

Original Research

Antiulcer activity of Property fruit extract of the *Cucumis trigonus* L.
In albino rats

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ABSTRACT:

The effect of alcoholic fruit extract of *Cucumis trigonus* L. (Family: Cucurbitaceae) was evaluated for the anti-ulcerogenic activity with aspirin induced ulceration in albino rats and various biochemical parameters viz., ulcer index, mucus production, total volume of gastric juice, pH of gastric juice were analyzed. A qualitative phyto chemical analysis was carried out and the fruit extract was found rich in carbohydrates, flavonoids, fixed oils and fats. Although the fruit extract of all the concentrations were active in reducing the gastric acidity, the drug dosage at 400 mg/animal was found as the most effective in anti ulcer activity. The presence of flavonoids in the alcoholic fruit extract of the plant is being the responsible for their antiulcer properties.

Keywords:

Anti ulcerogenic activity, gastric juice, phytochemicals, *Cucumis trigonus*.

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INTRODUCTION

This research has been carried out to explore the hidden truths and to utilize the healing property of *Cucumis trigonus*. Regardless of incredible development in the field of synthetic drugs and antibiotics during the 20th century, plants still constitute one of the major sources of drugs in modern medicine throughout the world. Recently, demand for plant based drugs have increased tremendously owing to their effectiveness and less side effects (Malairajan *et al.*, 2006; Siti *et al.*, 2009).

Peptic Ulcer Diseases (PUD) encompassing gastric and duodenal ulcer are the most prevalent gastrointestinal disorders (Valle, 2005). It is one of the major ailments affecting about 60% of adults and nearly 80 % of children in tropical countries (Bakhru, 1999). Peptic ulcer is believed to cause because of the increased gastric acid and pepsin secretion, a reduced mucosal defense or a combination of these two abnormalities. The predisposing factors for peptic ulcer, ulceration includes *Helicobacter pylori* infection, non steroidal anti inflammatory drugs, cigarette smoking, diet, stress, alcohol and diseases such as chronic pancreatitis and chronic obstructive pulmonary diseases (Soll, 1990).

Plant extracts are the most attractive source since longtime and a large number of plants have been shown to produce promising anti ulcerogenic effects (Akhtar *et al.*, 1992). *Cucumis trigonus* L. is an indigenous medicinal herb belonging to the family Cucurbitaceae. Several studies have shown that the plant is used to treat various gastro intestinal problems. Germano *et al.* (1998) investigated that *Cucumis trigonus* was used in the traditional medicine of Mali for the treatment of gastric and duodenal ulcers. Vela *et al.* (1997) suggested that *Cucumis trigonus* is used in the folk medicine to treat gastric and intestinal disturbances. The freeze - dried aqueous extract of the whole plant tested in rodents up to the dose of 2000 mg/ Kg animal not produced any toxicity. The present investigation was

designed to investigate the anti ulcerogenic effect of the ethanolic fruit extract of the plant *Cucumis trigonus* in albino rat models.

MATERIALS AND METHODS

Collection and identification of plant material

Disease free, healthy *Cucumis trigonus* plants were collected from agricultural areas of Thanjavur, Tamilnadu, India. The mature fruits were collected carefully and then stored in clean aerated containers. The plant was identified with the help of regional floras (Gamble, 1967 and Mathew, 1983).

Preparation of the plant extract

Ethanolic extract of the plant material was prepared according to the Indian Pharmacopoeia (Anonymous, 1966). The mature fruits were dried in shadow. The dried fruits pieces were subjected to Soxhlet extraction. The extract was concentrated to dryness in rotary evaporator under reduced pressure and controlled temperature. The extract was put in air tight containers and stored in refrigerator. The extract was undergone for phytochemical screening and pharmacological activity.

Qualitative phytochemical investigation

Qualitative phytochemical analyses were done by using the methodology of Kokate *et al.* (1995). Alkaloids, carbohydrates, tannins, phenols, flavonoids, gums, mucilage, phytosterol, proteins, fixed oils, fats, volatile oil and saponins were qualitatively analyzed.

Experimental animals

Wister strain albino rats of either sex weighing 200-250g were purchased from Jay Nine Biotech. Centre, Thanjavur. The animals were maintained on a standard diet and water *ad libitum*. The food was withdrawn 24 hours before the experiment but animal was allowed to free access of water. To avoid coprophagy the rats were fasted with wire-bottomed cages.

Table 1. Qualitative phytochemical screenings of the ethanolic fruit extract of *Cucumis trigonus* L.

