

Research Article

Ant ulcer Potential of *Cucumis melo* Var. *momordica* (Roxb.), Duthie & Fuller Fruits in Experimental Animal

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ABSTRACT

Purpose: This study was aimed to evaluate the antiulcer potential of *Cucumis melo* var. *momordica* (Roxb.) Duthie & Fuller, fruits.

Methodology: Ethanol and pylorus ligation induced ulcer methods were used in rats for the study. The aqueous and ethanolic extracts (200 and 400 mg/kg body weight) were administered orally and effects on volume of gastric secretions, ulcer index, total and free acidity were evaluated.

Findings: A significant dose dependent reduction ($P < 0.05$) in the acid parameters like ulcer index, gastric volume, free and total acidity and elevation in pH were observed after treatment with 200 and 400 mg/kg extracts in Pylorus ligation and ethanol-induced ulcer models. This reduction in acid-secretory parameters and ulcer score suggesting that acid inhibition accelerates ulcer healing.

Conclusion: Based on our findings, we presume that the anti-secretory properties of both extracts were responsible for its anti-ulcer potency. These findings suggest the potential for use of *Cucumis melo* extract as an adjuvant in the treatment of gastric ulcer.

Keywords: Antiulcer, *Cucumis melo* var. *momordica* (Roxb.) Duthie & Fuller, ethanolic extract pylorus ligation.

INTRODUCTION

Peptic ulcer disease is a common cause of morbidity and in severe conditions; it is also a cause of mortality among individuals. The most common causes are extensive use of non steroidal anti-inflammatory drugs, stress conditions and *Helicobacter pylori* invaded cells of gastric mucosa¹. It results when the balance between belligerent and preventive factors are disturbed. Belligerent factors include acid, pepsin, bile, *H. pylori* infection, and preventive factors include gastric mucus, bicarbonate secretion, availability of prostaglandins at the sites, nitric oxide, innate resistance of the mucosal cells and high mucosal blood flow². Ulcers of gastric mucosa appears in those parts of gastrointestinal tract which are exposed to pepsin and gastric acid, these are stomach and duodenum. Some other reasons for the development of gastric ulcers include severe illness, severe emotional disturbance, shock, post-surgical complications, poor digestion and elimination, improper metabolism, mental stress and physical restlessness and some foods that are difficult to digest enhance the development of ulcers.

The locations where ulcers occur commonly are stomach and just few centimeters of the duodenum where the stomach joins. The gastric lesions may be found in single or it may be in multiples. Histamine is a potent secretagogue in

gastric mucosa that promotes acopious secretion of acid from parietal cells which also increases the output of pepsin and intrinsic factors³. Although the secretion of gastric acid also is evoked by signals of the vagus nerve in association with enteric hormone gastrin, presumably by activation of M₃ and CCK₂ receptors on the parietal cell, acetylcholine and gastrin also stimulate histamine release from the entero chromaffin-like cell. In the beginning of ulcers cause's hemorrhagic eruptions of the gastric mucosa and this extend into the interior of the mucosa. Most common symptoms associated with ulcer are, anorexia, nausea, dyspepsia, abdominal pain, diarrhea, Anemia, GI hemorrhage and perforation. NSAIDs may increase the frequency or severity of ulcers.

From the ancient period natural products obtained from herbal⁴ and animal sources⁵ were used to treat all types of disorders of gastrointestinal tract. There are a huge number of plant derived drugs being used for the treatment of acute as well as chronic diseases of gastrointestinal tract. There are number of studies related to the treatment of stomach disorders like flatulence, belching and pain, concerned with acute and long term stomach problems. There is a great abundance of plants used in traditional medicine reported to have antiulcer properties, after possible manipulations in the chemical structure, provide new and improved antiulcer drugs. A number of works are being done on Cucurbitaceae family of kingdom Plantae, they have a great potential as anti-inflammatory⁶, antioxidant⁷, wound healing⁸ and against other disorders. Some members of Luffa and Cucumbers in different organic extracts shows marked effect as ulcer protective and inhibitor⁹.

Cucumis melo var. *momordica* (Roxb.) Duthie & Fuller, a plant that is cultivated and wildly grown in humid areas near the rivers at many parts of India and belongs to family Cucurbitaceae. The ancient literature mentioned that the plant is used in eye infection, ulcers, bronchitis, kidney troubles and chronic fever. Chemically the fruit contain a saponin (stigmasta-7-16-25(26-3-O-β-D-lucopyranosyl(1→5)-O-β-D-xylofuranoside. Curcumin and Leptodermin

are also reported in the fruits. Since there is not much scientific evidence explored for this plant with respect to pharmacological aspect, hence we have made an attempt to explore this plant scientifically for its antiulcer activity as this plant is used traditionally for this activity¹⁰.

