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EVALUATION OF ANTI-ULCER ACTIVITY OF ETHANOLIC EXTRACT OF SEEDS OF *HORDEUM VULGARE* IN RATS

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ABSTRACT
The present study was aimed to evaluate the anti-ulcer activity of ethanolic extract of *Hordeum vulgare* on ulcer formation and gastric acid release in rats. Pylorus ligation method was used to induce gstric ulcers in rats. Five groups of male wistar albino rats were used in the study. Group 1 serves as normal control group, Group 2 serves as ulcer control, Group 3 treated with 100mg/kg of test compound and Group 4 treated with 200mg/kg of test compound and Group 5 treated with 3.6mg/kg of Omeprazole and serves as standard control group. Before treatment with test and standard drugs ulcer was induced by tight ligation of pylorus portion of stomach. Immediately after pylorus ligation test and standard drugs were administered. After 19 hours rats were sacrificed and ulcer index, percentage protection was determined. The stomach contents were analyzed for gastric acid volume, gastric acid pH and gastric acidity. Both Omeprazole and test compound showed significant reduction ($P< 0.01$) in ulcer index, gastric acidity and increase in gastric acid pH. Test compound has showed more significant effect ($p<0.05$) over Omeprazole in decreasing the gastric acid volume. Treatment with test compound showed significant protection against ulcers induced by pylorus ligation. Histological studies revealed the ulcer control group exhibited severe damage of gastric mucosa compared to rats treated with test compound and omeprazole which showed gastric mucosal protection. The present findings suggest that test compound promotes ulcer protection as ascertained grossly and histologically as compared to the ulcer control group. In conclusion test compound possesses anti ulcer property which might be due to its antisecretory activity.

KEYWORDS: *Hordeum vulgare*, pylorus ligation, Ethanol, antiulcer activity, ulcer index, gastric acid volume.

INTRODUCTION
Gastric or peptic ulcer disease is the most common ulcer of an area of the gastrointestinal tract that is usually acidic and thus extremely painful. Gastric ulcers occur due to imbalance between agressive (acid, pepsin and *Helicobacter pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors). Peptic ulcer disease is a chronic pathology that affects millions of people worldwide. It is believed that 10% of the population will develop this condition at some point in their lives (Zapata-Colindres*et al.*, 2006). Peptic ulcers are usually classified by their anatomic location, such as gastric or duodenal ulcers, and increased gastric acid is the main cause. There is a strong association between H. pylori infection and duodenal ulcers. H. pylori causes an inflammatory response in the gastric mucosa, with increased production of cytokines (Mercus *et al.*, 2013) and influx of neutrophils and macrophages into the gastric mucosa with release of leukotrienes (LT) and reactive oxygen species, which makes the defense of the mucosa and stimulates of ulcer formation process (Prabhu and Shivani, 2014). There are two main approaches for treating peptic ulcer. The first deals with reducing production of gastric acid and the second reinforcing gastric mucosal protection. Although a number of anti ulcer drugs are available, proton pump inhibitors are most potent inhibitors of acid secretion available today.

From the times immemorial, drugs of natural origin served as excellent source to attain and maintain good health and worked as potential to heal wide range of diseases. In the developed countries, there are several lines of studies about *Hordeum vulgare* and its importance in the treatment of different diseases by health experts and pharmacological organizations. *Hordeum vulgare* consists of 12.98% nitrogenous substances, 15% water, 2.70% lipids, 6.74% gum, 3.2% sugar, 59.95% starch and also some carbohydrates, alkaloids, and amino acids. Barley (*Hordeum vulgare* L., *H. volgare*) is an important miscellaneous grain and a widely used cereal, because of its dietary health advantages, ready availability and low costs. Barley is mostly known for its high amount of dietary

fiber such as β-glucan that may decrease the risk of coronary heart disease (Lee et al., 2010). Barley leaves have also a high antioxidant activity that might be useful in metabolic syndrome prevention or therapy, as well as diseases caused by oxidative stress damage. This property is mainly attributed to saponarin, a flavonoid with potent antioxidant activity found in young green barley leaves (Kamiyama and Shibamoto, 2012). Barley is a rich source of magnesium, a mineral that acts as a co-factor for more than 300 enzymes, including those involved in glucose metabolism and insulin secretion. The biological effect of *Hordeum vulgare* has been attributed to Hordinine and its metylated ester. With regard to the several beneficial effects mentioned for *Hordeum*, we hypothesized that the administration of this substance can be effective in the improvement of gastric ulcer. Therefore, the aim of this study was to investigate the anti-ulcer activity of this substance in rats.

