Research Article

Activity of *Basella alba* in the Protection of Peptic Ulcer on Experimental Rat Model

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ABSTRACT

Peptic ulcer is a common GI disorder affects up to 20% of the world population. Treatment of it remains challenging due to the limited effectiveness and side effects of the currently available drugs. Hence, natural medicinal compounds are becoming popular potential candidates in preventing and treating peptic ulcers. Flavonoids the phytoconstituents exhibit gastro protective effects against peptic ulcer both in vivo and in vitro. In this study, we summarized the anti-ulcer functions of *Basella alba* which contain bioflavonoid in both pyloric ligation ulcer and aspirin induced ulcer model in rat. The results of the study indicates the ethanolic extract of *Basella Alba* (EEBA) has possess ulcer protective effects dose-dependently. The EEBA at 200 and 400 mg/kg dose levels showed significant lowering in ulcer index. There was no significant difference found between the dose of 400 mg/kg of EEBA and ranitidine treated animals.

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INTRODUCTION

Due to sedentary life style mainly feeding habits, peptic ulcer has been identified as the leading disease of 21stcentury. Ulcer formation is an interactive process resulting from an imbalance between aggressive (acid and pepsin) and defensive (mucosal) factors of gastrointestinal mucosa. Though multi factorial pathogenesis involved in mucosal damage, there is an evidence that chancesof injury to the gastric mucosa can increase when challenged repeatedly with many agents, including ethanol, acid, alkali, hyperosmolar solution, bile acids and nonsteroidal anti-inflammatory drugs over a periods of time. [1,2]

The treatment goals of peptic ulcers are relieve pain, manage ulcer healing and prevent complications and recurrence.[3] Although there is no optimal therapeutic regimen, the effective regimen consists of a proton pump inhibitor. However, the number of relapses is still high, and there are many side effects.[4]

For centuries, herbal products have been used traditionally for the treatment of a large range of ailments, including gastrointestinal disorders. The last two decades has witnessed a revival of interest in natural drugs and herbal products primarily due to the widespread belief that 'green' medicine is healthier than synthetic products. Medicinal plants possessing active principles like flavonoids, saponins, tannins, and terpenoids are found to have anti-ulcer activity. Some research paper has shown the efficacy of flavonoids in the protection of peptic ulcer both in vitro and in vivo.[5-8]

The plant*Basella alba*(fam- Basellaceae), commonly known as Indian spinach, contains so many phytoconstituents like caroteniids, organic acids, bioflavonoid, beta-sitosterol and lutpeol, protein, fat, carbohydrate, high calcium level and rich in vitamin-A, thiamine, Niacin, oxalic acid. *Basella alba* L. is a widely cultivatedvegetable with climbing growth habit. It is a succulent, branched, smooth, twining herbaceous vine, several meters in length. Stem are purplish or green. Leaves are fresher, ovate or heart-shaped,5 to12 cm long and purple when mature. It is traditionally used as an antidote, aperients, rubefacient astringent, demulcent, diureticandlaxative. It also used in the treatment of inflammation, atherosclerosis, stroke, heart disease, diabetes mellitus, multiple sclerosis, Parkinson's disease, Alzheimer's disease, etc.. Moreover, leaves of the plant exhibited its potentiality of recovering male infertility. [9-11]

Therefore, the present study was designed to investigate the protective effect of *Basella alba*plantin peptic ulcer.

MATERIALS AND METHODS

Collection of the plant materials: The whole plant of *Basella alba* was collected locally. The plant materials were authenticated at the department of Pharmacognosy, Royal College of Pharmacy and Health Sciences (R.C.P.H.S.), Berhampur. The plant materials were washed and dried under shade.

Successive extraction: The dried coarse powder of *Basella alba* plant materials (200g) were placed in the percolator of soxhlet apparatus and extracted successively with the solvent for 72 h. For defatting the plant materials petroleum ether (60-80°C) was used as solvent in initial step of extraction. The marc was air dried and subjected to further extraction by using ethanol. After completion of extraction, the marc was pressed to collect the excess of extract present. The solvent extract were dried using rotary vacuum evaporator and stored in desiccators until further use.

