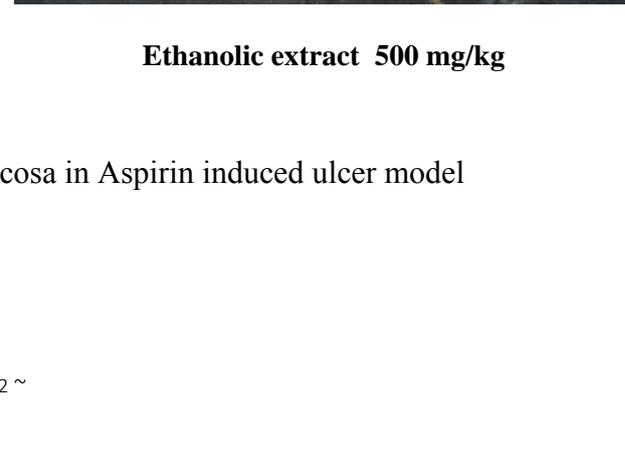


Fig 5: Gross appearance of gastric mucosa in Aspirin induced ulcer model

CONTROL (Saline treated Group)



Note: Severe injuries are seen in the gastric mucosa

Standard (Sucralfate treated Group)



Note: No ulcerations are seen in lansoprazole



Aqueous extract 250 mg/kg



Aqueous extract 500 mg/kg



Ethanolic extract 250 mg/kg



Ethanolic extract 500 mg/kg

Fig 6: Gross appearance of gastric mucosa in ethanol induced ulcer damage

5. Conclusion

It could be concluded that *Hibiscus rosa sinensis* has both gastroprotective and ulcer-healing properties by increasing gastric mucosa, prostaglandin synthesis and antioxidant activities.

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