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# ASSESSMENT OF ANTI-ULCER ACTIVITY OF ETHANOLIC EXTRACT OF *BORAGO OFFICINALIS* FLOWERS BY PYLORUS LIGATION IN RATS

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## ABSTRACTS

**Context:**
Ulcer an inflamed erosions in the mucus membrane lining the alimentary tract, present in around 10% of the population due to an imbalance between the aggressive (acid, pepsin and *Helicobacter pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors. In recent years, abundant work has been carried out on herbal medicine to elucidate their potential effectiveness in gastric ulcer prevention or management. **Objective:** To evaluate the anti-ulcer activity of ethanolic extract of *Borago officinalis* flowers by pylorus ligation induced ulcer. **Methodology:** Ethanolic extract of *Borago officinalis* flowers were subjected to the acute oral toxicity study as per ICH Topic S7A guidelines and OECD 423 guidelines and 250 mg/kg body weight of dose was selected for further study. In pylorus ligation induced ulcer model Ulcer index, ulcer preventive index, volume and pH of gastric fluid, free acidity and total acidity were evaluated. **Result:** The extract showed significant reduction in ulcer index, gastric volume, total acidity, free acidity and increase in pH of gastric juice and percentage protection as compared to control. This present study indicates that Ethanolic extract of *Borago officinalis* flowers have potential antiulcerogenic property. **Conclusions:**In conclusion the antiulcer properties of the extract may be accredited to the presence of phytochemicals like polyphenols, flavonoids and substances such as gamma-linolenic acid.

**KEYWORDS:** *Borago officinalis*, Pylorus ligation, ulcer index.

## INTRODUCTION

Peptic ulcers are defects in the gastric or duodenal mucosa that extend through the muscularis mucosa which mainly caused due to an imbalance between the aggressive (acid, pepsin, and *Helicobacter pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors. [1] The time trends in the epidemiology of peptic ulcer reflect complex, multifactorial etiologies with the lifetime risk for developing an ulcer is approximately 10%. [2] Acid suppression is the general pharmacologic principle of medical management of a peptic ulcer, using histamine-2 receptor antagonists, proton pump inhibitors, Antacids, $PGE_1$ analogues, Bismuth salts and potassium-competitive acid inhibitors. Unfortunately drugs used presently are associated with frequent side effects such as Histamine-2 receptor antagonists causes confusion, gynecomastia and impotence; proton pump inhibitors causes most common adverse effects such as diarrhea, itching, skin rash, dizziness, headache, muscle aches and a higher than normal rate of respiratory infections; Aluminum containing antacids are associated with constipation, aluminum-intoxication, osteomalacia, and hypophosphatemia whereas $PGE_1$ analogues causes Cramps, diarrhea, abdominal pain, menstrual disorders (including heavy bleeding and severe cramping). Because of the incidence of adverse effects with synthetic medicines, herbal plants stand out as being exceptional for its ethnic, ethobotanical and ethnopharmaceutical use. Various Plants extracts and phytomedicines are used for treatment or prevention of peptic ulcers by various mechanisms like antioxidant, cytoprotective, antisecretory action.[3]

*Borago officinalis* is an annual herb which is cultivated for medicinal and culinary uses belonging to the family *Boraginaceae.* The primary phytoconstituents of *Borago officinalis* flowers include mucilage, tannin, saponins, essential oil, alkaloid (pyrrolizidine), vitamin C, calcium and potassium. The plant reported to contain essential fatty acids, linoleic acid and gamma-linolenic acid. It is used for the treatment of various diseases such as pre-menstrual pain, multiple sclerosis, rheumatoid arthritis, asthma multiple sclerosis, diabetes, heart diseases,

arthritis, eczema bronchitis, palpitations, cramps and diarrhea. It has been reported that borage has antipyretic, adaptogenic, anxiolytic, aphrodisiac, antispasmodic, demulcent, hypocholesterolemic, antihypertensive and diuretic effects. [4][5] The present study was intended to assess the antiulcer activity of *Borago officinalis* flowers for the treatment or prevention of peptic ulcers.