Yield of each extract of *Basella alba* was calculated. Also the color and consistency of the extract were tabulated.[12]

Preliminary Phytochemical screening: The ethanolic extract of *Basella alba*(EEBA) wasanalyzed for presence of different phytoconstituents by the method of qualitative phytochemical analysis.[13- 15]

Pharmacological Study:

Animals: Young female albino mice weighing 22-28 g were used for acute toxicity study and wister albino rats weighing 180-200 g of both sexes were used for other in vivo methods. All the animals were procured from the animal house of R.C.P.H.S., Berhampur. Animals were maintained under controlled room temperature (22 ± 2°C) and humidity (55 ± 5°C) with a 12:12 hour light: dark cycle. The animals were fed with standard laboratory food diet made in-house and pure drinking water ad libitum.

The animal experiments were conducted according to the ethical norms approved by CPCSEA and the ethical clearance was granted by Institutional Animal Ethics Committee of R.C.P.H.S., Berhampur..

Acute toxicity studies:

The acute oral toxicity study of theEEBA was carried out as per the OECD guidelines423. Thirty six number of young female albino mice were divided into six groups (n=6). Administration of stepwise doses of EEBA, i.e. 100, 500, 1000, 1500, 2000 mg/kg through oral route, to tested animals and observed the signs of toxicity. The control group received 5 ml/kg of distilled water orally.

The animals were observed continuously for the first 4 h and occasionally up to 24 h and at the end of 72 h, after administration of dose, for recording mortality or signs of toxicity, if any. [16,17]

Antiulcer and cytoprotective studies:

Antiulcer and cytoprotective studies were carried out by using Pyloric ligation (PL)-induced gastric ulcers and Aspirin (ASP)-induced ulcers in rats.

PL-induced ulcer[16-20]: Thirty number of healthy albino rats were divided into five groups (n=6) and were treated as per the following schedule (Table 1).

Table 1: Schedule of drug treatment for pyloric ligation induced ulcer in rats

Group	Group name	Treatment	Dose
Group- 1	Control	Distilled water	5 ml/kg
Group- 2	Standard	Ranitidine	50 mg/kg
Group- 3	Test 1	EEBA-100	100 mg/kg
Group- 4	Test 2	EEBA- 200	200 mg/kg
Group- 5	Test 3	EEBA- 400	400 mg/kg

All the scheduled drugs were administered by oral route for 5 days prior to induction of ulcer by pyloric ligation. The rats were fasted for 24 h but water was allowed *ad libitum*. The pylorus-ligation method was carried out in anaesthetized rats and were kept in a separate cage. Water was withheld from one hour before pylorus ligation and till the end. After 19 h the rats were sacrificed, abdomen was opened and the stomach was taken out carefully. The stomach was then cut open along the greater curvature and the contents of the stomach was collected in a graduated centrifuge tube by gave a small cut to the pyloric end just above the ligated portion. Centrifuged the contents of the stomach at 3000 rpm for 10 min and analyzed the contents for:

a) Volume of gastric content,

b) pH of gastric content with pH meter.

c) Free acidity and total acidity: Separate the supernatant liquid and titrate against 0.01N NaOH using Topfer's reagent and phenolphthalein as indicators. Volume of NaOH which turns the solution to yellowish orange corresponds to free acidity. Continue the titration till the solution turns pink and the total volume of NaOH corresponds to total acidity. Acidity (mEq/l/100g) can be calculated as:

$$\text{Acidity} = \frac{\text{Vol. of NaOH} \times \text{Normality} \times 100}{0.1} \text{mEq/l/100g}$$

The mucosa of stomach was washed slowly by saline water. Properly stretched and pinned the stomach on a wax plate. The ulcers were observed with the help of magnifying glass and scored the intensity of ulcer as: 0 – Normal stomach, 1 – Superficial mucosal erosions, 2 – Deep

ulcers, 3 – Penetrated or perforated ulcers. Mean ulcer score of each group is expressed as ulcer index.

ASP-induced ulcer[17-23]**:**Gastric ulceration may be produced in rat by certain drugs. NSAIDs like Aspirin, Indomethacin, and Ibuprofen inhibit the endogenous prostaglandin production and consequent loss of gastric mucosal defense. The ability of the test drug to protect against the ulceration is observed. Aspirin -induced ulcer is an important model for identifying drugs that could be effective in NSAID induced gastropathy. The experimental protocol was designed for evaluation of antiulcer and cytoprotective studies by ASP-induced ulcer method.

