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# Phytochemical screening and Evaluation of antiulcer activity of hydroalcoholic extract of *Tagetes Patula* flowers

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**ABSTRACT**

The main aim of the study was to determine the phytoconstituents present in flower of *Tagetes Patula*. The present study was therefore carried out to evaluate the anti-ulcer activity of Hydroalcoholic (70% methanol) flower extract of *Tagetes Patula* in rats. The effect of *Tagetes Patula* extract on gastric ulcer in rats in indomethacin induced gastric ulcers model and ethanol-induced models was studied. Therefore, this study validates its anti-ulcer use in Ethiopian folk medicine. Further investigations on isolation of specific phytochemicals and elucidating mechanisms of action are needed.

**Keyword:** Stomach Ulcer, *Helicobacter pylori (H. pylori)*, *Tagetes Patula* flower extract.

## 1. Introduction

### 1.1 Peptic ulcer

Peptic ulcer is a common disease throughout the world. It represents one of the major health problems, both in terms of morbidity and mortality. Gastric hyperacidity and ulcer are very common causing human suffering today. It is an imbalance between damaging factors within the lumen and protective mechanisms within the gastro duodenal mucosa. Although prolonged anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation, the mechanism is still very poorly understood.

Peptic ulcer is a broad term which includes ulcers of digestive tract in the stomach or the duodenum. Earlier it was believed that one developed this type of ulcers due to stress and spicy food. However, recent research has shown that these are just the aggravating factors. The causative agent is infection

caused by the bacteria H. pylori or reaction to certain medicines like non-steroidal anti-inflammatory drugs (NSAIDs). Symptoms of peptic ulcers include abdominal discomfort and pain. Other symptoms include weight loss, poor appetite, bloating, nausea, and vomiting. Some may also experience blood in stool and vomit,and black stools that indicate gastrointestinal bleeding.

More recently the role of mucosal factors in peptic ulceration has receivedconsiderable attention, and the term "cytoprotection" has been introduced to encompass the physiological processes which protect gastric mucosa from acid-pepsin digestion. Most of these cytoprotective mechanisms are related, at least in part, toendogenous prostaglandin secretion. The usual medical treatment for peptic ulcer is either by the inhibition of acid secretion or by neutralization of the acid. The neutralization of gastric acid can be done by antacid administration but their effectiveness is only for a brief period. Muscarinic antagonists such as atropine or pirenzipine are effective inhibitors of acid production. The histamine $H_2$-receptor antagonist cimetidine, ranitidine and famotidine act as potent inhibitors (70-80%) of acid secretion. Complete inhibition of parietal cells acid secretion by receptor antagonist is difficult because of complexity of known receptors on parietal cells and a variety of second messenger signaling system coupled to these receptors which involve adenylate cyclase coupled with histamine receptor and intracellular $Ca^{2+}$ with acetylcholine receptors. Thus, the most successful and desirable therapy is to inhibit the enzyme responsible for acid secretion. Gastric $H^+$-$K^+$ ATPase of the parietal cell is the $H^+$ ion pump responsible for acid secretion in the stomach and has been identified as a pharmacological target for the development of drug to treat ulcers. Long lasting inhibition of the $H^+$-$K^+$ ATPase by drugs such as omeprazole, lansoprazole and timoprazole has been shown effective in the treatment of peptic ulcer disease. However, such agents irreversibly inactivate the ATPase and the return of acid secretion following such as inhibition requires *denovo* synthesis of new pump. This isthe drawback of such type of inhibitors, because acid secretion is only achieved when new ion pumps are synthesized. This can be overcome by the use of reversible inhibitors of $H^+$-$K^+$ ATPase which may allow greater control over the duration of suppression of acid secretion. Other drugs, such as prostaglandins, carbenoxoline and sucralfate, stimulates mucus production. The negative charge conferred on mucous, particularly by its sulphate radicals, has resulted in to the development of new compound like the basic aluminum sucrose, octasulfate (sucralfate), which has properties similar to mucus. Thus, sucralfate and other cytoprotective drugs are effective in the management of peptic ulcer disease by their predominant actions on mucosal defensive factor.