## MATERIALS AND METHOD

### Collection of Plant Material

*Borago officinalis* flowers were collected from the local medicinal plant supplier and were authenticated by Dr. P. Veera Reddy, Professor, Anantha Lakshmi Government Ayurvedic Medical College, Warangal, Telangana, India.

### Drugs and Chemicals

Omeprazole was procured from Ranbaxy Research Laboratories, India and Tween 80 was obtained from S.D Fine Chemicals, India. All other reagents used for the experiments were of high analytical grade.

### Preparation of Plant Extract

The *Borago officinalis* flowers were shade dried and it was then powdered in an electrical processo. 50 gram of dried powder material was extracted in a soxhlet apparatus with 200 ml. of ethanol. The ethanolic extract was distilled, evaporated and then dried in vacuum. The resultant extract was kept in desiccator and stored in a refrigerator for carrying out acute toxicity study and antiulcer activity.

### Animals Used

Female Wistar rats weighing about 180-200 grams were used in experiments. Rats were housed in polypropylene cages with not more than three animals per cage and kept under standard condition of 12 hours light / dark cycle; relative humidity 48%; temperature 25± 3°C and had free access to standard rat pellet diet (Hindustan Lever Ltd., India) and water *ad libitum*. All the animals were acclimatized to laboratory condition for 7 days before beginning of experiments. The experimental procedures were appraised and approved by Institutional animal ethical committee and experiments conducted according to CPCSEA.

### Acute toxicity studies

The acute toxicity study to carry out the gross behavioral effects and safety effects of the *Borago officinalis* flowers was carried on rats weighing about 180- 200 as per ICH Topic S7A guidelines and OECD 423 guidelines. [6][7] Overnight fasted mice received the test extract at a dose of 5 mg/kg bodyweight orally and mortality was observed for first 24 hours, with special attention for the first 4 hours and daily then, for a total of 14 days. If no mortality was observed for any rats, then the procedure was repeated again with doses of 50, 300 and 2000 mg/kg orally. The extract was well tolerated by the rats without any explicit signs of toxicity.

### Methodology: Pylorus- ligation induced gastric ulcer[8]

A simple and reliable method for production of gastric ulceration in the rats is based on ligature of the pylorus. The animals were divided into three groups containing six rats in each group. After having free access to water and food ad libitum, rats were fasted for 48 hours. The animals were divided into three groups containing six animals each group. Group 1was treated as control and administered with 1ml of 1% Tween 80 (p.o), Group 2 was treated with ethanolic extract of Borago officinalis flowers (250mg/kg, p.o) and Group 3 was treated with Omeprazole (25mg/kg, p.o) for fourteen days. On fifteenth day under ether anesthesia midline incision was made below the Xiphoid process and without damaging the blood supply pylorus was ligated. The abdominal walls was closed by interrupted sutures, animals were allowed to stabilize. The rats were deprived of both food and water during the postoperative period.

#### *Determination of Ulcer Index*

The stomachs of rats were opened along the greater curvature, then washed in warm water, and examined under a 3-fold magnifier. The number of ulcers is noted and the severity recorded with the following scores: 0-no ulcer; 1-superficial ulcers;2- deep ulcers and 3-perforation.

An ulcer index $U_I$ was calculated by the following formula

$$U_I=U_N+U_S+U_P x10^{-1}$$

Where $U_N$= average of number of ulcers per animal; US = average of severity score; UP = percentage of animals with ulcers

$$\text{Ulcer Preventive Index(\%Protection)} = \frac{\text{Ulcer Index in control} - \text{Ulcer Index in treated}}{\text{Ulcer Index in control group}} \times 100$$

#### *Determination of gastric volume*

Four hours after the operation animals were sacrificed, abdomen was opened and a ligature was placed around the esophagus close to the diaphragm. The stomach was opened the contents of the stomach were collected in the centrifuge tubes and volume of gastric juice was noted down.