Thirty number of healthy albino rats were divided into five different groups (n=6) and were treated as per the following schedule (Table 2).

Table 2: Schedule of drug treatment for aspirin induced ulcer in rats

Group	Group name	Treatment	Dose
Group- 1	Control	Distilled water	5 ml/kg
Group- 2	Standard	Ranitidine	50 mg/kg
Group- 3	Test 1	EEBA-100	100 mg/kg
Group- 4	Test 2	EEBA- 200	200 mg/kg
Group- 5	Test 3	EEBA- 400	400 mg/kg

All the above scheduled drugs were administered by oral route for 5 days prior to induction of ulcer. Aspirin (500 mg/kg, p.o.) was administered orally on the day of experiment with the help of a feeding needle and animals were sacrificed after 6hr of administration by overdosing with ether. Immediately afterwards abdomen wasopened, cardiac end and pyloric end of stomach was ligated and the stomach was taken out carefully. The stomach was then cut open along the greater curvature. The mucosa of stomach was washed slowly by saline water. Properly stretched and pinned the stomach on a wax plate. The ulcers were observed with the help of magnifying glass and scored the intensity of ulcer as: 0 – Normal stomach, 1 – Superficial mucosal erosions, 2 – Deep ulcers, 3 – Penetrated or perforated ulcers. Mean ulcer score of each group is expressed as ulcer index.

Statistical analysis: The group means were considered to be significantly different at 5% level of significance, $P < 0.05$ by using one-way ANOVA (nonparametric), followed by Bonferroni's multiple comparison test. The values were expressed as mean ± SEM.[12]

RESULTS AND DISCUSSION

Percentage of yield (w/w), colour and consistency of different extracts:

The extract of *Basella Alba* was filtered and then it was concentrated by distilling off the solvent to obtain the crude extract. The extractive values, color are tabulated below(Table 3).

Phytochemical studies:

Preliminary qualitative phytochemical screening of EEBA showed the presence of alkaloid, phenolic, tannins, saponins flavones and flavonoids, and flavones and flavonoids are responsible for most of pharmacological activity by their antioxidants activity.

Acute toxicity studies:

The results of acute toxicity study was recorded in the Table 4.

The acute toxicity study exposed that the EEBA did not show any signs of toxicity or mortality even at the dose level of 2000 mg/kg body weight. The LD50 dose of 2000 mg/kg and above is considered low toxicity.[21] Overall results, suggested the LD50 value is greater than 2000 mg/kg, therefore, 1/5th (400 mg/kg), 1/10th (200 mg/kg) and 1/20th (100 mg/kg) of 2000 mg/kg were used for further pharmacological investigations.[24]

PL- induced ulcers:

Pyloric ligation induced gastric ulcers in rats were carried out and the results are recorded on the Table 5 and 6.

Table 4: Acute toxicity studies of EEBA

Treatment	Dose (mg/kg)	No. of Mice	No. of Death	signs of toxicity	LD_{50}
Control (Distilled water)	10 ml/ kg	6	0	-	-
Ethanol extract of *Basella Alba*	100	6	0	-	> 2000 mg/kg
	500	6	0	-	
	1000	6	0	-	
	1500	6	0	-	
	2000	6	0	-	

Table 5: Effect of EEBA on gastric secretion of Pyloric ligated rats :

Treatment	Dose (mg/kg)	Volume (ml/100g)	Gastric pH	Total Acidity (µEq/ml)
Control	-	4.19 + 0.12	2.14 + 0.95	109 + 3.92
Ranitidine	**50**	1.76±0.31*	4.79± 0.11*	28.64 ± 2.31*
EEBA	**100**	3.52± 0.16	2.58 ± 0.28	89.20 ± 2.20
EEBA	**200**	2.90 ± 0.10*	2.82 ± 0.23*	63.18± 2.30*
EEBA	**400**	#2.02 ±0.70*	#3.96 ± 0.02*	#39 ± 2.86*