MATERIALS AND METHODS

Collection of Plant material

The fruits of *Cucumis melo* var. *momordica* (Roxb.) Duthie & Fuller were collected from the river side area of District Allahabad, Uttar Pradesh, India in the month of September 2016 and authenticated from Botanical Survey of India, Allahabad by taxonomist Dr. G. P. Sinha with voucher specimen no. GC 950221. Collected fruits were washed with water and sliced with a sharp edged knife without unpeeling. Sliced fruits were dried in tray drier at optimum temperature till all the moisture was evaporated and powdered.

Crude drug Analysis: The Moisture content, ash values, swelling index, extractive values with various reagents were determined as per the standard procedures¹¹.

Preparation of extract: The dried fruit powder (300gm) was macerated with petroleum ether to remove the fatty substances. Marc was exhaustively extracted with ethanol (95%) by maceration method. The extract was filtered and the marc is extracted again with water by hot percolation method. Both extracts (ethanolic and aqueous) concentrated on rota vapour at 10,000 rev/min. separately. The concentrated extracts were further lyophilized under vacuum to get uniform powder of both the extracts and kept in air tight container. The residue of ethanolic and aqueous extracts was found to be 4 g (1.33%) and 6 g (2%) respectively. Both the extracts were further subjected to preliminary phytochemical screening to find out various secondary metabolites by adopting standard procedure.

Phytochemical Screening

Preliminary phytochemical screening of both extracts was carried out by standard procedure¹² which revealed the presence of various chemical constituents mentioned in table 2.

Experimental animals

Healthy adult Wistar albino rats weighing about 150-200 g were used for the pharmacological activity. The experimental animals were housed in well cleaned polypropylene cages for seven days prior to oral administration at United Institute of Pharmacy, Allahabad animal house. Standard conditions (12/12 h light and dark) and temperature $25 \pm 3^\circ\text{C}$ has been maintained. Humidity (35-60%) was adjusted and proper monitoring was performed by hygrometer. All the animals were fed with pellet diet for rats and water *ad libitum*. The protocol of this study was approved by Institutional Animal Ethical Committee of United Institute of Pharmacy, Allahabad, India with (No. UIP/IAEC/2014/April/23).

Oral acute toxicity study: The oral acute toxicity study was performed as per the OECD- 423 guidelines. The extracts were suspended in Tween-80 (0.1%w/w) and administered orally at doses in a wide range of 5-2000 mg/kg. The concentration was adjusted as the dose does not exceed 1 mL/kg body weight of the rats. The extract was devoid of any toxicity in animals when given in dose up to 2000mg/kg. For further pharmacological study, dose of 200 mg/kg and its double strength of 400 mg/kg of body weight have been selected¹³.

Experimental induction of ulcer

Pylorus ligation induced ulcer

The adult albino wistar rats of either sex weighing between 150g and 200 g were selected for study. The animals were grouped into seven, each group consisting six animals. The rats were fasted for 24 hours, allow to free access of water and *ad libitum*. The groups were divided follows; Group I: served as control – 0.1% of Carboxy Methyl Cellulose(CMC) in a dose of 1ml/kg through oral route oral route, Group II: Served as Positive control –Ranitidine 50 mg/kg, Group III: Served as ethanolic test extract- CMEE 200 mg/kg, Group IV: Served as ethanolic test extract- CMEE 400 mg/kg, Group V: Served as aqueous test extract- CMAE 200 mg/kg and Group VI: Served as aqueous test extract – CMAE 400 mg/kg. Administration of drug was undertaken with, the

standard drugs and extracts were administrated (twice daily for two consecutive days) orally by using oral feeding needle. The aftermost dose of standard drugs and extracts were administrated one hour prior to pyloric ligation. The rats were anaesthetized using ketamine:xylazine (80:5 mg/kg) by single syringe. The abdomen was opened through a midline epigastric incision of approximately 1cm cut, the pylorus was exposed and stomach was untouched. A thread was then tied around the pylorus to stop the gastric flow from stomach to duodenum. It was so carefully performed that no blood vessel was harmed during this process. The animals were starved for during the post-operative period. Ketamine injection and cervical dislocation was used to sacrifice the animals, 4 h after the ligation. The stomach was excised considerably; keeping the esophagus closed, opened from the greater curvature and collected the gastric juice. The stomach was washed with saline and the stomach affixed on dissection table. The ulcer index was calculated and the lesion was counted with the aid of hand lens. The collected gastric juice was centrifuged and various physicochemical and biochemical parameters were studied on the gastric fluid such as the free acidity, total acidity, volume and pH.

