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EVALUATION OF GASTRO-PROTECTIVE EFFECTS OF *FLEMINGIA STROBILIFERA R.Br. (FABACEAE) ROOT EXTRACT*

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To evaluate the gastro-protective activity of the chloroform root extract of *Flemingia Strobilifera R.Br* in rodents. Gastro-protective effect was evaluated by Indomethacin induced ulcer in rats. Other anti-ulcer related activities of the extract such as the effect on gastrointestinal motility, and the activity on contraction evoked by standard agonists on isolated guinea pig ileum preparation were also determined. Increasing concentrations of the chloroform extract of flemingia Strobilifera did not produce spasmogenic effect on the isolated guinea pig ileum preparation, but produced a dose-related inhibition of contractile responses to histamine and acetylcholine with IC_{50} 12.5 µg/ml and 34.95µg/ml respectively. In Indomethacin induced ulcer model, administration of the extract of *F. Strobilifera* at a dose 15mg and 30mg/kg body wt. reduced the ulcer indices significantly ($p < 0.001$) as compared to the control. The standard drug Ranitidine also showed similar effect, but was more effective compared to both the doses.The chloroform extract of the root *Flemingia Strobilifera possess* gastroprotective activity could justify folklore use of the plant in peptic ulcer diseases.

Keywords: Anti-ulcer activity, *Flemingia Strobilifera*, motility activity, Peptic ulcers

INTRODUCTION

Peptic ulcer disease (PUD) is a spectrum of diseases consisting of gastritis, gastric ulcers, and duodenal ulcers[1]. It is known to occur when the endogenous defense mechanisms of the protective mucosal barrier have failed to sufficiently counteract the aggressive factors (hydrochloric acid, pepsin, and *Helicobacter pylori*) and is characterized by gnawing or burning sensation in the abdomen[2]. Duodenal ulcers occurs more frequently (about 80 % of PUDs) than gastric ulcers. The life time prevalence of PUDs is about 10 %[3]. PUDs are recurrent and most clinical studies have shown that approximately 50 % of all ulcer patients will have recurrence within one year of diagnosis. Although advances have been made in the treatment of PUDs due to an increased understanding of the mechanisms associated with the development of ulceration in the gastrointestinal tract, the morbidity and mortality toll is still very high. In the United States, for example, a study estimated about 6500 deaths each year on ulcer-related complications[4]. The available drugs for the management of PUDs are associated with high relapse rates and limiting side effects[5, 6, 7]. Validation of the efficacy and harnessing of medicinal plants used in folk medicine for the treatment of peptic ulcer diseases is a very promising approach to overcome the limitations of orthodox medicines. Already, there is an avalanche of scientific evidences in support of the efficacy of medicinal plants in the management of ulcers of different etiologies [6,8]. The treatment of peptic ulcer with plant products used in folk medicine and the protection of induced gastric ulcer in laboratory animals using medicinal plants was reported[4]. Generally plant flavonoids have been found to be effective against ulcer in experimental animals[5] and exhibit several biological effects.[6]

F. strobilifera R.Br, an important medicinal plant, is commonly known as Kusrunt found in Sind, Rajputana, Bengal, South India and Andaman's.[9] Previous chemical studies showed that flavonoids, flavonoid glycosides, chalcones, epoxychromenes and pterocarpans were the main constituents found in this genus of Flemingia Strobilifera R.Br.[9,10] Hence the present study was undertaken with the aim to assess the antiulcer activity from *Flemingia Strobilifera* claimed by traditional system of medicine.

MATERIAL AND METHODS:

Collection of Plant material:

The roots of the plant *Flemingia Strobilifera R.Br.* belonging to family *Fabaceae* were collected from the Western

Table I. Effect of CEFS root against Indomethacin induced ulcer.

Treatments	**Drug** (mg/kg)	**Ulcer index**	**GSH** (µmol/100mg)	**Total protein** (µg/100mg)	**MDA** (µmol/gm)
Control	1ml/100g.	7.83 ± 0.105	1.694 ± 0.093	2.48 ± 0.239	3.65 ± 0.114
Standard	25	2.16±0.586***	3.046 ± 0.254**	4.91 ± 0.257**	2.35 ± 0.260*
CEFS	15	4.16 ±0.380***	2.673 ± 0.194*	2.98 ± 0.261*	3.13 ± 0.139ns
CEFS	30	3.0 ± 0.707***	3.27 ± 0.269***	4.53 ± 0.155**	2.22 ± 0.285**

Values expressed as mean ± SEM, n=6, ANOVA followed by Tukey's Kramer post hock test, * p<0.05, ** p<0.01, *** p<0.001 when compared with control.

Table-II: Effect of CEFS root on guinea pig ileum in presence of Histamine.

