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Phytochemical and anti-ulcer investigations of 95% ethanolic-benzene-chloroform leaf extract of *Hibiscus tiliaceus* Linn. in albino rat model

Mukesh Chandra Sharma*, Smita Sharma[a], Dharm Veer Kohli[b]

School of Pharmacy, Devi Ahilya Vishwavidyalaya, Khandwa Road, Indore (M.P), India
[a]Department of Chemistry, Yadhunath Mahavidyalya Bhind (M.P), India
[b]Department of Pharmaceutical Sciences, Dr. Hari S. Gour University, Sagar (M.P), India

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Abstract

The present study was undertaken to evaluate the fresh leaf extract of *Hibiscus tiliaceus* Linn. (Malvaceae) for its anti-ulcer activity in pyloric ligation (PL induced gastric ulcer models in Albino rats. Methanol, petroleum ether and chloroform extract of *Hibiscus tiliaceus* Linn. (Malvaceae) was explored for antiulcer and gastroprotective activity using acute gastric lesion induced by cold restraint stress and pylorus ligation in rats. The anti-ulcer activity of extract of the leaves *Hibiscus tiliaceus* was evaluated by using models of acute gastric lesions induced by cold restrain stress and pylorus ligation in rats. Animals pretreated with doses of 150 mg/kg and 250 mg/kg of Methanol, petroleum ether and chloroform extract showed significant reduction in lesion index, total affected area and percentage of lesion in comparison with control group in the cold restraint stress-induced ulcer and pylorus ligation models.

Key words: *Hibiscus tiliaceus* , Formulation,Phytochemical, Pylorus ligation

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Introduction

Hibiscus (Malvaceae) is a genus of herbs, shrubs, and trees; its 250 species are widely distributed in tropical and subtropical regions of the world. About 40 species occur in India. Many species belonging to this genus have been used since ancient times as folk remedies for various disorders [1]. *Hibiscus tiliaceus* L. (Malvaceae), commonly known as “bola” is a mangrove plant growing in tropical Asia and abundant in littoral forests and mangrove forest margins of atolls and high islands [2]. In folk medicine, the leaves of this plant used to treat fevers, soothe coughs, ulcer, wounds and various skin diseases[3]. The

various phytochemical isolated from plant are hibiscusin, hibiscus amide, vanillic acid, P-hydroxybenzoic acid, syringic acid, P-hydroxybenzaldehyde, scopoletin, N-tras-feruloyltyramine, N-cis-feruloyltyramine, β-sitosterol, stigmasterol, β-stigmasteronone, hibiscolactone, hibiscones, hibiscoqinones, lapachol, gossypol, gossypetin, manosononones, hyperoside, kaempferol, quercetin, gossypitin, gossytrine, para-coumaric and fumaric acid [4,5]. Since plant is used traditionally in treatment of painful illnesses like ulcer and wound, it became worthwhile to evaluate its anti-inflammatory and antinociceptive activities.Gastric hyperacidity and gastro duodenal ulcer is a very common global problem today. It is now generally agreed that gastric lesions develop when the delicate balance between some gastro protective and aggressive factors are lost. Major aggressive factors are acid, pepsin, *Helicobacter pylori* and bile salts. Defensive factors mainly involve mucus bicarbonate secretion and prostaglandins. Hypersecretion of gastric acid is a pathological condition, which occurs due to uncontrolled secretion of hydrochloric acid from the parietal cells of the gastric mucosa through the proton pumping H+K+ATPase. Even the normal rate of acid secretion may cause ulceration in the breached mucosa when some gastro protective factors are lost. The modern approach to control gastric ulceration is to inhibit gastric acid secretion, to promote gastroprotection, block apoptosis and stimulate epithelial cell proliferation for effective healing. Most of the antisecretory drugs such as proton pump inhibitors (omeprozole lansoprazole, etc.) and histamine H2-receptor blocker (ranitidine, famotidine, etc.) are extensively used to control increased acid secretion and acid related disorders caused by stress, NSAID's and *H. pylori*; but there are reports of adverse effects and relapse in the long run. On the contrary most of the herbal drugs reduces the offensive factors and are proved to be safe clinically effective, having better patient tolerance, relatively less expensive and globally competitive. Plant extracts, however, are some of the most attractive sources of new drugs and have been shown to produce promising results in the treatment of gastric ulcers [6].From the source of literature documentation and relevant traditional approaches on plant drugs. The present investigation was carried out to investigate the chemical and therapeutically potential by evaluating phytochemical and anti-ulcer profile of the fresh leaf extract of *Hibiscus tiliaceus* L. (Malvaceae) is being reported here.