MATERIALS AND METHODS

Plant material and extraction

Hordeum seeds were collected from local residential area. Then it was recognized by botanist from Hyderabad Central University. Immediately, it was washed thoroughly with running tap water and cut into small pieces. Then the plant material was shade dried at temperature 21-24°C and ground mechanically into a coarse powder and stored in an airtight container. Powdered plant material (150 g) was macerated with 400 ml of distilled water at 21-24°C temperature for 3 days with frequent shaking. After 3 days, the extracts were filtered and to the marc part 300 ml of the solvent was added and allowed to stand for next 2 days at same temperature for second time maceration (re-maceration) and after two days, again filtered similarly. The combined filtrates (macerates) were evaporated *in vacuo* at 40°C and the dry extract obtained was stored in a vacuum desiccator for future use. All the test samples were administered by oral lavage in a volume of 1 ml/100 g body weight once a day to each rat. (Phaechamud et al 2008)

Chemicals

Omeprazole (OMEZ 20mg) was gift by Dr. Reddy's laboratories, Solan, India. All the chemicals were obtained from SD fine Chemicals, India.

Experimental Animals

Adult male wistar rats weighing between 200±20g (Mahaveer enterprises Hyderabad) were used in the study. The animals were acclimatized to standard laboratory conditions of temperature ($27^{o}C \pm 1^{o}C$) and maintained on 12:12 h light: dark cycle in animal house. They were housed in elevated wire cages and provided with regular rat chow (Standard pellet diet – Nutrivet life sciences, Pune, India) and distilled water *ad libitum* for 2 weeks. The animal care and experimental protocols were in accordance with CPCSEA / IAEC.

Method of Induction of Gastric ulcers

Pylorus ligation induced gastric ulceration model was used to induce ulcers in rats (Manoj *et al*., 2003). In briefly, the animals were divided in to 5 groups containing six in each and kept in metabolic cages. For the induction of gastric ulcers, the albino rats were fasted for 48hrs but water was allowed *ad libitum* prior to pylorus ligation. Under light ether anesthesia abdomen was opened by a small midline incision below the xiphoid process, then the stomach was ligated in the pyloric region. The stomach was placed carefully and the abdominal wall was closed by interrupted sutures. Rats were administered with ethanolic extract of *Hordeum vulgare* and standard drug (Omeprazole) immediately after pylorus ligation.

Study design

About 30 Male Wistar albino rats were taken and divided into 5 groups with 6 in each. Drugs and vehicle were administered through oral route. **Group 1:** Treated with 0.5% sodium CMC (Normal control group); **Group 2:** Ulcer induced using pylorus ligation method (Ulcer control group); **Group 3:** Treated with *Hordeum vulgare* with a single dose of 100mg/kg (Test1); **Group 4:** Treated with *Hordeum vulgare* with a single dose of 200mg/kg (Test2); **Group 5:** Treated with Omeprazole 3.6mg/kg (standard control group).

Collection and Analysis of stomach contents

The 19h after pylorus ligation, rats were killed with over dose of ether and the stomach was dissected out and cut open along the greater curvature. The stomach contents were drained out and centrifuged at 1,000 rpm for 10 minutes. The supernatant was collected and analyzed for gastric acid volume, gastric acid pH and gastric acidity.

Gastric acid volume

The total amount of gastric acid present in the stomach after pits dissection was collected in centrifuged tubes. The tubes were centrifuged at 1000 rpm for 10min and the gastric volume was directly read from the graduation on the tubes (Amit *et al*., 2011).

Gastric acid PH

The pH was estimated using Indikrom pH strips (Glaxo India Limited, India) with pH ranges of 2.0-4.5 and 5.0-8.5 with a difference in range of 0.5. Or using digital pH Meter pH 142(Cyber Labs, Mumbai, India) (Bhave *et al*.,2006).

Determination of total acidity

Total acidity was measured by diluting 1ml of gastric juice with 1ml of distilled water in a 50ml conical flask, two drops of phenolphthalein indicator was added to it and titrated with 0.01 N NaOH until a permanent pink color exists. The volume of 0.01 NaOH consumed was noted. The total acidity was expressed as below (Dalputre *et al*., 2011).

Determination of free acidity

Gastric juice was titrated with 0.01N NaOH using Topfer's reagent (Dimethylaminoazobenzene) instead of Phenolphthalein indicator until canary color was observed. The volume of 0.01N NaOH consumed was noted. The free acidity was calculated by the same formula used for the determination of total acidity (Dalputre *et al*., 2011).

Measurement of ulcer index

Immediately after the animals were sacrificed, their stomachs were dissected out, incised along the greater curvature and the mucosa were rinsed with cold normal saline to remove blood contaminant if any. Tissues were kept overnight in 10% formaline solution. Next day the ulcers were examined under a magnifying lens and measured with a scale. The ulcer index is calculated by using the formula (Moumita *et al*., 2010).