Ulcer-induction by absolute ethanol

The rats fasted for 24 h before the experiment but were allowed free access to drinking water till 2 h before the experiment. Gastric ulcer was induced by providing orogastric intubation of absolute ethanol (5 ml/kg). The groups were divided follows; Group I: served as control – 0.3% of Carboxy Methyl Cellulose (CMC) (1ml/kg, oral route), Group II: Served as Positive control –Ranitidine 50 mg/kg, Group III: Served as ethanolic test extract- CMEE 200 mg/kg, Group IV: Served as ethanolic test extract- CMEE 400 mg/kg, Group V: Served as aqueous test extract- CMAE 200 mg/kg and Group VI: Served as aqueous test extract – CMAE 400 mg/kg. One hour after this pre-treatment all the groups of rats were administered with absolute ethanol (5 ml/kg) in order to induce gastric ulcers 14. The rats were euthanized 60 min later under an overdose of xylazine and ketamine anesthesia and their stomachs were immediately excised carefully;

keeping the esophagus closed, opened along the greater curvature and collected the gastric juice. The collected gastric juice was purified by using centrifuge apparatus, which was subjected to various physicochemical and biochemical parameters. The free acidity, total acidity, volume and pH were recorded in the general laboratory procedure.

Statistical analysis: All values were reported as mean $\pm$ SEM. The statistical significance of differences between groups was assessed using one-way ANOVA. A value of $p < 0.05$ was considered significant.

RESULTS

Crude drug analysis: Physicochemical parameters like foreign matter, percentage of moisture content, total ash, acid insoluble ash, water soluble ash, swelling index, ethanol soluble extractive and water soluble extractive were determined and depicted in Table 1.

Table 1: Crude Drug analysis of Powdered Drug

Parameter %	w/w
Foreign matter	Nil
Moister content	9.5
Total ash	26.92
Water soluble ash	21.0
Acid insoluble ash	10.7
Extractive value in Petroleum ether	1.5
Extractive value in Ethanol	7.1
Extractive value in Water	21
Swelling index	4.04

Preliminary phytochemical screening: Preliminary phytochemical screening revealed the presence of carbohydrates, proteins, glycosides, steroids, and saponin in both the extracts, whereas tannin, phenolic compounds, and fats are absent in aqueous extracts. Results are mentioned in table 2.

ANTIULCER ACTIVITY

Pyloric ligature induced gastric ulcers in rats

Both extracts produced a dose dependent and

Table 2: Preliminary phytochemical screening of plant extracts

Test Performed	Name of Test	Ethanollic	Aqueous
Test for Flavonoids	FeCl ₃ Test	--	+
Test for	Molisch test	+	+
	Benedict test	+	+
Protein	Biuret test	+	+
	Millons Test	+	+
Amino acids	Ninhydrin test	-	+
Glycosides	Legal's test	+	+
	Bromine water test	+	+
Steroids	Salkowski test	+	+
	Lieberman burchard	+	+
Tannins	Gelatin test	+	-
Phenolic compounds	Lead acetate test	+	-
	Acetic acid	-	-
Fats	Filter paper test	+	-
Alkaloids	Hagers test	-	-
	Murexide test	-	-
	Ammonium renicate	-	-
Saponin	Foam test	+	+

significant ($P < 0.05$) reduction in the ulcer index. Here also, maximum protection was seen in the ranitidine treated group. Higher doses of ethanolic extract (400 mg/kg body weight) were more efficacious than higher dose of aqueous extract in reducing the ulcer index in the treated animals. The volume of gastric secretion and total acidity was significantly reduced in all drug treated groups as compared to control. Both extract produced a dose dependent reduction of gastric juice volume and total acidity. Gastric pH was also found to be significantly increased in all drug treated groups as compared to control with maximum increase being produced by ranitidine. The results are mentioned in table 3.

Ethanol induced gastric ulcers in rats

Both extracts showed a dose dependent protection against ethanol induced ulcers in rats. Maximum protection was seen in the ranitidine treated group. Even though ethanolic extract at dose of 400 mg/kg produced a significant reduction of ulcer index, whereas all the tested doses produced a decrease in

ulcer index as compared to the control. The volume of gastric secretion and total acidity was significantly reduced in all drug treated groups as compared to control. Ethanolic extract produced a dose dependent reduction in gastric juice volume and total acidity, but maximum reduction in these parameters was produced by ranitidine. Gastric pH was also found to be significantly increased in all drug treated groups as compared to control, with maximum increase being produced by ranitidine. The results are mentioned in table 4.

