Anti-ulcer activity of methanolic extract of *Hibiscus cannabinus* (Leaves) in wistar strain rats

Silambujanaki P*, Chitra V, Hari DurgaPrasad K, Raju D, Sankari M, Soni D

Department of Pharmacology, SRM College of Pharmacy, SRM University, Kattankulathur, Tamil Nadu, India

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Abstract

The anti-ulcer activity of methanolic extract of leaves of Hibiscus cannabinus (Malvaceae) MEHC was investigated by Pylorus ligation and Indomethacin (8mg/kg;p.o) induced ulcer models in wistar rats. Ulcer index was used as the common parameter to determine the activity. MEHC at doses of 1.6 & 3.2g/kg p.o produced significant inhibition of the gastric lesions produced by Pylorus ligation & indomethacin. It also significantly (P<0.05) reduces gastric volume, free acidity and ulcer index as compared to control. The data obtained suggests that MEHC possess potent anti-ulcer activity ie.,antiulcerogenic and ulcer healing properties, which might be due to its antisecretory activity. The presence of flavanoids in the extract supports its activity. Hibiscus cannabinus (Malvaceae) commonly known as Mesta is cultivated all over India. In ayurvedic medicine, leaves are used in the treatment of dysentery and bilious, blood and throat disorders. The seed possess pain reducing effect when applied externally. It effectively delayed the onset of cataract formation.

Keywords: *Hibiscus cannabinus,* Pylorus ligation, Indomethacin induced ulcer model, ulcer index.

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INTRODUCTION

Gastric ulcer, one of the most widespread, is believed to be due to an imbalance between aggressive and protective factors[1]. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acid, food ingredients, bacterial products (*Helicobacter pylori*) and drugs[2]. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility[3]. Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor 'PAF', leukotrienes, endothelins, bile or exogenous factors

223

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including NSAIDs) or stimulating the mucosal defences (mucus, bicarbonate, normal blood flow, prostaglandins(PG), nitric oxide)[4]. The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently there is no cost-effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product sources. In this work, the anti-ulcer activity of the leaves of *Hibiscus cannabinus* was evaluated in wistar rats by pylorus ligation and indomethacin induced ulcer model.

Hibiscus cannabinus(Family:Malvaceae) is popular in the western world as kenaf. It is known by various names in India such as Bimli, Deccan hemp, Gogu, Channa, Ambadi Gongura, Sunkura and Sunbeeja. It is an ancient crop, domesticated in Western and Southern Africa and Western Asia. It is fast growing, woody to herbaceous plant reaching 4-5m in height. It has a deep-penetrating taproot with deep-seated laterals. The leaves are alternate, long petiole, with 3-7 toothed lobes[5,6]. In ayurveda, the leaves are used for dysentery, diseases of blood, bile and throat. The seeds are used for its analgesic, aphrodiasic, appetizer, carminative, diuretic, demulscent, antispasmodic and tonic property. The flowers are used for biliousness and constipation[7,8]. *Hibiscus cannabinus* leaf contains Rectin and isoquercitin. The flowers contain Hibiscatin, Gossipin, Gossipitrin, Glycoside cannabiscitrin, Aglycone cannabiscitin[9]. The reported pharmacological activites are Antioxidant, Anticancer, Hypoglycemic, Anti-arthritic and Diuretic activity.

RESULTS AND DISCUSSION

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanisms. To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucosal production, stabilising the surface epithelial cells or interfering with the prostaglandin synthesis[15].

