ANTI ULCER ACTIVITY OF LEAVES OF *GMELINA ARBOREA* PLANT IN EXPERIMENTALLY INDUCED ULCER IN WISTAR RATS

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Summary

In Maharashtra, the juice of the leaves was used to remove foetid discharges and worms from ulcers and used as an folk medicine.Anti ulcer activity of leaves of *Gmelina arborea* plant in experimentally induced ulcer in wistar rats.The effect of hydroalcoholic extracts of leaves of *Gmelina arborea* on gastric ulcers was evaluated by using different experimental models such as aspirin induced ulcer, pylorus ligation induced ulcers, ethanol induced ulcers and cold restrain stress induced ulcers. In all these models the common parameter determined was ulcer index. The hydroalcoholic leaf extracts of *Gmelina arborea* (286mg/kg and 667mg/kg) showed a significant effect on ulcer induced by different ulcer induced models. The leaf extract of *Gmelina arborea* was effective in ulcer healing. It was concluded that leaves of *Gmelina arborea* increases healing of gastric ulcers and also prevent the development of gastric ulcers in rats.

Key words: *Gmelina arborea*; gastric ulcer; gastric secretion; gastric cytoprotection.

Introduction

Peptic ulcer is a lesion of gastric or duodenal mucosa occurring at a site where the mucosal epithelium is exposed to aggressive factor. In spite of the vast amount of research on ulcer, the cause of chronic peptic ulceration is still not clear[1]. For more than a century, peptic ulcer disease has been a major cause of morbidity and mortality. The pathophysiology of peptic ulcer has been centralized on an imbalance between aggressive and protective factors in the stomach such as acid–pepsin secretion, mucosal barrier, mucus secretion, blood flow, cellular regeneration, prostaglandins and epidermal growth factors[2, 3]. Gastric and duodenal ulcers are illnesses that affect a considerable number of people in the world and some authors consider gastric ulcer as a new “plague” of the 21st century[4].

Progression of peptic ulcer disease can result in serious complications, including hemorrhage, which occurs in approximately 20% of patients, and perforation, which occurs in approximately 7%, other possible sequelae include penetration to an adjacent organ and obstruction resulting from inflammation and edema or fibrotic scarring near the gastroduodenal junction[5]. Therapies are directed at decreasing gastric acidity, enhancing the lower esophageal sphincter or stimulating esophageal motility[6]. However no scientific study on the anti-ulcer activity of this plant has been reported. Thus the present investigation was undertaken to study the anti-ulcer activity of leaves of *Gmelina arborea* in experimentally induced ulcer in wistar rats.

Materials and Methods

Collection of Plant

Plant material used in this study consisted of the leaves of *Gmelina arborea*, collected from surrounding area of Ahmednagar, Maharashtra, India. The plant was authenticated by Forest department Ahmednagar.

Preparation of the plant extract

The leaves were dried in shade at room temperature. The dried leaves were powdered by using grinder, to coarse powder and this powder was packed into soxhlet column and extracted with petroleum ether (60-80°C) for 24 h. The same marc was successively extracted with chloroform (59.5-61.5°C) and afterwards with hydroalcohol (70 %) for 24 h. The extracts were concentrated under reduced pressure (bath temperature 50°C). The dried extracts were stored in airtight container.

Animal used

Albino Wistar rats either sex weighing between 150-250 g were used. Institutional animal ethics Committee approved the experimental protocol; animals were housed under standard conditions of temperature (24 ± 2°C) and relative humidity (30-70 %) with a 12:12 light: dark cycle. The animals were given standard diet supplied by Pranav agro industries Ltd. (Sangli, Maharashtra, India) and water *ad libitum* all procedures involving animals were carried out under the institute ethics committee approval (997/c/06/CPCSEA).

Toxicity studies

Toxicity studies of the hydroalcoholic leaf extract were carried out in Swiss Albino mice of either sex weighing between 20-25 g. the LD_{50} of the hydroalcoholic leaf extract was found to be 2000 mg/kg (i.p. and p.o.).

Antiulcer activity

The antiulcer activity was carried out by using the following models

- Aspirin induced ulcer
- Ethanol induced ulcer
- Cold restrain induced ulcer and
- Pylorus ligation induced ulcer

Aspirin induced ulcer

HLEGA 286 and 667 mg/kg and H_2-receptor blocker, ranitidine, in the dose of 20 mg/kg were administered orally daily, respectively, for five days for ulcer protective studies. ASP in dose of 20 mg/kg was administration to the animals on the day of the experiment and ulcers were scored after 4 h. The animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0ml of 0.9 %NaCl and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach. Ulcer index was then calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach[7-9].