Materials and Methods

Plant material

The fresh leaves of *Hibiscus tiliaceus L. (Malvaceae)* were collected from Bhind, Madhya Pradesh India.

Preparation of Extract

The fresh leaf part of *Hibiscus tiliaceus* Linn. about 1000 gms were homogenized successively and extracted with the with the Methanol, petroleum ether and 95% Absolute Ethanolic-Benzene-Chloroform Extraction as a solvent for 72 hours room temperature. The whole extract was collected 5 liter conical flask and filtered. Then it is concentrated to a dry mass by using vacuum distillation. A greenish black waxy residue was obtained. The percentage yield of prepared extract was around 5.0 %w/w.

Drugs and Chemicals

Aqueous solution of *Hibiscus tiliaceus* was prepared in distilled water and was administered orally. Omeprozole (OMZ) was procured from Cadila Pharmaceuticals Ltd, Gujarat India. Eighteen healthy albino rats were equally divided into three groups containing 6 rats each and treated as follows: Group -I: Control Vehicle (5% w/v Acacia, 5 ml / kg-1),Group -II: Omeprazole (reference drug) (20 mg kg-1) ,Group -III: Methanol extract of leaves of *Hibiscus tiliaceus* Linn. dissolved in 5% w/v Acacia (150 mg kg-1)

Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out on the petroleum ether, chloroform and ethanolic extracts of *Hibiscus tiliaceus* for qualitative identification [6, 7].

Phytochemical Screening

For the phytochemical examination of methanol extract of leaves of *Hibiscus tiliaceus* Linn. was performed by the standard methods [8-10].

Animals

Albino rats of either sex weighing about 150-250gm provided by the Animal Housing, polyethylene-walled cages in groups of five, with food and water. The animals were kept on a 12 h light 12 h dark regime at 23^0C prior to the experiments.

Experimental design

Gastric ulcer induced by pylorus ligation.

Albino rats were housed in individual cages and fasted (water allowed) for 72 hours prior to pyloric ligation, care being taken to avoid coprophagy. Under light chloroform anaesthesia the abdomen is opened by a small midline incision below the xiphoid process; pyloric portion of the stomach is slightly lifted out, and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach is replaced carefully, and the abdominal wall closed by interrupted sutures. The drugs are administered orally two hours prior to pyloric ligation. They are deprived of both food and water during the postoperative period, and are sacrificed at the end of six hours after operation. Stomach is dissected out and the contents are drained into a tube and this is subjected to gastric juice was collected for performing gastric secretion study and analysis for pH and for free and total acidity. The stomach is then cut open along the greater curvature and the inner surface is examined for any ulceration. The mean ulcer size was calculated by dividing the total length (in mm) of ulcers for all the animals divided by total number of animals [11].

Gastric secretion study.

The volume of gastric juice obtained by pyloric ligation was expressed in terms of ml/150 gms of body weight. Total acidity, free acidity and dissolved mucous substances of gastric juice were measured.

Effect of leaves on acid secretion studies.
The effect of Petroleum ether extract of leaves of *Hibiscus tiliaceus* L on different offensive factors like free and total acidity, which play a crucial role in the pathogenesis of gastric ulcers, was studied by collecting gastric juice from stomachs in PL model. As shown in Table-2, treatment with MEIC at a dose 100 and 200 mg kg-1 has significantly reduced the free and total acidity ($P < 0.01$ and $P < 0.001$) respectively when compared to Omeprozole, the reference drug.

Total Acidity
A volume of 2 ml. Diluted gastric juice was titrated with 0.1 M sodium hydroxide run fro a micro burette using phenolphthalein as indicator and the acidity was expressed as mg. HC1/150 gm body weight of rat.

Free Acidity
It is determined in similar manner using topfer's reagent as indicator and sodium hydroxide was run until canary yellow colour was observed.