Serial No	**Drug added** (µ/ml)	**Response** (mm)	**% Response** (Mean & SEM)	**IC_{50}** (µg/ml)
1.	Histamine (2)	31.66	100.00 ± 0.00	12.5 µg /ml
2.	Histamine (2) + CEFS (5)	20.66	64.88 ± 9.320ns	
3.	Histamine (2) + CEFS (10)	14.66	47.52 ± 9.608*	
4.	Histamine (2) + CEFS (20)	10.66	35.08 ± 10.843**	
5.	Histamine (2) + CEFS (40)	6.66	21.66 ± 9.775***	
6.	Histamine (2) + CEFS (80)	1.66	9.55 ± 3.445***	

Values expressed as mean ±SEM, n=3, ANOVA followed by Tukey's Kramer post hock test,*p<0.05, **p<0.01, *** p<0.001 when compared with Histamineresponse.

Table-III: Effect of CEFS root on guinea pig ileum in presence of Acetylcholine.

Serial No	**Drug added** (µg/ml)	**Response** (mm)	**% Response** (Mean & SEM)	**IC_{50}** (µg/ml)
1.	Acetylcholine (5)	33.00	100.00 ± 0.00	34.95 µg /ml
2.	Acetylcholine (5) + CEFS (5)	29.66	88.87 ± 4.459ns	
3.	Acetylcholine (5)+ CEFS (10)	26.00	77.79 ± 3.115ns	
4.	Acetylcholine(5) + CEFS (20)	22.33	66.92 ± 4.091**	
5.	Acetylcholine (5)+ CEFS (40)	14.00	41.41 ± 4.343***	
6.	Acetylcholine(5) + CEFS (80)	5.00	24.50 ± 2.929***	

Values expressed as mean ±SEM, n=3, ANOVA followed by Tukey's Kramer post hock test, **p<0.01, *** p<0.001 when compared with Histamine response.

Ghats of Maharashtra in the month of July 2009. The plant was authenticated by Dr. Jawahar Raveendran, Conservative Research & Action group, FRLHT, and preserved a specimen sample of the same in the herbarium section of the FRLHT, Bangalore-64, with the voucher No. 100154 for future reference.

Preparation of plant extracts:

The collected roots were cut in to require size and air dried then extracted with chloroform at 50^0C and the extract so obtained was filtered. The procedure was again repeated five times using adequate amount of chloroform at an interval of 3 days. The filtrate was evaporated to dryness to get residue. Then the residue was transferred to a china dish and evaporated on thermostat controlled water bath at 40ºC, stored in a refrigerator until further use. The amount of extract collected was 50 gm w/w from the dried powdered root of *Flemingia Strobilifera R.Br.*

Animals:

Albino wistar rats (150-200g) and adult guinea pigs (250-300 g) of either sex used for the study were obtained from Drug testing laboratory and Institution of veterinary science Bangalore, Karnataka. The animals were acclimatized for 7 days and housed under standard conditions of temperature (25 ± 2°C) and 12-h light/dark cycle. After acclimatization the animals were used for experiments. The protocol for the study was approved by institutional animal Ethics committee (Reg. no.152/1999/CPCSEA) as per the CPCSEA guidelines.

Acute Toxicity Studies:

The acute toxicity was determined on virgin female albino wistar rats by fixed dose method of OECD Guide line no 420 given by CPCSEA.[11] Groups of 6 rats were administered test drug by oral route in the range of 2000-300 mg/kg and mortality was observed after 24 hr. The safe dose was found to be 300 mg/kg body weight. The doses selected for the study were15mg and 30mg/kg body weight ($1/20^{th}$, $1/10^{th}$ of the maximum safe dose) respectively.

Indomethacin induced ulcer model:

Albino wistar rats of either sex weighing 150– 200 g were maintained in animal house and they were divided in to 4 groups of 6 animals in each. The weight range of the animals was equally distributed throughout the groups. They were acclimatized to housing conditions at least one week prior to use. Then they were fasted for a period of 24 hrs, allowing them free access to drinking water. The animals of group I were pretreated with vehicle and the animals of group II were pretreated with standard i.e. Ranitidine 25mg/kg. Similarly the animals of group III and IV were pre-treated with CEFS root 15 mg and 30 mg/kg respectively. After 60 minutes of the respective treatments, the animals of group I-IV were administered with Indomethacin (30mg/kg p o). After 4 hr of Indomethacin administration, rats were sacrificed, stomach was taken out, cut opened along the greater curvature, washed with normal saline and examined for ulcer.[12] The other parameters such as reduced Glutathione level,[13] Lipid peroxidation[14] and Total protein content[15] were evaluated in the stomach tissue.