### 1.2. Causes of ulcer

#### 1.2. 1. *Helicobacter pylori* gastritis

Two Australian researchers discovered the bacterium *Helicobacter pylori* and deciphered its role in gastritis and peptic ulcer disease, have been awarded this year's Nobel Prize in Physiology or Medicine. They revealed that gastritis, and ulceration ofthe stomach or duodenum, were the result of infection with some curved Gram negative bacilli.

### 1.2.2. Acid-pepsin secretion

It has been reported that about 50% of gastric ulcer patients are pepsin and acid hypersecretors. It is the first line of mucosal defense to prevent bacterial colonization and reduced their ability to entrance in the mucosal layer.

### 1.2.3. Mucus secretions

Mucus secretion is a crucial factor in the protection of gastric mucosa from the gastric lesions and has been regarded as an important defensive factor in the gastric mucus barrier. A decrease in the synthesis of sulphated mucus glycoprotein has been implicated in the aetiology of gastric ulcer.

### 1.2.4. Gastritis

Gastritis is inflammation (irritation) of the stomach lining. This may be caused by many factors including infection, alcohol, particular medications and some allergic and immune conditions. Gastritis can be either acute (with severe attacks lasting a day or two) or chronic (with long-term appetite loss or nausea). In many cases, gastritis has no symptoms (asymptomatic). Some forms, including chronic atrophic gastritis, have been associated with an increased risk of stomach cancer. Treatment options include avoiding exposure to known irritants and taking medication to reduce the amount of gastric juices.

### 1.2.5. Local irritants

Numerous other factors causing this disease include smoking habits, alcohol consumption, coffee drinking and familial occurrences of peptic ulcers in patients with gastric or duodenal ulcer. Epidemiologic studies suggest that smokers are about twice as likely to develop peptic ulcer disease as non-smokers. Smoking increase gastric acid secretion and duodenogastric reflux and decreases both gastroduodenal prostaglandin production and pancreatic duodenal bicarbonate production.

### 1.2.6. Dietary factors

Various types of food stimulate mucosal defense factors in experimental models. Incidence of peptic ulcer disease has decreased due to increase in the use of dietary essential fatty acids since the beginning of 20 century. Intake and handling of rice in various areas of the world may also explain peptic ulceration, as fresh rice oil in animal experiments protect against gastric ulceration, but stored oil is ulcerogenic. Salt increases mortality from gastric but not duodenal ulcer.

### 1.2.7. Psychological factors

Conventionally, peptic ulcer disease has also been considered to be a stress- associated psychosomatic disease. Importance of emotional disturbances due to stresshas long been shown to be a consideration in the pathogenesis of this disease. There is evidence that psychological stress induces many ulcers and impairs response to treatment. This stress probably functions most often as a cofactor with *H. pylori*. It may act by stimulating the production of gastric acid or by promoting behavior that causes a risk to health.

### 1.2.8. Endogenous mediators

Several endogenous mediators or substances, including lipid metabolites, neuropeptides, biogenic amines, reactive oxygen species and free radicals have been identified and reported to be involved in the induction of gastrointestinal lesions.

### 1.2.9. Platelet-Activating Factor (PAF)

PAF is one of the most potent ulcerogens (Rosam et al., 1986). The mechanism involved in PAF induced ulceration is the sequestration of nuetrophil aggregates in stomach, vasoconstriction, generation of free radicals and release of lysosomal enzymes.

### 1.2.10. Thromboxane A2 (TXA2) and Leukotriens (LTC4/D4)

TXA2 and LTC4/D4 are derived from arachidonic acid through the action of enzyme cyclooxygenase and lipooxygenase respectively. Vasoconstriction may be causative factor in the $TXA_2$ mediated gastric mucosal ulceration which predisposes the mucosa to disruption by local irritants. Leukotrienes induce vasoconstriction in the vascular bed in the rat submucosa that leads to tissue necrosis in the stomach.