Ulcer index = Area of ulcers * 100/Total stomach area.

Measurement of percentage inhibition of ulcers

Percentage inhibition of ulceration was calculated as below (Samuel *et al*., 2011):

$$\% \text{ Inhibition of Ulceration} = \frac{(\text{Ulcer index}_{\text{Control}} - \text{Ulcer index}_{\text{Test}}) \times 100}{\text{Ulcer index Control}}$$

Histopathological examination

For histopathological examination the stomach tissues were removed from the animals and placed in a fixative to evaluate the histopathological changes. In brief, fresh stomach tissues previously trimmed to approximately 2 mm thickness were placed in plastic cassettes and immersed in neutral buffered formalin for 24 h. The fixed tissues were processed routinely and then embedded in paraffin blocks, sectioned with microtome (0.7 µ thickness) deparaffinized and rehydrated using standard techniques. The extent of protection by test compounds against the damage caused by ulcers was evaluated by assessing morphological changes in stomach sections stained with hematoxylin and eosin using standard techniques and photographed.

Statistical analysis

All data are expressed as mean ± SD. The significance was determined by using Student's unpaired t-test. For comparison treated group with control group, One-way analysis of variance (ANOVA) followed by Dunnet test was performed. P value less than 5% (P <0.05) was considered to be statistically significant.

RESULTS

In the present study, we have used pylorus ligation method for the induction of ulcers in rats and the test compound was administered orally to evaluate for the antiulcer activity. The following results were found with the above experimentation.

Volume of Gastric acid

Treatment with test compound in rats resulted in a decrease in volume of gastric acid at 19h post treatment. The decrease in gastric volume in test compound treated group was found to be dose dependent and statistically significant (table 1). No significant change (P>0.05) was observed after treatment with Omeprazole 3.6mg/kg and test compound 20mg/kg.

Table: 1 Volume of gastric acid in animals of different groups.

Groups	Volume of gastric acid (ml)
Normal control	-
Ulcer control	2.89±0.47
Test (100mg/kg)	2.32±0.21*
Test (200mg/kg)	1.93±0.23**
Omeprazole(3.6mg/kg)	1.27±0.21**

*All the values are expressed as Mean ± SD; *P<0.05, **P<0.01 vs Ulcerative control.*

Gastric acid pH

Similar to the Omeprazole, treatment with test compound to rats resulted in an increase gastric acid pH values and the results were showed in table 2.

Table: 2 pH of gastric acid of different groups of animals.

Groups	Gastric acid pH
Normal control	-
Ulcer control	3.07±0.87
Test (100mg/kg)	3.90±0.25*
Test (200mg/kg)	4.97±0.14**
Omeprazole(3.6mg/kg)	6.02±0.32**

*All the values are expressed as Mean ± SD; *P<0.05, **P<0.01 vs Ulcerative control.*

Acidity

Similar to the Omeprazole, treatment with test compound to rats resulted in a decrease in free acidity and total acidity values at 19h post treatment. The decrease free acidity & total acidity values are found to be significant in group of rats treated with Omeprazole 3.6mg/kg ($p<0.01$), Test (100mg/kg)($p<0.05$) and test 250mg/kg ($p<0.01$) (table 3).

Table: 3 Gastric acidity of gastric acid of different groups of animals.

GROUPS	FREE ACIDITY	TOTAL ACIDITY
Normal control	-	-
Ulcer control	7.0±2.94	12.85±3.75
Test (100mg/kg)	5.8±1.41*	10.65±2.11*
Test (200mg/kg)	4.1±0.56**	7.6±1.83**
Omeprazole(3.6mg/kg)	2.5±0.35**	5.45±1.61**

*All the values are expressed as Mean ± SD; *P<0.05, **P<0.01 vs Ulcerative control*.

Ulcer Index

It is defined as the severity of damage caused by an ulcer inducing agent. The ulcer indices in all the group of treated rats were showed in table 4.Similar to the Omeprazole, treatment with test compound to rats resulted in a significant ($p<0.01$) and dose dependent decrease in ulcer indices at 19h post treatment.

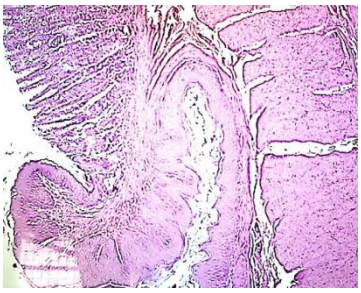
Table: 4 Ulcer indices of stomach tissues in animals of different groups.