#### *Determination of pH of gastric content*

An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and pH of the solution was measured using digital pHmeter.

#### *Determination of free acidity and total acidity*

The gastric contents were centrifuged at 1000 rpm for 10 min. One ml of the supernatant liquid was pipetted out and diluted to 10 ml with distilled water. Then free acidity of gastric juice was estimated by titration with 0.01 N sodium hydroxide using Topfer's reagent as an indicator, to the end point of yellowish orange colour. The volume of sodium hydroxide consumed was noted which indicates free acidity. Now 2 drops of phenolphthalein indicator was added and titration was

continued until a definite pink colour appears. The volume of sodium hydroxide required was noted and was taken as corresponding to the total acidity. The acidity is expressed as mEq/L by the following formula:

$$\text{Acidity} = \frac{\text{Volume of NaOH Consumedx Normalityx100 mEq/l}}{0.1}$$

## STATISTICAL ANALYSIS

Values were expressed as Mean ± standard error of the mean. The Significance of differences among the treated groups was evaluated using one way analysis of variance (ANOVA). The test followed by Dunnett's multiple comparisons test of significance. p values less than 0.05 were considered as statistically significant.

## RESULT

### Acute Toxicity Study

The ethanolic extract of *Borago officinalis* flowers was found to be safe at the maximum dose of 2000mg/kg body weight by Oral route. Even after 14 days, rats were found to be well tolerated with no mortality and no signs of toxicity. Hence the dose of 250 mg/kg was selected for the study.

### Antiulcer Activity of extract in Pylorus- ligation induced gastric ulcer

Oral administration of ethanolic extract of *Borago officinalis* flowers at 250mg/kg, orally produced a significant reduction in ulcer index of as compared to the ulcer control group. The Ulcer Preventive Index of 42.69 % was obtained, whereas that of preventive index of the reference standard drug (omeprazole) was 69.02% (Table 1).

**Effect of various extracts of ethanolic extract of *Borago officinalis* flowers against Pylorus ligation induced gastric ulcer in rats**

| S.No | Treatment | Ulcer index | Ulcer Preventive Index# | Volume of gastric fluid (ml/100 gm) | pH of gastric fluid | Total acidity (mEq/l/100g) | Free Acidity (mEq/l/100g) |
|---|---|---|---|---|---|---|---|
| 1 | Control | 19.79±0.89 | - | 7.44±1.53 | 2.38± 0.19 | 113.54±2.63 | 78.33±2.44 |
| 2 | Extract | 11.34±1.13** | 42.69 | 4.81± 0.67** | 3.77±0.22** | 79.67±2.58** | 46.49±1.82** |
| 3 | Omeprazole | 6.13±0.76** | 69.02 | 3.02± 0.58** | 4.69±0.38** | 63.24±3.84** | 32.67±1.34** |

#the ulcer preventive index for each group was calculated by comparison with the ulcer control group considered as 100% of gastric damage.

Values are mean ± S.E.M. (n=6) One way ANOVA followed by Dunnet's test. **P < 0.01 when compared to control.

## DISCUSSION

This study has been carried out to assess the antiulcer activity of ethanolic extract of *Borago officinalis* flowers by pylorus ligation method in rats. A peptic ulcer is erosion in the protective mucosal lining of the lower esophagus, stomach, or duodenum which results from an imbalance between factors that can damage the gastro-duodenal mucosal lining and defense mechanisms that normally limit the injury. The major destructive factors for peptic ulcer disease are gastric juice (including hydrochloric acid, pepsin, and bile salts refluxed from the duodenum*),H. pylori* infection of the gastric mucosa and habitual use of NSAIDs. Alcohol and smoking might influence susceptibility to ulcer disease. Some chronic diseases, such as emphysema, rheumatoid arthritis, and cirrhosis, are related with the development of peptic ulcers. Psychological stress could also be a risk factor for peptic ulcer disease.[9]