Table I: Effect of MEHC on various parameters in Pyloric ligation induced Gastric ulcer model

| Group	Treatment	Ulcer index	Protection (%)	P^H of gastric juice	Gastric juice (ml)	Free acidity meq/ltr	Total acidity meq/ltr
I	Control (Pyloric ligation)	15.8±1.4	-----	2.4±.20	9.4±.20	97.8±1.4	117.8±.24
II	Omeprazole (20 mg/kg)	2.4±.05*	84 %	4.9±.15*	2.4±.18*	32.8±2.4*	57.8±1.4*
III	MEHC (1.6g/kg)	3.7±.05	76 %	3.6±.20	4.4±.12	47.8±1.4	67.8±.38
IV	MEHC (3.2g/kg)	2.7±.06*	82 %	4.5±.18*	3.9±.15*	37.8±1.4*	62.8±1.4*

In pyloric ligation induced ulcer model, oral administration of MEHC showed significant reduction in ulcer index, gastric volume, free acidity, total acidity as compared to the control group. The extract also showed protection index of 76 % and 82 % at the dose of 1.6 and 3.2 g/kg respectively in comparison to control whereas omeprazole showed 84% protection index (Results are tabulated in Table-I). Histopathological changes on pylorus ligation model were shown in figure 1. The causes of gastric ulcer by pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid. According to Shay et

224

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al., the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid[16,11].
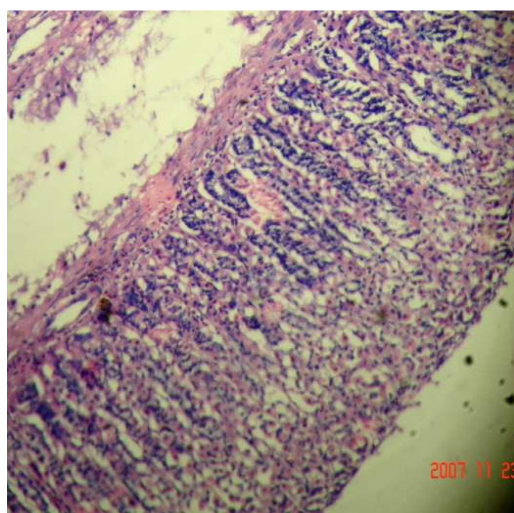
Figure 1: Histopathology of pyloric ligation induced ulcer model (Hematoxin&Eosinx100)
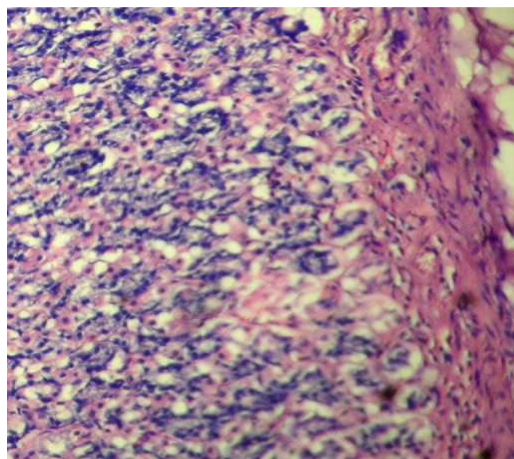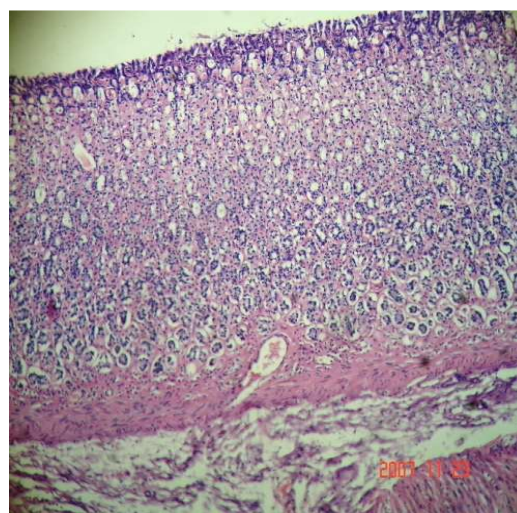