DISCUSSION

This study was aimed to investigate antiulcer potential of fruits of *Cucumis melo* var. *Momordica*. The physicochemical parameters like total percentage of ash value, acid insoluble ash, water soluble ash and percentage yield of extractives in different solvents are constant features of a part of plant which may constitute individual drug. These reports would be of much significance in genuineness of drug sample. Swelling index of drug is quite large; it explains the water absorption capacity of the drug.

The etiology behind pylorus ligation induced ulcer includes increase in the acid secretion, which in turn cause increase in gastric volume, low pH, and increase in free and total acidity resulting into increase in ulcer index. Ulcer index is the number of lesions formed on gastric mucosa. The digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for induction of ulceration. Moreover accumulation of gastric acid and pepsin leads to autodigestion of gastric mucosa and breakdown of gastric mucosal barrier.ue to increase in this gastric acid, pepsin secretion and their accumulation in stomach, increases the peptic activity in pylorus ligation model.

Ethanol produces necrotic lesions in the gastric mucosa by reducing the secretion of bicarbonate and production of mucus, increasing vascular permeability and decreasing non-protein sulfhydryl groups of gastric mucosa¹⁵. Since The acute ulcer induction technique ethanol was used to study the cytoprotective activity of plant extract, which materializes cytoprotection by a reduction or absence

Table 3: Antiulcer activity of both extracts in pylorus ligation model

Treatment	Gastric volume	Free acidity	Total acidity	Ulcer index	Percent inhibition	pH
Control	3.005±0.56	53.33±1.38	92±1.99	5.81±0.13		2.63±0.31
Ranitidine 50mg/kg	1.21±0.03	24.88±0.89	41.05±1.69	1.37±0.09	76.44	5.6±0.26
CME 200mg/kg	1.63±0.05	42.16±0.95	76.41±1.22	3.71±0.11	36.16	5.53±0.16
CME 400mg/kg	1.39±0.01***	31.81±1.78***	50.99±0.76***	2.32±0.16***	60.06***	5.45±0.13***
CMAE 200mg/kg	1.76±0.05	45.98±0.66	76.08±1.18	3.84±0.16	33.9	4.98±0.09
CMAE 400mg/kg	1.48±0.02 ***	33.23±1.47 ***	56.64±1.09 ***	2.8±0.21***	51.8***	4.54±0.2***

The data's represented as Mean ± SEM for six rats per group. P<0.05 considered significant compared to control group.

Table 4. Antiulcer activity of both extracts in ethanol induced ulcer model

Treatment	Gastric volume	Free acidity	Total acidity	Ulcer index	Percent inhibition	pH
Control	5.6±0.17	58.04±1.09	92±1.99	6.8±0.46		2.83±0.13
Ranitidine 50mg/kg	2.30±0.11	28.01±0.74	47.66±0.81	1.8±0.08	73.52	6.06±0.32
CME 200mg/kg	4.91±0.17	51.64±2.08**	76.41±1.22	5.35±0.12	21.32	4.85±0.10
CME 400mg/kg	3.13±0.09***	33.73±.95***	55.46±1.86 ***	1.9±0.14***	72.05***	5.2±0.14***
CMAE 200mg/kg	5.28±0.15	53.65±1.35	84.35±1.41	5.36±0.13	21.17	4.35±0.14
CMAE 400mg/kg	3.55±0.15***	36.68±1.52***	60.48±1.52***	2.25±0.15***	66.91***	2.25±0.15***

The data's represented as Mean ± SEM for six rats per group. P<0.05 considered significant compared to control group.

of lesions. This model was used because it is more or less similar to that observed in acid hyper secretion cases in human species mechanism. In this study, we have found that the gastric mucosa shows not only significant reduction in ulcer index but also reduction in inflammatory condition around the ulcer site. Total acidity parameter was found to be extremely low because the drug neutralizes various types of acids present in stomach. As far as free acidity is concern, this is due to the secreted hydrochloric acid. There is a marked increase in pH of the gastric fluid, pH rises and reaches near the neutral point. It is observed that the pH change takes place in a smaller dose level as well as in larger dose also but the difference is very less. Gastric volume governs the excessive secretions during the ulcer conditions and in both the models there is an appreciable decrease in gastric volume, this is due to the efficacy of the drug against the gastric secretions which are suppressed by the drug. The results of the present study suggest that the ethanolic and aqueous extract of fruits of *Cucumis melo* may have protective effect on gastric ulceration in dose dependent manner.

CONCLUSION

Based on our findings, we presume that the anti-secretory properties of both extracts in different concentrations were responsible for anti-ulcer activity in different extents. These findings suggest the potential for use of *Cucumis melo* extract as an adjuvant in the treatment of gastric ulcer. Further studies to identify the active moieties and elucidation of the mechanism of action are recommended.

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