A score for the ulcer was made as follows:

- 0.5: Hemorrhage
- 1: Streaks
- 2: Spot ulcer
- 3: Sever ulcer
- 3: Sever steaks
- 4: Erosions
- 5: Perforation.

Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer protection was determined as follows

$$\% \text{ Protection} = \frac{\text{Control mean ulcer index}}{\text{Control mean ulcer index} - \text{test mean ulcer index}} \text{ X } 100$$

Ethanol induced ulcer

The gastric ulcers were induced in rats of either sex weighing between 150-250 g by administrating absolute ethanol (8 ml/kg). They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The rats were divided in to four groups each containing six animals and fasted for 24h and allowed free access to water. The first group received control vehicle only and the second group 286 mg/kg body weight, third group 667 mg/kg body weight received hydro alcoholic extract of Gmelina arborea and fourth group received Standard OMZ in the dose of 20 mg/kg were administered orally daily to different groups for five days. On the sixth day of experiment the drugs were administered orally 30 min prior to the oral administration of absolute ethanol. The animals were anaesthetized 6 h latter with ether and stomach was incised along the greater curvature and ulceration was scored[10-12].

The number of ulcers and the length of each ulcer were measured. A score for the ulcer was made as mentioned above.

Cold restrain stress induced ulcer

HLEGA 286 and 667 mg/kg and H_2 receptor blocker, ranitidine, in the dose of 20 mg/kg were administered orally daily to different groups for five days. Rats were deprived of food, but not water, for about 18 h before the experiment. On day six, the experimental rats were immobilized by strapping the fore and hind limbs on a wooden plank and kept for 2 h, at temperature of 4-6^{o}C. two hours latter, the animals were sacrificed by cervical dislocation and ulcers were examined on the dissected stomach as described above[10-13].

A score for the ulcer was made as mentioned above.

Pylorus ligation induced ulcer

After 45 min of HLEGA (286 mg/kg and 667 mg/kg body weight) and OMZ (20 mg/kg body weight) treatment for five days, on day sixth, pyloric ligation was done to pyloric end of rats of respective groups under thiopental sodium anesthesia at a dose of 45 mg/kg of body weight. Ligation was done without causing any damage to the blood supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during post-operative period. After 4 h of surgery, rats were sacrificed and ulcer scoring was done. Gastric juice was collected and gastric secretion studies were performed. A score for the ulcer was made as mentioned above[13-15].

Statistical analysis

Mean values ± S.E.M. were calculated for each parameter. For the determination of significant intergroup differences, each parameter was analyzed separately and one-way analysis of variance (ANOVA) was carried out.

Results & Discussion

Ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as reduction in gastric volume, decrease in free and total acidity. HLEGA has decreased the intensity of gastric mucosal damage induced by ulcerogenic agents in

a dose dependent manner. It showed antiulcerogenic activity in all the models, each of which induces ulcer through a different mechanism. HLEGA showed the ability to reduce significantly the severity of ulceration of stomach induced by absolute ethanol. The results of histopathological investigation revealed that the pretreatment with HLEGA absolutely prevented the ethanol-induced congestion, hemorrhage, edema, necrosis, inflammatory and dysplastic changes, erosions and ulceration in the gastric mucosa of rats.

The stomach appearance was normal. The incidence of ethanol-induced ulcers predominant in the glandular part of stomach was reported to stimulate the formation of leukotriene C4 (LTC4), mast cell secretory products[16], and reactive oxygen species resulting in the damage of rat gastric mucosa[17]. HLEGA has shown significant protection index of 93.27% ($P < 0.01$) and 98.51% ($P < 0.01$) with the dose of 286 and 667 mg/kg respectively in comparison to control, the high dose effect is almost same as that of standard drug omperazole (97.9%). In ethanol model, ulcers are caused due to perturbations of superficial epithelial cells, notably the mucosal mast cells leading to the release of the vasoactive mediators including histamine, thus causing damage to gastric mucosa[18]. Mucosal blood flow has been attributed to be an important factor in the damage caused by alcohol and is modulated by prostaglandin[19]. The effectiveness of HLEGA protection against mucosal damage caused by ethanol is indication of its effect on prostaglandins.