1a) Section of gastric mucosal layer Shows normal appearance in Control
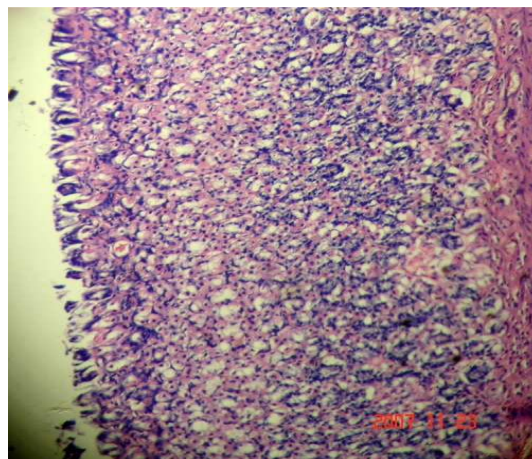
1b) Pylorus ligation group shows mucosal ulceration and inflammation

1c) Omeprazole (20 mg/kg) shows almost normal appearance

1d) MEHC (3.2g/kg) shows almost normal appearance

Table II: Effect of MEHC on various parameters in Indomethacin induced gastric ulcer

Group	Treatment	Ulcer index	% Protection
I	Control(vehicle)	12.6±.08	-----
II	Omeprazole (20 mg/kg)	3.5±.07*	72 %
III	MEHC(1.6g/kg)	5.7±.05	54 %
IV	MEHC(3.2g/kg)	4.2±.04*	66 %

*Values are expressed as mean ± SEM, n=6; Significant at * p<0.05 compared to control group.*

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The MEHC and omeprazole significantly decreased the total acidity and free acidity which suggests that MEHC possess antisecretory effect. Its anti-ulcer activity is further supported by the histopathological study which shows protection of mucosal layer from ulceration and inflammation.

In indomethacin induced model, the control animals produced characteristic lesions in the glandular portion of rat stomach which appeared as elongated bands of thick, black & dark red lesions. Methanolic extract of *Hibiscus cannabinus* treated animals showed significant protection index of 54% and 66% with the dose of 1.6and 3.2g/kg respectively in comparison to control (Results are tabulated in Table-II). In indomethacin induced model, ulcers are caused due to inhibition of synthesis of endogenous cytoprotective Prostaglandin(PG). The excess gastric acid formation by prostaglandin includes both increase in mucosal resistance as well as decrease in aggressive factors, mainly acid and pepsin. Inhibition of PG synthesis by indomethacin coincides with the earlier stages of damage to the cell membrane of mucosal, parietal and endothelial cells. Indomethacin induced gastric lesion formation may be due to stasis in gastric blood flow because of vascular congestion and mucosal capillary necrosis and this contributes to the development of the hemorrhage and necrotic aspects of tissue injury[17]. Indomethacin directly acts on gastric epithelium and produces lipid peroxidation. The extract shows protection against characteristic lesions produced by indomethacin and also showed significant decrease in ulcer index. The cytoprotection offered here might be due to significant antisecretory property of the extract and could have inhibited indomethacin induced inhibition of PG synthesis. The preliminary phytochemical studies revealed the presence of flavanoids; various flavanoids have been reported for its anti-ulcerogenic activity. So the possible mechanism of anti-ulcer activity might be due its flavanoid content[18,19]. Further studies are needed for their exact mechanism of action on gastric acid secretion and gastric cytoprotection.

MATERIALS AND METHODS

Plant: The leaves of Hibiscus cannabinus were collected from the garden around Tambaram, during the month of November. It was identified and authentified by Prof. P.Jayaraman Ph.D., Director-Plant Anatomy Research Centre (PARC), Tambaram, Tamilnadu, India.

Animals: Wistar albino rats of either sex weighing between 150-250 gm were used. Institutional Animal Ethical Committee approved the experimental protocol; animals were maintained under standard conditions as per CPCSEA norms. Albino rats used in this study were obtained from SRM College of Pharmacy Animal house. The animals were housed in Poly propylene cages and maintained at 24°C ± 2°C under 12h light/ dark cycle and were fed *ad libitum* with standard pellet diet and had free access to water.