### 1.2.11. Histamine

Histamine is present in large quantities, about 40 micrograms per wet weight, in the oxyntic mucosa of humans and other mammals. Histamine has been found in the gastric wall and it is powerful stimulant for gastric secretion. However, excessive release of histamine by histamine release or by injection of aqueous solution of histamine produces gastric and duodenal ulcer.

### 1.2.12. Serotonin (5-hydroxytryptamine, 5HT)

More than 90% of endogenous 5-hydroxytriptamine is found in the gastrointestinal tract. 5-HT is stored in endocrine cells and enteric neurons in the gut. It is established that 5-HT possesses ulcer producing properties. Serotonin has been shown to be involved in the ethanol and reserpine induced ulceration. These ulcerogens release 5-HT from gastric mucosa which causes reduction in gastric blood flow and mucus depletion.

## 2. MATERIAL AND METHOD

### 2.1 Collection of plant material

The plant material was collected from local area of Bhopal.

### 2.2 Storage

Dried flower were preserved in plastic bags and closed tightly and powdered as per the requirements.

### 2.3 Extraction by maceration process

100 gram flower of *Tagetes patula* were exhaustively extracted with hydroalcoholic extract solvent (methanol 70%) and using drug – solvent ratios (1:2) using maceration process (10hrs). Finally the percentage yields were calculated of the dried extracts. The percentage yields of each extract were calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

**2.4 Phytochemical Screening**

The chemical tests were performed for testing different chemical groups present in extracts (Khandelwal, 2005; Kokate, 1994). The *Tagetes patula* Flower extract acquire was subjected to the precursory phytochemical analysis following standard methods by Khandelwal and Kokate. The extract was screened to identify the presence of various active principles of Alkaloids, glycosides, Diterpenes, saponins, flavonoids and phenol.

**2.5 Estimation of total Phenolic and flavonoid Content**

**2.5.1 Total Phenolic content estimation**

**Procedure:** The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. 2 ml (1mg/ml) of this extract was for the estimation of Phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

**2.5.2 Total flavonoids content estimation**

**Procedure:** Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. 3 ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

**2.6 *In Vivo* antiulcer activity**

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments.

**Experimental designs**

**Indomethacin induced gastric ulcers model**

Group –1: Control

Group –2: Cimetidine (Standard)

Group –3: Hydroalcoholic flower extract of *Tagetes patula* **(**100mg/kg, p.o.)

Group –4: Hydroalcoholic flower extract of *Tagetes patula* (200mg/kg, p.o.)

**Ethanol induced gastric ulcers model**

Group –1: Control

Group –2: Cimetidine (Standard)

Group –3: Hydroalcoholic flower extract of *Tagetes patula* **(**100mg/kg, p.o.)

Group –4: Hydroalcoholic flower extract of *Tagetes patula* (200mg/kg, p.o.)

The animals were fasted for 24 h prior to the experiment. Under anaesthesia, ulcers were induced by applying indomethacin (5 mg/kg. p.o.) over the anterior serosal surface of the stomach for 60 seconds. The animals were treated with Cimetidine (100 mg/kg, p.o.), low dose of Hydroalcoholic flower extract of *Tagetes patula* (100 m/kg p.o.) or high dose of Hydroalcoholic flower extract of *Tagetes patula* (200 m/kg p.o.) [once daily, for 5 days after the induction of ulcer, while the control group received only the vehicle. The rats were sacrificed on the $5^{th}$ day, the stomachs removed and cut open along the greater curvature (Khare *et al.*, 2008). The ulcer index was determined using the formula:

$$\text{Ulcer index} = 10/X$$

Where X = Total mucosal area/Total ulcerated area.

Based on their intensity, the ulcers were given scores as follows:

0 = no ulcer,

1 = superficial mucosal erosion,

2 = deep ulcer or transmural necrosis,

3 = perforated or penetrated ulcer.