Table 6: Effect of EEBA extract on pyloric ligation induced gastric ulcers

Group	Treatment	Dose(mg/kg)	Ulcer index	%Protection
I	**Control**	-	16.24 2.63	-
II	**Ranitidine**	50	4.42 0.42*	72.78
III	**EEBA**	100	12.93 1.27	20.38
IV	**EEBA**	200	8.86 2.53*	45.44
V	**EEBA**	400	#5.79 0.95*	64.34

Table 7: Effect of EEBA extract on Aspirin-induced gastric ulcers

Group	Treatment	Dose (mg/kg)	Ulcer index	%Protection
I	**Control**	-	14.84 1.12*	-
II	**Ranitidine**	50	3.36 0.79*	77.35
III	**EEBA**	100	10.24 0.97	30.99
IV	**EEBA**	200	6.69 1.85*	54.91
V	**EEBA**	400	#4.18 1.52*	71.83

*All of the above results were expressed as mean ± SEM, n=6, *P< 0.05; compared Standard and Test groups vs Control group, '#'- Indicates there is no significant difference between standard and test drug at P< 0.05 significant level.*

The Shay model of peptic ulcer in rat is a simple, reproducible and highly predictable model for the screening and evaluation of antiulcer drugs. Pylorus-ligated ulcers may be developed due auto digestion of gastric juiceby excess secretion of acid and pepsin, decrease mucosal blood flow and break down of mucosal barrier.

The pyloric ligated animals of control group had ulcer index of 16.24. Also volume of gastric juice was 4.19 ml/ 100 g, pH of gastric juice was 2.14 and total acidity was 109µEq/ml. It was revealed that, the ranitidine (50 mg/kg) treated animals showed significant lowering in ulcer index (i.e. %protection was72.78%).It also significantly reduced the gastric volume and total acidity; and increased the gastric pH as compared to control group. The ethanolic extract of *Basella Alba* was found to possess ulcer protective effects dose-dependently against Pylorus-ligation. The EEBA at 200 and 400 mg/kg dose levels showed significant lowering in ulcer index and also significantly reduced the gastric volume and total acidity; and increased the gastric pH. There was no significant difference found between the dose of 400 mg/kg of *EEBA* and ranitidine treated animals.

ASP-induced ulcer

Aspirin induced ulcer in rats were carried out and the results are recorded on the Table 7.

NSAIDs like aspirin enhance acid secretion, cause mucosal damage by interfering with prostaglandin synthesis and breaking up of the mucosal barrier. The control group animals had ulcer index of 14.84. It was found that, the ranitidine (50 mg/kg) treated animals showed significant lowering in ulcer index (i.e. %protection was 77.35%).The ethanolic extract of *Basella Alba*has showed ulcer protective effects dose-dependently. The EEBA at 200 and 400 mg/kg dose levels showed significant lowering in ulcer index. There was no significant difference found between the dose of 400 mg/kg of *EEBA* and ranitidine treated animals.

CONCLUSIONS

The results of the present work provide evidence that ethanol extract of *Basella alba* have antiulcer and cytoprotective activities in a dose dependent manner. In future, extensive studies may be carried out to establish the mechanism of actions of *Basella Alba*. The clinical evaluation of ethanol extract of *Basella Alba* on patients with peptic ulcer will throw more light on safety and efficacy of these plants.