The animals were given standard diet supplied by Pranav Agro Industries Ltd. Sangli. The composition of the diet are protein 10%, Arachis oil 4%, Fibers 1%, Calcium1%, Vitamin A 1000 IU/gm and Vitamin D 500 IU/gm.

Extraction: The leaves of *Hibiscus cannabinus* were shade dried and reduced to coarse powder in a mechanical grinder. The powdered material obtained was then subjected to successive extraction by Hot Percolation Method using petroleum ether and methanol as solvents in a soxhlet extractor. The extract obtained was evaporated at 45°C to get a semisolid mass. The extract thus obtained was subjected to phytochemical analysis and used for further study[10].

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Experimental Method

Pyloric Ligation in Rats: Animals were divided into five groups, each consisting of six animals.
Group I - Control group received distilled water orally.
Group II – Negative Control (Pyloric ligated)
Group III & IV – Test groups received MEHC 1.6 & 3.2g/kg;p.o respectively.
Group V – Standard group received Omeprazole (20 mg/kg;p.o)

After 45 min of the respective drug treatment, pyloric ligation was done by ligating the pyloric end of the stomach of respective groups under anaesthesia. Ligation was done without causing any damage to the blood supply of the stomach. After recovery, the animals were stabilized in individual cages and deprived of water during post-operative period. After 4h of surgery, rats were sacrificed and ulceration was scored. Gastric juice was collected and studied[11,12].

Indomethacin induced Ulcer Model: The animals were divided into four groups of six animals. The group I received Indomethacin(8mg/kg) and served as control. The group II, III & IV received the extract (1.6, 3.2g/kg) and Omeprazole(20mg/kg) respectively. The test, standard and control vehicle were administered orally in two doses at an interval of 15 hours. Indomethacin (8mg/kg;p.o) was administered by oral needle in two doses after 30min of administration of each dose of test compound to all groups. One hour after the second dose of the indomethacin all the rats were sacrificed. The number of ulcer spots in the glandular portion of the stomach was counted in all groups of animals and the ulcer index was calculated.

Scoring of ulcer was made as follows

Normal stomach................ (**0**)   Hemorrhagic streak.................... (**1.5**)
Red coloration................ (**0.5**)   Ulcers........................ (**2**)
Spot ulcer................ (1)   Perforation............... (3)

$$\text{Ulcer index} = \frac{\text{Number of Ulcer spots}}{\text{Total Mucosa}} \times 100$$

Ulcer index was thus calculated by adding the total number of ulcers per stomach and the total severity of the ulcers per stomach.

The percentage of ulcer protection was determined as follows:-

$$\% \text{ Protection} = \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

The acidity of the gastric juice was determined as follows:

$$\text{Acidity} = \frac{\text{volume of NaOH x Normality of NaOH x 100}}{0.1} \quad \text{mEq/litre[13].}$$

Statistical Analysis: The values are expressed as mean ± S.E.M, and statistical significance between treated and control groups was analyzed using of One way ANOVA, followed by Dunnett's test where $P < 0.05$ was considered statistically significant.

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Histopathological Evaluation: The gastric tissue samples were fixed in neutral buffered formalin for 24 h. Sections of gastric tissue were used for histopathological study. The tissues were fixed in 10% buffered formalin and were processed using a tissue processor. The processed tissues were embedded in paraffin blocks and about 5-μm thick sections were cut using a rotary microtome. These sections were stained with hemotoxylin and eosin using routine procedures. The slides were examined microscopically for pathomorphological changes such as congestion, hemorrhage, edema and erosions using an arbitrary scale for the assessment of severity of these changes[14].

CONCLUSION

Thus we conclude that the methanolic extract of *Hibiscus cannabinus* leaves possess anti-ulcer activity.

Acknowledgement

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