The results are presented in table 3. HLEGA has shown significant effect in aspirin induced ulcer model with a protection index of 73.62% ($P < 0.01$) and 79.62% ($P < 0.01$) with the dose of 286 and 667 mg/kg respectively in comparison to control, whereas standard drug ranitidine was 92.32%. Aspirin has been reported to produce ulcers by both local and systemic effects[20].

Table 1 Gastroprotective activity of HLEGA on different ulcer-induced models:

Treatment	Aspirin induced ulcer		Ethanol induced ulcer		CRS induced ulcer		Pylorus ligated ulcer	
	Ulcer index	% Protection	Ulcer index	% Protection	Ulcer index	% Protection	Ulcer index	% Protection
Vehicle	217.6±13.472	--	295.8±24.231	--	161.5±27.884	--	209.5±15.828	--
HLEGA 286mg/kg	57.4±8.811**	73.62	19.9±5.806**	93.27	63.3±6.595**	60.8	54.2±3.720**	74.13
HLEGA *667*mg/kg	44.9±4.302**	79.36	4.4±1.259**	98.51	56.2±14.412**	65.2	23.3±1.972**	88.87
Ranitidine 20mg/kg	16.7±2.782**	92.32	§	§	15.5±5.927**	90.4	§	§
Omeprazole 20mg/kg	§	§	6.2±0.9301**	97.9	§	§	15.1± 5.464**	92.79

Values are mean ± S.E.M. *p<0.0001 extremely significant, **P< 0.01 very significant, ***<0.05 significant as compared to control group.
-- The group treated for the control vehicle.
§ The group does not treat for the specified test.

Table no. 2 Effect of HLEGA on Gastric volume, free acidity and total acidity of Pylorus ligation induced-ulcer

Group	No. of Animals used	Gastric Juice ml	Free acidity mEq/ltr	Total Acidity mEq/ltr
Control	5	2.8±0.1414	20±0.5040	123±4.817
Omeprazole 20mg/kg	5	2.4±0.3271***	9±0.4219***	67±3.830***
HLEGA 286mg/kg	5	1.2±0.1378***	8±0.3564***	87±3.391***
HLEGA 667mg/kg	5	2±0.1140***	9±0.1732***	25±0.9675***

Values are mean ± S.E.M. *p<0.0001 extremely significant, **P< 0.01 very significant, ***<0.05 significant as compared to control group.

Table no. 3 Effect of HLEGA on total hexoses, total protein, total pepsin and total mucin content of pylorus ligation induced-ulcer

Group	Total Hexoses mg	Total Protein mg/ml	Total Pepsin µgm/ml	Total mucin mg/g tissue weight
Control	0.9±0.02345	145.62±17.201	135±1.924	0.15±0.01817
Omeprazole 20mg/kg	2±0.2302***	239.79±5.409***	10±1.000***	0.25±0.006944***
HLEGA 286mg/kg	1±0.04517***	114.056±5.258***	95±2.702***	0.2±0.007071***
HLEGA 667mg/kg	4±0.2062***	136.17±3.526***	50±2.864***	0.2±0.01095***

Values are mean ± S.E.M. *p<0.0001 extremely significant, **P< 0.01 very significant, ***<0.05 significant as compared to control group.

Aspirin causes direct irritant effect and mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion by increasing the H+ ion transport/back diffusion of H+ ions, resulting overproduction of leukotrienes and other products of 5-lipoxygenase pathway[7]. It decreases mucin, surface active phospholipids bicarbonate secretion, mucosal proliferation and also produces damage by formation of free radicals[21]. The possible protective effect of HLEGA against aspirin-induced gastric lesions could be due to prevention of direct irritation, increased mucus secretion and due to its 5-lipoxygenase inhibitory effect. The results are summarized in table 1.

Animals subjected to restraint plus cold for 3 h showed the presence of considerable ulcerogenicity in the form of hemorrhagic mucosal lesions in the stomach, which were confined to the glandular segment only. There was also evidence of intraluminal bleeding in the stomach of these animals. Treatment with extract produced a significant dose-dependent inhibition of intraluminal bleeding and ulceration (Table 1). Stress plays an important role in aetiopathology of gastroduodenal ulceration. Stress-induced ulcers are probably mediated by histamine release with enhancement in acid secretion, a reduction in mucous production and generation of free radicals etc. Increase in gastric motility, vagal overactivity[22,23], mast cell degranulation[23] decreased gastric mucosal blood flow[24] and decreased prostaglandin syntheses[7] are involved in genesis of stress-induced ulcers. The gastroprotective action of HLEGA against stress-induced ulceration could be due to its histamine antagonistic, anticholinergic and antisecretory effects. HLEGA has shown significant protection index of 60.8% ($P < 0.01$) and 65.2% ($P < 0.01$) with the dose of 286 and 667 mg/kg respectively in comparison to control, whereas standard drug ranitidine 20 mg/kg was 90.4%.