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REFERENCES

1. Torres OJ, Santos OJ, Moura RS, et al. Activity of Orbignya phalerata and Euterpe edules in the prevention and treatment of peptic ulcer in rats. ABCD Arq Bras Cir Dig. 2018; 31(3), 1-4.
2. Amandeep K, Ranica S, Sunil K. Peptic Ulcer: A Review on Etiology abd Pathogenesis. IRJ of Pharmacy. 2012; 3 (6): 34-40.
3. Gadekar R, Singour PK, Chaurasiya PK, Pawar RS, Patil UK. A potential of some medicinal plants as an antiulcer agents. Pharmacogn Rev. 2010; 4(8): 136.
4. Umamaheswari M, Asokkumar K, Rathidevi R, Sivashanmugam AT, Subhadradevi V, Ravi TK. Antiulcer and in vitro antioxidant activities of Jasminum grandiflorum L. J Ethnopharmacol. 2007; 110 (3): 464–470.
5. Maity P, Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. The use of neem for controlling gastric hyperacidity and ulcer. Phytother Res. 2009; 23(6): 747-755.
6. Pinto LA, Cordeiro KW, Carrasco V, Carollo CA, Cardoso CA, Argadoña EJ, de Cássia Freitas K. Antiulcerogenic activity of Carica papaya seed in rats. Naunyn-Schmiedeberg's Archives of Pharmacol. 2015; 388(3): 305–317.
7. Melese E, Asres K, Asad M, Engidawork E. Evaluation of the antipeptic ulcer activity of the leaf extract of Plantagolanceolata L. Phytother Res. 2011; 25(8): 1174-1180.
8. Zewdu1 WS, Aragaw TJ. Evaluation of the anti-ulcer activity of hydromethanolic crude extract and solvent fractions of the root of Rumex nepalensis in rats. J of Exp Pharmacol. 2020;12: 325-337.
9. Islam MS, Rahi MS, Chowdhury AJ, Rahman MH, Israt J, Amin R, Kazi MFH, Reza MA. In Vivo Anticancer Activity of Basella alba Leaf and Seed Extracts against Ehrlich's Ascites Carcinoma (EAC) Cell Line. Evidence-Based Complementary and Alternative Medicine. 2018; 1-11.
10. Serafim C, Araruna ME, Júnior EA, Diniz M, Lima CH, Batista L. A Review of the Role of Flavonoids in Peptic Ulcer (2010–2020). Molecules. 2020; 25: 5431.
11. Aboshi T, Ishiguri S, Shiono Y, Murayama T. Flavonoid glycosides in Malabar spinach Basella alba inhibit the growth of Spodoptera litura larvae. Bioscience,

Biotechnology, and Biochemistry. 2018; 82,(1): 9–14.

12. Panigrahi G, Panda C, Patra A. Extract of Sesbania grandiflora ameliorates hyperglycemia in high fat diet-streptozotocin induced experimental diabetes mellitus. Scientifica. 2016; 1-10.
13. Kokate CK. Practical Pharmacognosy, 4th ed. NewDelhi: Vallabh Prakashan; 1994. p. 107-111.
14. Kiritkar KR, Basu BD. Indian Medicinal Plant, 3rd ed. Delhi. Sri Satguru Publication; 2000. p. 2587-2590.
15. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments, 11th ed. Pune: NiraliPrakashan; 2000. p. 149-156.
16. Ghosh MN. Fundamentals of Experimental Pharmacology. 3rd ed. Kolkata: Hilton & Company;2005. p. 175-197.
17. Panigrahi G, Patra A. Experimental Pharmacology- I, II and III (Bridges the Gap Between Animal Models and Computer Simulation Models). 1st ed. Pune: NiraliPrakashan; 2019.
18. Vogel HG. Drug discovery and evaluation: Pharmacological Assays. Vol II, 3rd ed. New York: SpringerVerlag Berlin Heidelberg; 2008. p. 1103-106.
19. Goel RK, Sairam K, Dorababu M, Prabha T, Rao ChV; Effect of standardized extract of Ocimum sanctum Linn. on gastric mucosal offensive and defensive factors; Indian J Exp Biol. 2005 Aug;43(8):715-21.
20. Goel R K, Govinda Das D and Sanyal A K: Effect of vegetable banana powder on changes induced by ulcerogenic agents on dissolved mucosubstance in gastric juice. Indian J Gastroenterol 4 (1985) 249-251.
21. Sanyal A K, Debnath P K, Bhattacharya S K and Gode K D: The effect of cyproheptadine on gastric activity: An experimental study, In: C.J. Pfeiffer (Ed.), Peptic ulcer, edited by C J Pfeiffer, Munksgaard, Copenhagen (1971); 312-318.
22. Sanyal A K, Mitra P K and Goel R K: A modified method to estimate dissolved mucosubstances in gastric juice. Indian J Exp Biol 21 (1983) 78-80.
23. Shanmugasundaram P, Venkataraman S. Hepatoprotective and antioxidant effects of Hygrophilaauriculata (K. Schum) Heine (Acanthaceae) root extract. Journal of Ethnopharmacol. 2006; 104: 124-128.