## 3. RESULTS AND DISCUSSION

### 3.1 Result of Percentage Yield

**Table No. 3.1: % Yield of hydroalcoholic extract of *Tagetes Patula* flowers**

| . No. | Solvent | % Yield (w/w) |
|---|---|---|
| 1. | hydroalcoholic (30:70) | 7.3 % |

**3.2 Results obtained from qualitative chemical tests**

**Table – 3.2: Quantitative chemical tests of extract of *Tagetes Patula* flowers**

| S. No. | Constituents | *Tagetes Patula* (flowers) |
|---|---|---|
| **1.** | Alkaloids | |
| | *i. Mayer's test* | -ve |
| | *ii. Dragendorff's test* | +ve |
| **2.** | Carbohydrates | |
| | *i. Molisch's test* | -ve |
| | *ii. Fehling's test* | -ve |
| | *iii. Benedict's test* | -ve |
| **3.** | Flavonoids | |
| | *i. Ferric-chloride test:* | +ve |
| | *ii. Alkaline reagent test:* | +ve |
| | *iii. Shinoda's test* | +ve |
| **4.** | Proteins | |
| | *i. Biuret's test* | -ve |
| **5.** | Saponins | |
| | *i. Foam test* | -ve |
| **6.** | Steroids | |
| | *i. Salkowski test* | -ve |
| | *ii.Liebermann-burchard reaction* | -ve |
| **7.** | Amino acid | |
| | i. *Xanthoprotic test* | -ve |
| **8.** | Glycosides | |
| | *i. Legals test*: | +ve |
| | *ii. Killer Killiani test:* | +ve |
| **9.** | Tannins | |
| | *i. Gelatin test* | +ve |
| **10** | Phenol | |
| | *i. Ferric-chloride test:* | +ve |

**+ ve – Present, - ve – Absent**

### 3.3 Result of Total Phenolics and total flavonoids content

**Table -3.3: Estimation of total phenolics and total flavonoids content**

| S. No | Extract | Total phenolic content | Total flavonoids Content |
|---|---|---|---|
| **1** | Hydroalcoholic | 0.816 | 0.321 |

### 3.4 Results of *In Vivo* antiulcer activity

**Table -3.4: Anti-ulcerogenic effect of Hydroalcoholic extract *Tagetes Patula* flowers against ulcerogenic agents in rats (Ulcer index)**

**(INDOMETHACIN INDUCED GASTRIC ULCERS MODEL)**

| Groups | Treatment and dose | Ulcer index |
|---|---|---|
| **Group-1** | Control | 3.50 ± 5.0 |
| **Group-2** | Cimetidine (100 mg/kg, p.o.) | 1.50 ± 5.0*** |
| **Group-3** | Hydroalcoholic extract *Tagetes Patula* flowers (100 mg/kg, p.o.) | 2.71 ± 5.0** |
| **Group-4** | Hydroalcoholic extract *Tagetes Patula* flowers (200 mg/kg, p.o.) | 2.10 ± 5.0*** |

Values are expressed as mean ±S.E.M. (n = 6).

Percent inhibition calculated as compared to control group.***$P < 0.001$, ** $P < 0.01$, * $P < 0.05$ (One-way ANOVA followed by Tukey's post hoc test).