After 45 min of treatment with a hydroalcoholic extract of Gmelina arborea, pylorus ligation of rats for 4 h resulted in accumulation of gastric secretory volume, and increase in titrable acidity and ulceration (Table 2). HLEGA has also depicted significant effectiveness ($P < 0.01$) in pylorus ligation induced gastric ulcer model. It was showing protection index of 74.13% and 88.87% at the dose of 286 and 667 mg/kg respectively in comparison to control whereas standard drug Omeprazole has shown 92.79% protection. Pyloric ligation induced ulcers caused due to imbalance between offensive and defensive mucosal factors[25] are ideal model to infer the mechanism by which a drug works as an anti-ulcerogenic agent. Pylorus ligation induced gastric ulcers occur because of an increase in acid-pepsin accumulation due to pyloric obstruction and subsequent mucosal digestion and breakdown of the gastric mucosal barrier[25,26]. A copious amount of mucus is secreted during superficial damage and provides favorable microenvironment in repair. Hence estimation of acid secretion, pepsin 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, HLEGA (286mg/kg) has reduced the free acidity by 60% which is comparable to standard drug Omeprazole (55%), HLEGA (667mg/kg) has reduced the free acidity by 45% which is comparable to standard drug Omeprazole (55%), whereas total acidity reduction by HLEGA (286mg/kg) (29.26%) is slightly less than that of Omeprazole (45.52%), total acidity reduction by HLEGA (667mg/kg) (79.67%) is slightly high than that of Omeprazole (45.52%). Significantly pepsin activity reduced by HLEGA (286mg/kg, 667mg/kg) to 29.62%, 62.96% is less than the reduction conferred by Omeprazole (92.59%). At the same time HLEGA (286mg/kg) showed increase in defensive mucus secretion by 33.33%, which is again less than that of Omeprazole (66.66%) (Table 3), HLEGA (667mg/kg) showed increase in defensive mucus secretion by 33.33%, which is again less than that of Omeprazole (66.66 %?) (Table 5).

The HLEGA (286mg/kg) showed increase in total hexoses by 11.11%, which is very less than Omeprazole (122.22%), The HLEGA (667mg/kg) showed increase in total hexoses by 344.44%, which is very high than Omeprazole (122.22%).

Overall, HLEGA has shown a substantial and significant protection against gastric ulcers in all the models. This protective effect might have been mediated by both anti-secretory and cytoprotective mechanisms. Moreover, further insight into the precise mechanism of action is essential to exploit the complete potency of HLEGA and increase its usage in contemporary medicine.

Conclusion

The qualitative phytochemical study reveals the presence of alkaloids, carbohydrates, Phytosterols, cardiac glycosides, Saponins, tannins and flavonoids. The present study demonstrated that the hydroalcoholic extract of leaf of *Gmelina arborea* possess anti-ulcer property. This protective effect might have been mediated by both anti-secretory and cytoprotective mechanisms. The above effects of it may also be due to the presence of tannins and flavonoids in the extract.