***In vitro* pharmacological studies**

The effects of the extracts on isolated guinea pig ileum preparation were studied. Segments of the tissues, 2-3 cm long, were suspended in 20 ml organ bath filled with Tyrode solution of composition (mM/L): NaCl-136.7, KCl-2.7, CaCl2-1.8, NaHCO3-11.9, MgCl2-1.0, Na2HPO4-0.4, and glucose-5.5 maintained at 37 ± 1 °C and aerated with air. The preparation were set up under a resting tension of 0.5 g and allowed to equilibrate for 60 min during which the bathing fluid was changed every 10 min. At the end of equilibration period, the extracts were tested for any spasmogen or spasmolytic activity by adding increasing concentrations of each of these extracts (5–1000 µg/ml) on guinea pig ileum preparation. The effects of the extracts on sub maximal responses of standard drugs (ACh and Histamine) were also determined and the IC50 calculated for each treatment. The contact time for the

activity of each extract was 120 seconds while the standard spasmogen acted for 30 seconds in a 3 minute time cycle. Responses were determined in triplicate and recorded with the help of polyrite-4, (Model-201, Medicare). The adequate concentration of the extracts required to block the submaximal responses (100 %) of standard drugs (ACh and Histamine hydrochloride) were determined and the IC_{50} value was calculated.[16]

Statistical analysis: Results were expressed as mean ± SEM, n=6. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey's Kramer post hock test. $P < 0.05$ was considered significant.

RESULT:

Chloroform extract of flemingia strobilifera root (CEFS) was found to be safe up to 300 mg/kg body weight when administrated orally in female Wistar rats.

Oral administration of Indomethacin produced characteristic lesions in the glandular portion of the rat stomach. Pretreatment with CEFS root reduced the characteristic lesions in a dose dependent manner as shown in the fig-I. Table-I shows that CEFS root produced dose dependent ($P<0.001$) protection of gastric mucosal ulceration when compared with the control. Pretreatment with CEFS at a dose of 15 and 30 mg/kg body wt. increased the gastric mucosal GSH level, total protein content significantly ($P<0.01$, 0.001) as compared to control group. Whereas there is significant ($P<0.01$) reduction in gastric mucosal MDA levels in the animals pretreated with 15 and 30 mg/kg body wt. of CEFS roots when compared to control. Standard drug treated group also showed significant activity as compared to control group.

The extracts have no inherent spasmogenic activity on the isolated guinea pig ileum. Increasing concentrations of the extract did not produce contractile response of the isolated guinea pig ileum, but produced a dose-related inhibition of contractile response to histamine and acetylcholine. The concentration of extract which produced 50 % inhibition of the maximal response to histamine and Ach, IC50 values are shown in Table II, III.

The histopathology of gastric mucosa of rats reveals a significant reduction in gastric erosion and lesions in CEFS root treated group, which is similar to that in the Ranitidine treated group as compared to the control group (Figure II).

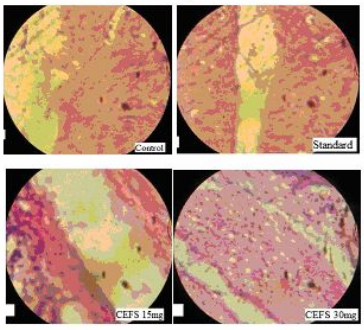
DISCUSSION:

The different therapeutic options available for the treatment of ulcers are either employed to inhibit gastric secretions or to enhance mucosal defense. These anti-ulcer therapeutic strategies are aimed at balancing the mucosal aggressive factors against mucosal protective factors. These therapies are intended to relief the patient from the ulcer pain, to facilitate the healing of the lesions, to prevent reoccurrence and the development of associated complications.[17] Traditional folklore medicine practice has claimed a lot of success in the use of medicinal plants in the management of peptic ulcer diseases and these has encouraged our recent screening of CEFS (Chloroform extract of *Flemingia Strobilifera*) root for gastro-protective properties with a view to isolating potent and safe antiulcer drugs from medicinal plant. The present study indicate that Chloroform extract of *Flemingia Strobilifera (*CEFS) root showed ulcer protective effects against Indomethacin induced ulcer in rats and motility activity on Guinea pig ileum.