Groups	Ulcer Index	% Protection
Normal control	-	-
Ulcer control	20.5±9.42	-
Test (100mg/kg)	12.95±3.13*	36.8%
Test (200mg/kg)	7.5±0.41*	63.4%
Omeprazole (3.6mg/kg)	2.50±1.82*	87.8%

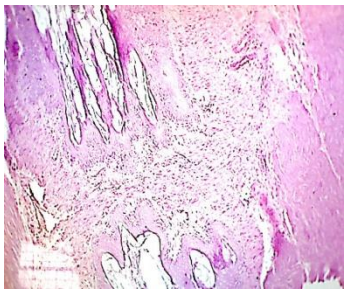
*All the values are expressed as Mean ± SD; *P<0.01 vs Ulcerative control*.

Histopathological analysis

The analysis of histopathological examination of rat stomach in different groups is as follows: **Normal control group (Fig 1a)**: There is no disruption to the surface of mucosal epithelium and no infiltration of mononuclear cells of the submucosal layer. **Ulcer control group (Fig 1b)**: There is severe disruption to the surface epithelium of mucosa with leucocyte infiltration of the submucosal layer. **Test group (100mg/kg) (Fig 1c)**: There is mild disruption to the surface of epithelial mucosa with leucocyte infiltration of the submucosal layer. **Test group (200mg/kg) (Fig 1d)**: There is no disruption to the surface of mucosal epithelium and no infiltration of mononuclear cells of the submucosal layer. **Standard contol group (3.6mg/kg of Omeprazole) (Fig 1e)**: There is mild disruption to the surface of epithelial mucosa with leucocyte infiltration of the submucosal layer.



1a



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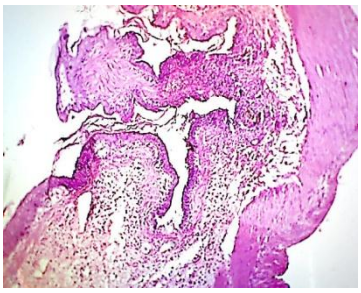

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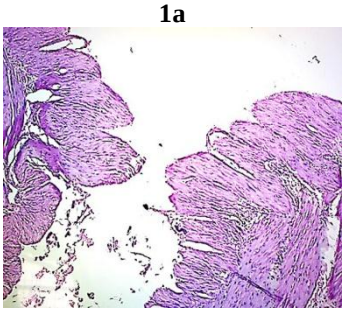

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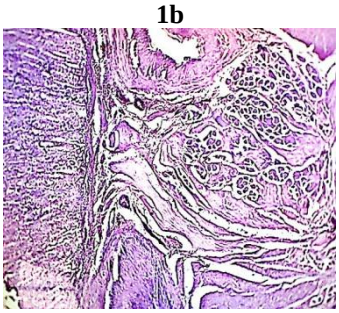

1e

DISCUSSION

The etiology of peptic ulcer is mainly because of an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms (Goel & Bhattacharya, 1991). To regain this balance, different therapeutic agents are used to inhibit the gastric acid secretion or to increase the mucosal defense mechanisms by increasing mucus production or interfering with prostaglandin synthesis (Akhtar et al., 1992). In this study the antiulcer effect of *Hordeum vulgare ethanolic extract* (test compound) was evaluated against the gastric lesions-induced by pylorus ligation. The test compound prevented the mucosal lesions induced by meliorated pylorus ligation-induced gastric damage. Pylorus ligation produces the mucosal damage by interfering with the gastric mucosal resistance. The causes of gastric ulcers after pylorus ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion. The secretion and accumulation of gastric acid are the two factors responsible for the production of gastric ulcer by the pylorus ligation (Manoj *et al.*, 2003). Omeprazole a proton pump inhibitor is used as a standard drug in the present study. It reversely inhibits the gastric acid pump which is the final common pathway for acid secretion in response to all varieties of stimuli. In the present study, it was confirmed the antiulcer activity of test compound by its inhibitory effects on ulcer index, gastric acid volume, free acidity and total acidity. Test compound contains alkaloids like hordinine which increased the pH of the gastric contents and thus exerted its gastroprotective effect by inactivation of pepsin. In support of the results, the histopathological examination of the stomach tissues revealed the protection of gastric mucosa and inhibition of leucocytes infiltration of gastric wall in rats treated with test compound when compared to ulcer control group. In view of all the results, it was confirmed the antiulcer activity of the test compound in rats.

CONCLUSION

Hordeum vulgare ethanolic extract of seed is found to be an efficacious agent in reducing the ulcer index, increased volume of gastric acid, increased gastric acidity caused by pylorus ligation in statistically significant manner. It also significantly protect the gastric mucosa against pylorus ligation-induced gastric injury. Such protection was shown by the reduction of mucosal epithelial disruption as well as inhibition of leucocytes infiltration of submucosal area. These observations proved its antiulcer activity. But further study is needed in order to understand the precise mechanism and side effects.

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