Mucosal defenses include a mucus bicarbonate layer secreted by surface mucus cells forming a viscous gel over the gastric mucosa; the integrity of tight junctions between adjacent epithelial cells; and the process of restitution, whereby any break in the epithelial lining is rapidly filled by adjacent epithelial and mucosal stromal cells migrating and flattening to fill the gap. [10] Pylorus ligation in rats causes ulceration by accumulation of acidic gastric juice in the stomach which in turn causes auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier. In the present study omeprazole is used as a standard drug to compare the efficacy of plant extract. Omeprazole is a selective and irreversible proton pump which suppresses stomach acid secretion by specific inhibition of the $H^+/K^+$-ATPase system found at the secretory surface of gastric parietal cells. [11] We have observed from our study that significant reduction in ulcer index, gastric volume, total acidity, free acidity and increase in pH of gastric juice and percentage protection in ethanolic extract of *Borago officinalis* flowers in pylorus ligated ulcer model when compared to control rats. This study indicates that ethanolic extract of *Borago officinalis* flowers exhibit antiulcer activity by suppressing damage to gastric mucosal layer by aggressive factor mainly gastric acid.

## CONCLUSION

Approximately 50% of patients experience a recurrence within a year if anti-ulcer medication is stopped. The present observations provide evidence that ethanolic extract of *Borago officinalis* flowers can be used for the treatment, prevention of peptic ulcers or as an adjuvant therapy for management of peptic ulcer. Further studies are required for their exact mechanism of action on gastric acid secretion and gastric cytoprotection.

## REFERENCES

1. Bashir Ado Ahmad, Mahadeva US Rao, Abdurrazaq Muhammad, et al. Reviews of herbal and their secondary metabolites in the treatment of ulcerative colitis and peptic ulcer. Journal of Applied Pharmaceutical Science, 2014; 4(08): 080-090.
2. Snowden FM. Emerging and reemerging diseases: a historical perspective. Immunol. Rev., 225(1): 9–26.

3. Rahul A W, Gajanan B B, Rahul S. S, et al. A Review on Medicinal Plants with Anti-Ulcer Activity. Journal of Pharmacognosy and Phytochemistry, 2013; 2(1): 235-240.
4. Mayank Gupta, Swati Singh. *Borago Officinalis* Linn. An Important Medicinal Plant Of Mediterranean Region: A Review. *International Journal of Pharmaceutical Sciences Review and Research., 2010;5(01):* 005.
5. Mohammad Abu Bin Nyeem, Mohammad Sadul Haque, Mohammad Abdul Hoque, et al. Phytoconstituents and pharmacological activity of Gauzaban (*Borago officinalis* Linn): A review. International Journal of Food Science and Nutrition, 2017; 2(1): 148-152.
6. ICH Topic S 7 A, Safety Pharmacology Studies for Human Pharmaceuticals. Note for guidance on safety pharmacology studies for human pharmaceuticals. [Cited on 16 December 2016]. Available from: www.ema.europa.eu/docs/en_GB/document_library/.../09/WC500002831.pdf
7. OECD/ OCDE, OECD Guideline for Testing of Chemicals, Acute Oral Toxicity- Acute Toxicity Class Method, 423. Adopted 17th December, 2001. [Cited on 16 December 2016]. Available from: https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf
8. Shay H, Komarow SA, Fels SS, et al. A simple method for the uniform production of gastric ulceration in the rat. Gastroenterol., 1945; 5:43–61
9. Peptic Ulcer. McCance, Kathryn L. Huether, Sue E. Pathophysiology: The Biologic Basis for Disease in Adults and Children, ed 6. Mosby, Inc., 200; 1464-1465
10. BMJ, Royal College of Physician. Peptic Ulcer Disease. [Updated: 2015, Oct 15; Cited: 2017, Feb 21] Available from: http://bestpractice.bmj.com/best-practice/monograph/80/basics/pathophysiology.html
11. Jai Moo Shin, and George Sachs. Pharmacology of Proton Pump Inhibitors. Curr Gastroenterol Rep., 2008; 10(6): 528–534.