Anti-inflammatory drug like Indomethacin administered in toxic doses (30 mg/kg), produce visible gastric ulcers in animals. Indomethacin is a potent inhibitor of prostaglandin biosynthesis.[18] Prostaglandins are known to play an important role in maintaining mucosal integrity. An Increase in certain endogenous prostaglandins can enhance gastric mucosal resistance to ulcerogenic agents.[19] The mechanisms involved in prostaglandin action are multiple, including stimulation of mucus and bicarbonate output,[20] gastric mucosal blood

flow,[21] decreasing gastric motility, increasing the release of endogenous mediators of gastric injury vasoactive amines and leucotrienes and also due to the stimulation of cellular growth and repair.[22] Indomethacin inhibits COX1 there by inhibits the prostaglandin synthesis, consequently lipooxygenase pathway is enhanced liberating leukotrienes and these leukotrienes are reported to have a role in ulcerogenesis. In addition there is some evidence that NSAIDs may induce ulcer by causing the back diffusion of H+ ion in to mucosal cells.[23] Therefore the gastro protective effect of the CEFS (Chloroform extract of Flemingia Strobilifera) may be due to its ability to inhibit the synthesis of prostaglandins/leukotrienes. In addition chloroform extract of root of Flemingia Strobilifera was significantly effective in protecting gastric mucosa against ulcerogenic model of the study. Hence, it may be inferred that chloroform extract of root of Flemingia Strobilifera affords effective protection to gastric mucosa against various insults.

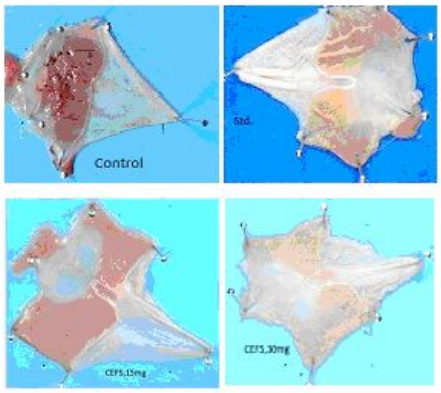
Figure II. Rats stomach mucosa (T.S. of the mucus membrane).



Control- Ulcerated rat stomach; *Standard*- Ranitidine, 25 mg/kg treated rat stomach and CEFS root, 15 and 30 mg/kg treated rat stomach.

Peptic ulcer and gastritis have multietiopathogenetic factors. It is widely accepted that a major under lying factor of this disorder is the generation of free radicals. There is substantial evidence that oxygen derived free radicals play an important role in the pathogenesis of the injury of various tissues, including the digestive system.[24,25] In addition, involvement of oxygen derived free radicals such as the superoxide anion, hydrogen peroxide, and hydroxyl radical are well established in the pathogenesis of ischaemic injury of gastrointestinal mucosa and in other models of mucosal damage induced by non-steroidal anti-inflammatory. Lipid peroxidation product of MDA is thought to reflect free radical mediated cell membrane damage. It is known that radical scavengers, such as alfa tocopherol, carotenoids and glutathione redox system, play a significant role in protecting membranes from oxidative damage. Depletion of gastric mucosal GSH may result in the accumulation of free radicals that can initiate membrane damage by lipid peroxidation. Salim et al. investigated the influence of free radical scavengers on the healing of gastric and duodenal ulcers resistant to therapy and found that antioxidative therapy stimulates the healing of therapy resistant ulcers.[26] In our present study the decrease in glutathione levels by Indomethacin induced ulcer was increased by CEFS root treatment indicating an enhanced antioxidant status, reduced lipid per oxidation and ulcer protection.

Figure I. No of lesion in the gastric mucosa.



Control- Ulcerated rat stomach; *Standard*- Ranitidine, 25 mg/kg treated rat stomach and CEFS root, 15 mg and 30 mg/kg treated rat stomach.

In addition, the antiulcer activities of the CEFS root may be attributed to its flavonoid content. This postulation is consistent with the earlier observations that flavonoids possess spasmolytic, antiulcerogenic[27] and antigastric activities as well as ability to inhibit acid secretion.[28] Most of these effects have been attributed to the influence of flavonoids on arachidonic acid metabolism, their vasoprotective action[29] and

their ability to interfere with the formation of histamine in the gastric mucosa.[30]

Histamine and Acetylcholine is believed to have an essential role in the pathogenesis of ulcer since these are potent stimulator of gastric acid secretion. Reduction in intestinal motility ameliorates ulcer pain and hastens the healing of ulcer wounds. The CEFS root reversibly binds histaminenergic and cholinergic receptors on the smooth muscles of Guinea pig ileum preparation and blocks them. The reduction in gastrointestinal motility by CEFS root may be related to the inhibition of contractile responses evoked by acetylcholine and histamine on isolated guinea pig ileum.

Histopathological study revealed that in CEFS root treated groups, the mucosa was found to be almost normal with mild muscularis mucosa. Ranitidine treated section showed the normal mucosa with no ulcer in the sub mucosa.

From the experimental study it can be concluded that chloroform extract of *Flemingia strobilifera R.Br.*root at 15 mg and 30 mg/kg, body wt. *p o* exhibited significant gastroprotective activity. Further study need to be done to elucidate the mechanism of action involved in the antiulcer activity.

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