References

1. Govindarajan R, Vijayakumar M, Singh M, Rao V, Shirwarkar A, Rawat AKS, *et al*. Antiulcer and antimicrobial activity of *Anogeissus latifolia*. J Ethanopharmacol 2006; 106: 57-61.
2. Lima ZP, Severi JA, Pellizon CH, Brito ARMS, Solis PN, Caceres A, *et al*. Can the aqueous decoction of mango flowers be used as an antiulcer agent?. J Ethanopharmacol 2006; 106(1): 29-37.
3. Toma W, Hiruma-Lima CA, Guerrero RO, Souza Brito ARM. Preliminary studies of *Mammea americana* L. Phytomedicine 2005; 12: 345-350.
4. Hiruma-Lima CA, Calvo TR, Rodrigues CM, Andrade FDP, Vilegas W, Brito ARMS. Antiulcerogenic activity of *Alchornea castaneaefolia*: Effects on somatostatin, gastrin and prostaglandin. J Ethanopharmacol 2006; 104: 215-224.
5. Steven WS. Pathogenesis and treatment of acid peptic disorders: Comparison of Proton pump inhibitors with other antiulcer agents. Clin Thes 1996; 18(1): 2-34.
6. Brunton LL, Lazo JS and Parker KL. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th ed. New York: McGraw-Hill Medical publishing Division; 2006.
7. Rao ChV, Ojha SK, Radhakrishnan K, Govindarajan R, Rastogi S, Mehrotra S, *et al*. Antiulcer activity of *Utleria salicifolia* rhizome extract. J Ethanopharmacol 2004; 91: 243-249.
8. Navarrete A, Trejo-Miranda JL, Reyes-Trejo L. Principles of root bark of *Hippocratea excelsa* (Hippocrataceae) with Gastroprotective activity. J Ethnopharmacol 2002; 79: 383-388.
9. Muniappan M, Sundararaj T. Anti-inflammatory and antiulcer activities of *Bambusa arundinacea*. J Ethanopharmacol 2003; 88: 161-167.
10. Sheeba MS, Asha VV. Effect of cardiospermum halicacabum on ethanol-induced gastric ulcers in rats. J Ethanopharmacol 2006; 106: 105-110.
11. Nwafor PA, Okwuasaba FK. Effect of methanolic extract of *Cassia nigricans* leaves on rat gastrointestinal tract. Fitoterapia 2001; 72: 206-214.

12. Sannomiya M, Fonseca VB, Da Silva MA, Rocha LRM, dos Santos LC, Hiruma-Lima CA, *et al*. Flavonoids and antiulcerogenic activity from *Byrsonima crassa* leaves extract. J Ethnopharmacol 2005; 97: 1-6.
13. Albiero ALM, Sertie JAA, Bacchi EM. Antiulcer activity of *Sapindus saponaria* L. in the rat. J Ethnopharmacol 2002; 82: 41-44.
14. Mesia-Vela S, Souccar C, Lima-Landman MTR, Lapa AJ. Pharmacological study of *Stachytarpheta cayennensis* Vahl in rodents. Phytomedicine 2004; 11: 616-624.
15. Kulkarni SK. Hand book of experimental pharmacology. 3rd ed. New Delhi: Vallabh prakashan; 1999.
16. Oates PJ, Hakkinen JP. Study on the mechanism of ethanol-induced gastric damage in rats. Gastroenterology 1988; 94(1): 10-21.
17. Peskar BM, Lange K, Hoppe U, Peskar BA. Ethanol stimulates formation of leukotriene C4 in rat gastric mucosa. Prostaglandins 1986; 31(2): 283-93.
18. Miller TA, Henagan. Indomethacin decreases resistance of gastric barrier to disruption by alcohol. Dig Dis Sci 1984; 29(2): 141-149.
19. Hollander D, Tarnawski A, Gergely H, Zipser RD. Sucralfate protection of the gastric mucosa against ethanol-induced injury: A prostaglandin-mediated process?. Scand J Gastroenterol, Supplement 1984; 19(101): 97-102.
20. Alvarez A, Pomar F, Sevilla MA, Montero MJ. Gastric antisecretory and antiulcer activities of an ethanolic extract of *Bidens pilosa* L. var. radiate Achult. Bip. J Ethnopharmacol 1999; 67: 333-340.
21. Scheiman JM, Dubois, Giardiello FM. NSAIDs, Eicosonoids and the Gastroenteric Tract. 25 vol, Sounders: Philadelphia; 1996.
22. Djahanguiri B, Taubin HL, Landsburg L. Increased sympathetic activity in the pathogenesis of restraint ulcer in rats. J Pharmacol Exp Ther 1973; 184: 163-168.
23. Cho CH, Ogle CW. Cholinergic-mediated gastric mast cell degranulation with subsequent histamine H1-and H2-receptor activation in stress ulceration in rats. Eur J Pharmacol 1979; 55(1): 23-33.
24. Hase T, Moss BJ. Microvascular changes of gastric mucosa in the development of stress ulcer in rats. Gastroenterology 1973; 65(2): 224-234.
25. Goel RK, Bhattacharya SK. Gastroduodenal mucosal defence and mucosal protective agents. Ind J Exp Biol 1991; 29(8): 701-714.
26. Sairam K, Rao CV, Dora Babu M, Vijay Kumar K, Agrawal VK, Goel RK. Antiulcerogenic effect of methanolic extract of *Emblica officinalis*: an experimentally study. J Ethanopharmacol 2002; 82(1): 1-9.

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