Sl. No.	Name of the compound	Name of the test	Status of the substance
1	Carbohydrates	i. Fehling's solution ii. Benedict's solution	+++ +++
2	Alkaloids	i. Mayer's reagent ii. Hager's reagent iii. Wagner's reagent	+ - -
3	Steroids	Chloroform + acetic acid +H ₂ SO ₄	-
4	Tannin and phenols	i. 10% Lead acetate ii. 5% Ferric chloride	- -
5	Saponin	Foam test	++
6	Fixed oils and fats	Spot test	+++
7	Gum and mucilage	Alcoholic precipitation	+
8	Proteins	Biuret test	+
9	Flavonoids	NaOH/HCL	+++

+++ High, ++ Medium, + Low, - Absent

Random grouping

The animals were randomly divided into the following groups and each group consists of five rats. All the animals received aspirin (200 mg/ Kg) once a day for a period of seven days. Group I received only water and served as control. Group II, III, IV and V were treated with the fruit extract *Cucumis trigonus* and Group VI was served as standard control and received Ranitidine (50 mg/ Kg animal) (Turner, 1965).

Drug administration

Various concentrations of the test drug viz., 100, 200, 300, 400 mg were administered orally twice a day at regular intervals (10:00 and 16:00h) respectively for seven days for ulcer protective studies. Normal and

standard control group of animals received distilled water and ranitidine respectively for the same administration period.

Aspirin + pylorus ligation (pl) induced gastric ulcer

Drugs were administered for a period of seven days as described above. On 7th day, the rats were kept for 18 h fasting and care was taken to avoid coprophagy. Animals were anaesthetized using ether, the abdomen was opened by a small incision below the xiphoid process and pylorus ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted silk sutures. The animals were deprived of water during the post operative period

Table 2. Anti ulcer genic effect of the ethanolic fruit extract of *Cucumis trigonus* in aspirin induced albino rats

Sl. No	Treatments	Drug Dosage mg/ Kg	Ulcer index	pH of gastric the juice	Mucus production	Volume of pepsin	Volume of gastric juice in ml.
1	Control	-	1.82±0.26	3.01±0.12	332.2±40.6	3.09±0.77	3.18 ±0.47
2	Group II	100	2.67±0.35	3.48±0.18	328.7±29.7	3.36±0.51	3.37± 0.46
3	Group III	200	2.12±0.49	3.34±0.22	339.5±37.3	3.27±0.35	3.29± 0.21
4	Group IV	300	1.43±0.54	3.26±0.12	347.1±45.8	3.19±0.29	3.15± 0.22
5	Group V	400	0.92±0.67	3.07±0.17	355.6±61.6	3.03±0.12	2.91± 0.09
6	Ranitidine	50	0.88±0.73	2.92±0.07	359.4±60.5	2.92±0.01	2.83 ±0.29

(Sanyal *et al.*, 1971). After 4 h, the animals were sacrificed by cervical dislocation and stomach was dissected out and the contents were collected for the analysis of biochemical parameters. Stomach was taken out and cut open along the greater curvature and ulcer in the glandular portion of stomach was scored. The ulcer index was scored, based upon the length and width of the ulcer area present in the glandular portion of the stomach (mm^2/rat) (Sairam *et al.*, 2001).

Collection of gastric juice

Gastric juice was collected from the pylorus ligated rats and the following ulcerogenic parameters were studied.

- i. Ulcer index
- ii. Estimation of total volume of gastric juice
- iii. Estimation of pepsin
- iv. Estimation of mucus production
- v. Determination of pH of gastric juice

Ulcer Index

Lesion size in mm was measured for each lesion along its greatest diameter and the score was assigned according to the following scale prescribed by Minano *et al.* (1987). 0 – no pathology, 1 – mucosal edema and petechiae, 2 – 1 to 5 small ulcers (1-2mm), 3 - > 5 small ulcers or 1 medium ulcer (3-4mm); 4 - > 2 medium ulcers or 1 large ulcer (>4mm) and 5 – perforated ulcers. The sum of the total activity score in each group was divided by the number of rats in that group and was expressed as the mean ulcer index.

Estimation of the total volume of gastric juice.

The gastric juice was collected 4 h after pylorus ligation and centrifuged for 5 min at 2000 rpm. The supernatant was collected and the volume of gastric juice was expressed as ml/100 g body weight. Total acid output was determined by titrating with 0.01 N NaOH, using phenolphthalein as indicator and expressed in $\mu\text{g}/\text{ml}$ (Debnath *et al.*, 1974).

Estimation of pepsin

The amount of pepsin was estimated by the

proteolytic activity of pepsin in gastric samples was analyzed by using hemoglobin as a substrate. The results were expressed in terms of the amount of liberated L – Tryosine.

Estimation of mucus production

Gastric mucus production was measured in rats that are subjected to pylorus ligation. The mucus covering of stomach was gently scraped using a glass slide and weighed immediately using a digital precision electronic balance and recorded as mg/100 g body weight (Oluwole *et al.*, 2007).

Determination of pH of gastric juice

The gastric content was aspirated and then centrifuged at 3000 x g for 15 min. The hydrogen ion concentration of the supernatant solution was measured with the help of a pH meter (Parmar, 1984).