Statistical analysis
The results were expressed as mean ± S.D. The data were also analyzed by ANOVA (one-way analysis of variance) using SPSS package. The statistical analysis was performed using Dunnett's T3 multiple comparison test for all parameters. The values were considered significant at the levels of $P < 0.09$, $P < 0.05$ and $P < 0.005$.

Results and Discussion

Phytochemical investigation the results of preliminary phytochemical investigation the presence of Steroids, Triterpenoids, Flavonoids, Glycosides, Reducing Sugar, Tannins and Saponins (Table1).The showed significant anti-ulcer effect against ulcers induced in both the models in a dose dependent manner. In PL induced ulcer model, at a dose of 150 and 250 mg kg-1 showed protection effect of 40.25% and 59.75%, respectively, where as Omeprazole showed protection index of 86.54 % at a dose of 35 mg kg-1 (Table 2).Pyloric ligation induced ulcers caused due to imbalance between offensive and defensive mucosal factors [12] are ideal model to infer the mechanism by which a drug works as an anti-ulcerogenic agent. PL-induced gastric ulcers occur because of an increase in acid-pepsin accumulation due to pyloric obstruction and subsequent mucosal digestion. A copious amount of mucus is secreted during superficial damage and provides favorable microenvironment in repair. Hence estimation of acid secretion and mucus secretion is a valuable part of the study to clarify the mechanism of action of the drug under trial. In the present study, we found that at dose of 200 mg/kg body weight significantly showed protection effect of 32.64 % and 69.06%, respectively, which are comparable to standard drug Omeprazole (79.65%). Omeprazole antagonized Pentagastrin, histamine and carbachol induced hyper acidity in gastric rats**.** It protects experimental animals from gastric ulceration induced by stress, pyloric ligation.

Table 1.Phytochemical group test for the methanol Extract of leaves of *Hibiscus tiliaceus*

S. No.	Phytoconstituents	Methanol Extract of leaves of *Hibiscus tiliaceus*
1	Alkaloid	-
2	Amino acid	-
3	Flavonoids	+
4	Glycosides	+
5	Tannins	+
6	Saponins	+
7	Gums	-
8	Triterpenoids	+
9	Steroids	-

Table 2: Effect of Methanolic extract of *Hibiscus tiliaceus* on ulcer index score against induced gastric ulcer in rats

Group	Treatment	Dose mg/ kg p.o.	Ulcer index
I	Control	5 ml/ 150ml	25.24 ± 0.43
II	Hibiscus tiliaceus	250	5.51 ± 0.01
III	Omeprazole (Standard)	80	7.2 ± 0.22

All values are mean ± S.E.M. (n = 6) per group. Statistical comparison was performed by using ANOVA followed by Dunnett t-test., p <0.01 were consider statistically significant when compared to control group.

Table 3: Gastroprotective activity of extract of *Hibiscus tiliaceus* on pylorus ligated ulcer

Treatment	Dose	Mean ulcer index	% protection	Gastric Juice ml	pH of gastric juice	Free acidity (µequiv./ml)	Total acidity (µequiv./ml)
Vehicle	5ml/150g	52.12±1.65	--	12.1± 0.32	2.4 ± 0.01	3.43 ± 0.02	23.11 ± 0.01
Omeprazole	150 mg/kg	22.04±0.51*	64.32 %	3.21 ± 0.02	2.1 ± 0.12	1.4 ± 0.01	11.07 ± 0.02
extract of *Hibiscus tiliaceus*	100 mg/kg	11.33 ± 0.16*	76.11 %	5.87 ± 0.08	6.8 ± 0.06	8.3 ± 0.33	9.33 ± 0.21

Results are expressed as Mean ± SEM (n = 6). Significant at *p<0.05 compared to control group.

Conclusion

Ulcers are caused due to imbalance between aggressive and defensive factors of the gastric mucosa. Pepsin and gastric acid make up the offensive factors, whose proteolytic effect is buffered by mucin secretion, mucosal glycoprotein, cell shedding, cell proliferation and prostaglandins[13]. Different therapeutic agents including plant extracts are used to inhibit the gastric acid secretion, or to stimulate the mucosal defense mechanism by increasing the mucus production protecting the surface epithelial cells or interfering with the PG synthesis[14].

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