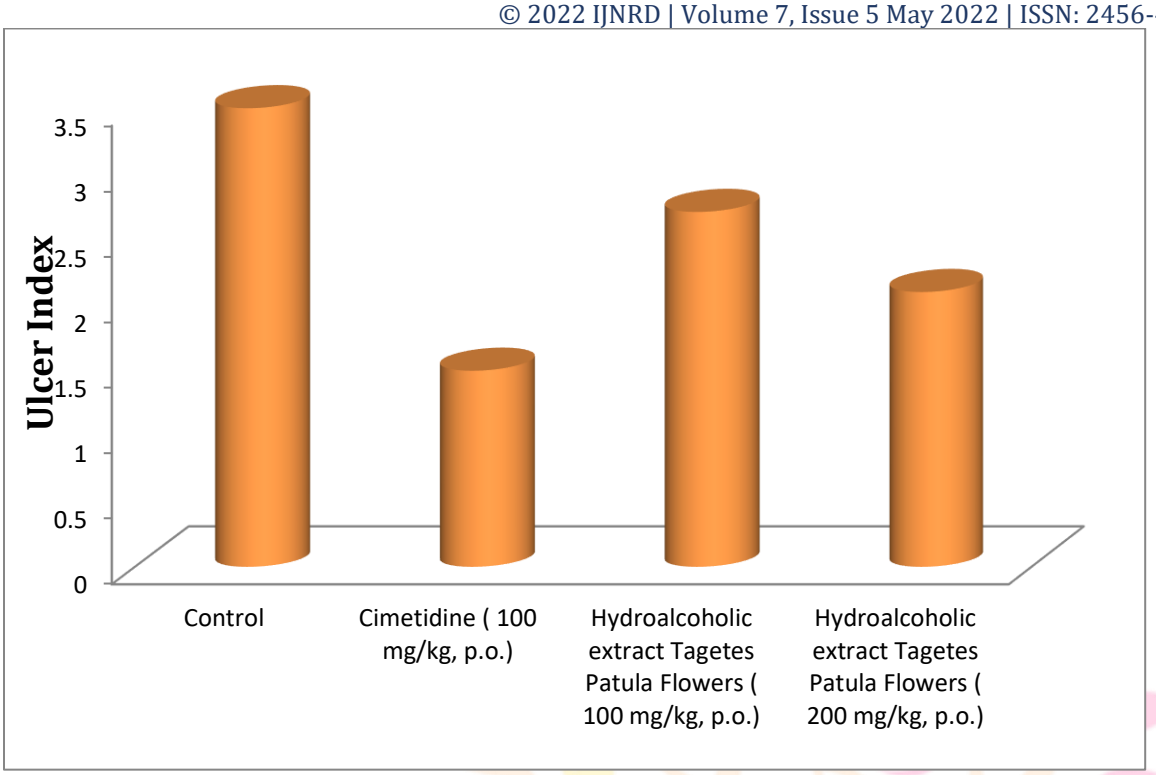


**Figure 3.2: Anti-ulcerogenic effect of Hydroalcoholic leaves extract *Tagetes Patula* against ulcerogenic agents in rats (Ulcer index)**

**Table -3.5: Anti-ulcerogenic effect of Hydroalcoholic extract *Tagetes Patula* flowers against ulcerogenic agents in rats (Ulcer index)**

**ETHANOL INDUCED GASTRIC ULCERS MODEL**

| Groups | Treatment and dose | Ulcer Index |
|---|---|---|
| **Group-1** | Control | 5.732±15 |
| **Group-2** | Cimetidine (100 mg/kg, p.o.) | 0.951±11 |
| **Group-3** | Hydroalcoholic leaves extract *Tagetes Patula* (100 mg/kg, p.o.) | 2.61±0.22 |
| **Group-4** | Hydroalcoholic leaves extract *Tagetes Patula* (200 mg/kg, p.o.) | 1.95±0.15 |