RESULTS

The preliminary phytochemical investigations on the ethanolic fruit extract of *Cucumis trigonus* revealed that the presence of alkaloid, gums, mucilage, protein, flavonoids. Tannin and phenol and steroids were absent. Moreover, flavonoid was found rich amount (Table - 1). The anti ulcerogenic effect of various concentrations of test drug *Cucumis trigonus* on aspirin induced albino rats along with their ulcerogenic parameters were recorded in Table - 2.

The gastric juice production in the disease induced control rats were 3.18 ml. The amount of gastric juice production in the test drugs concentrations 300 and 400 mg were 3.15 and 2.91ml respectively. The drug concentration at a level of 300 mg was found appropriately effective and reduced the gastric juice secretion.

The drug dosage, 400 mg/animal showed the gastric juice pH as 3.07, which was more or less equal to the pH of gastric juice (3.01) of the disease induced control rats (± 0.06)

The mucus production in disease induced control rat was 332.2mg/l. The difference in mucus production at

200, 300 and 400 mg dosages were found as 7.3, 14.9 and 23.4 mg/l. (Singh, 1997).

The ulcer index in disease induced control rats were 1.82. The ulcer index of the drug dosage 100, 200, 300 and 400 mg/animal was 2.69, 2.12, 1.43 and 0.92 respectively. A gradual decrease in ulcer index on increasing the concentrations of drug was observed. Standard control-ranitidine (50mg/animal) showed more or equal effect to the concentration of 400mg test drug.

DISCUSSION

Peptic ulcer is a gastrointestinal disorder caused mainly due to an imbalance between the aggressive factors like acid, pepsin, *Helicobacter pylori* and defensive factors like bicarbonate secretion, prostaglandins, gastric mucus and innate resistance of the mucosal cell factors. Normally, peptic ulcer develops when aggressive factors exceeds the defensive factors (Izzo and Borrelli, 2000). Due to the occurrence of many side effects by the use of synthetic drugs for many diseases, medicinal plants are considered as the main source of new drugs as they have less or no side effects. Herbal drugs are considered as economical, effective and relatively safe for the treatment of ulcer. Hence, extensive research studies are being carried out in search for potent antiulcer agents of plant origin (Vinay *et al.*, 2005).

According to the old hypothesis, acid secretion was thought to be the sole cause of ulcer formation and reduction in acid secretion was thought to be the major approach towards therapy. However, in the light of recent evidences this concept has changed. Now treatment of ulcer mainly targets the potentiation of the defensive system along with lowering of acid secretion.

Cucumis trigonus is known to possess various therapeutic properties and has been one of the most noteworthy plant mentioned in various medicinal system like Indian, Chinese, South American and possess various medicinal properties viz., analgesic, (Naik *et al.*, 1980), diuretic (Naik *et al.*, 1981) and anti-inflammatory

Preliminary phytochemical investigation on the fruit extract of the plant revealed the presence of flavonoids. Flavonoids and tannins have been reported to possess both antiulcer and anti-inflammatory activities (Goel *et al.*, 1988). Aquwa (1985) have reported that the gastro intestinal effect of the aqueous extract of *C. trigonus* was found to be having significant antiulcer activity. The presence of glycosides, saponins and tannins was also supported the present investigation.

Some flavonoids have been shown to increase the mucosal content of prostaglandins and mucus in gastric mucosa and also exhibit cytoprotective effect (Konturek *et al.*, 1986). The mucus production in the medicinal plant *Cucumis trigonus* at the dosage of 200 mg/animal onwards was very effective. The difference in mucus production at 200 mg dosages was 7.3 when compared with the control rats.

Cucumis trigonus prevent the mucosal lesions by decreased the total volume of gastric juice and was effective against pylorus ligation induced gastric ulcers. Pylorus ligation induced ulcer is caused due to the increase in acid pepsin accumulation and subsequent mucosal digestion. The anti-ulcer effect of the *Cucumis trigonus* in present investigation is due to its action on the gastric mucosal defensive factors.

CONCLUSION

The results of the study indicated that the test drug *Cucumis trigonus* fruit produced protection against gastric ulcers induced by aspirin and pylorus ligation animal model. The extract was able to decrease the acidity and increase the mucosal secretion and support the traditional use of the plant for the treatment of gastric ulcer. The anti ulcerogenic effect is probably due to the presence of bioactive compound, flavonoids.

The results of this study indicate that fruit extract of *Cucumis trigonus* have good potentials for use in peptic ulcer disease. Our results showed that this

medicinal plant could prevent ulcer in rats in a dose-dependent manner.

Therefore, efforts should be directed towards isolation and characterization of the active principles and elucidation of the relationship between structure and activity. Furthermore, detailed analysis of the active constituents of natural drugs should be directed towards clinical relevance.

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