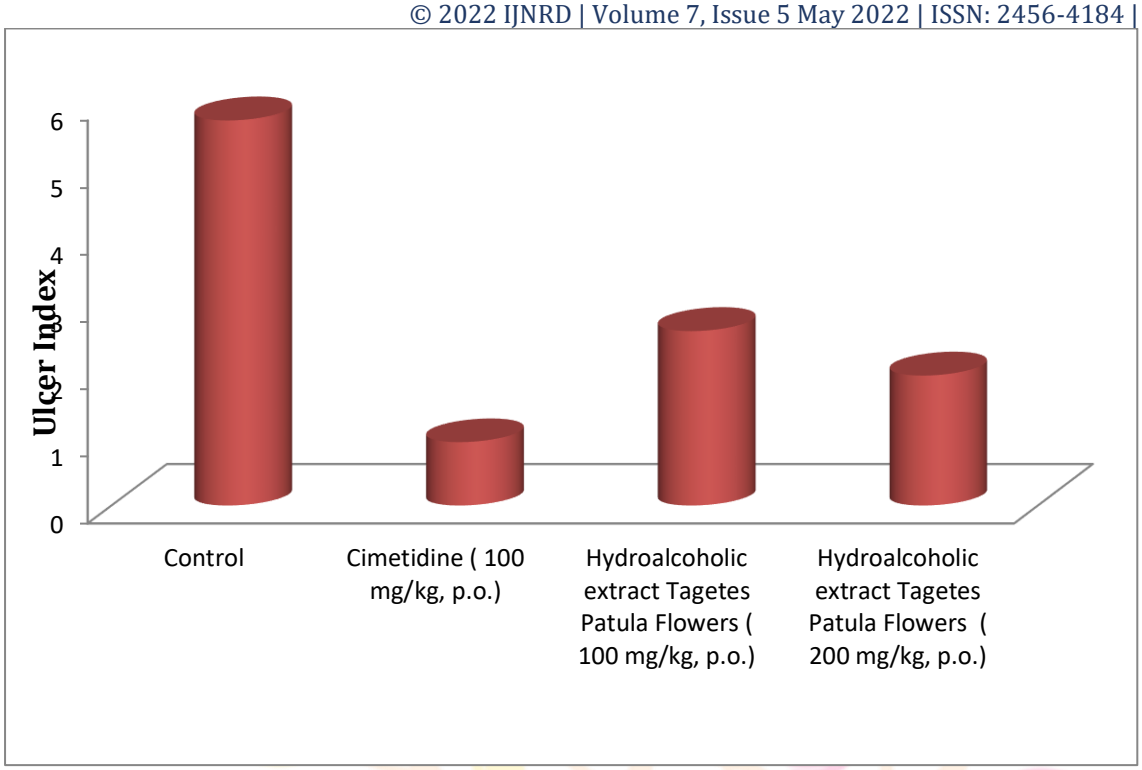


**Fig: 3.2 Anti-ulcerogenic effect of Hydroalcoholic leaves extract *Tagetes Patula* against ulcerogenic agents in rats (Ulcer index) ethanol induced gastric ulcers model**

The present study investigated the effect of Hydroalcoholic Flower extract *Tagetes Patula* on the ulcers. Hydroalcoholic Flower extract *Tagetes Patula* showed effect on the healing of gastric ulcers induced by indomethacin and ethanol. It acts through the inhibition of cell wall biosynthesis that leads to the death of the bacteria. Hydroalcoholic Flower extract *Tagetes Patula* was effective in reducing the ulcer area and the ulcer score. *Tagetes Patula* has an antiulcer effect. It increased healing of indomethacin and ethanol induced ulcer.

## CONCLUSION

Peptic ulcers are a deep gastrointestinal erosion disorder that involves the entire mucosal thickness, penetrating the muscular mucosa. For decades it was believed that gastrointestinal ulcerations were caused by the excessive secretion of gastric acid, but many patients presenting such ulcerations had normal acid secretion rates. The analysis and characterization of bioactive compounds from plants is important to ascertain their medicinal value. The present study investigated the effect of Hydroalcoholic flower extract of *Tagetes patula* on the ulcers. The phytochemical analysis showed that the *Tagetes patula* Flower extract contains a mixture of phytochemicals as Glycoside, Tannin, Alkaloids, Phenols and Flavonoids. The total phenolics and total flavonoids content for the Hydroalcoholic extract was found to be 0.81 6and 0.321mg/100g in flowers extract of *Tagetes patula*. Hydroalcoholic flower extract of *Tagetes patula*

showed effect on the healing of gastric ulcers induced by indomethacin. It acts through the inhibition of cell wall biosynthesis that leads to the death of the bacteria. Hydroalcoholic flower extract of *Tagetes patula* was effective in reducing the ulcer area and the ulcer score. It increased healing of indomethacin and ethanol induced ulcer.

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