

EVALUATION OF ANTI-ULCER ACTIVITY OF *CITRULLUS LANATUS* SEED EXTRACT IN WISTAR ALBINO RATS

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ABSTRACT

Citrullus lanatus is known to possess various therapeutic properties. The present study has evaluated the anti-ulcerogenic property of crude methanolic extract of *Citrullus lanatus* seeds in two different ulcer models in albino Wistar rats. The extract at 300 mg/kg body weight, once daily orally for 7 days has a significant effect in pyloric ligation (PL, 4 h ligation) and in water immersion (WS, 25 °C for 3 h) stress induced ulcer model, as it showed protection index of 57.33% and 63.38% respectively which is comparable to the standard drugs (Ranitidine 50mg/kg) and Omeperazole (20 mg/kg body weight) that have shown protection index of 64.47% and 70.59% in PL and WS model respectively. Furthermore the *Citrullus lanatus* has significantly decreased the gastric volume (53.55%), free acidity (57.02%) and total acidity (36.53%) in case of pyloric ligation model. Conclusively the ulcer protective effect of *Citrullus lanatus* may be due to its anti-secretory along with cytoprotective mechanism.

Keywords: *Citrullus lanatus*, Anti-ulcer activity, Cucurbitaceae, Pyloric ligation.

INTRODUCTION

Plant based drugs are one of the most important cultural and traditional parts of the people. Today, most of the world population depends upon plant based drugs for their primary health care needs.¹ World Health Organization (WHO) estimates that 80% of the people living in developing countries almost exclusively use traditional medicines plant. The Ethno-pharmacological approach provides the way for developing new drugs from plants sources, which have historical background.² Although modern medicines may be available, due to socio-economical, cultural and historical reasons, these drugs have maintained their importance.³ Based upon the literature survey and traditional uses of Cucurbitaceae family, it gives a great idea to the researchers. Cucurbits are the popular name of the family Cucurbitaceae, commonly known as gourd family. A family of about 110 genera and between 650 to 850 species. Some of its largely represented genera along with their number of approximately reported species include *Momordica* [bitter gourd], *Cucumis* [cucumber, muskmelon], *Cucurbita* [pumpkin, gourd, squash], *Lagenaria* [bottle gourd] and *Luffa* [sponge gourd].⁴ The fruits of cucurbits are very useful in terms of human health, i.e. purification of blood, remove of constipation, good for digestion, and give energy.⁵

The anti-ulcer potential of the *cucurbita pepo* fruit pulp has also been reported earlier.⁶ Peptic ulcer is a common global problem with increasing incidence and prevalence. It is one of the major gastro-intestinal disorders which occur due to many factors like free radicals induced stress⁷, reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl etc are considered to be the fundamental causative factor for the gastric lesions through oxidative damage.⁸ The recent approaches has made easy to treat these diseases by plant based drugs. There are many synthetic drugs, but these drugs are often encountered with several adverse effects such as diarrhea, headache, drowsiness, fatigue, and muscular pain.⁹⁻¹²

Therefore plant based medicines are now considered as an alternative approach to control the various diseases because it is considered to be safer and having no side effects due to the presence of natural ingredients.¹³⁻¹⁴ By taking these aspects in our mind the present objective is to investigate the *Citrullus lanatus* seeds for their anti-ulcer potential.

MATERIALS AND METHODS

Plant material

Citrullus lanatus seeds were purchased from the local grain market,

sector 26 in Chandigarh. The seeds were authenticated and the voucher specimen no: 0394 has been deposited in the Botanical and Environmental Science Department, Guru Nanak Dev University, Amritsar. For examination only healthy looking seeds were chosen (without mechanical damages and bacterial infection). The seeds were cleaned, washed, dried and carefully powdered in the grinder at room temperature. All samples were kept in tightened light-protected containers.

Extraction

The shade-dried seeds (500g) were coarsely powdered and extracted with methanol (3 × 700 ml) by simple maceration process at room temperature for 24 hrs. The solvent was completely removed by rotary evaporator at 35 °C under reduced pressure and the crude extract obtained was suspended in water and partitioned with n-Hexane (3 × 150 ml). Then the water layer was collected and the water was removed completely, the crude extract obtained was stored in the refrigerator. The crude extract was further used for the antiulcer potential.

Phytochemical Screening

The methanolic extract of *Citrullus lanatus* was subjected to qualitative chemical examinations for the presence of alkaloids, carbohydrates, flavonoids, proteins, saponins, sterols, triterpenoids, coumarins glycosides, anthraquinones and tannins according to standard procedures.¹⁵ Further isolation and characterization of pure compounds from the extract is in progress.

Animals

Wistar albino rats (160-180 g) of either sex were purchased from Sanjay Biologicals, Amritsar for experimental study. They were acclimated to standard animal house conditions such as temperature (24.0±1.0 °C), relative humidity: 55-65% and 12hrs light/12hrs dark cycle. They were allowed free access to standard dry pellet diet (Hindustan Lever, Gwuahati, India) and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee of CPCSEA, (Committee for the Purpose of Control and Supervision of Experiments on Animal) registration no. 874/ac/05/CPCSEA.

Drugs and Chemicals

Ranitidine, Omeperazol were obtained as free samples from Jackson Laboratories, Amritsar. Punjab. Pentobarbitone (Neon pharmaceuticals). The other solvents and chemicals like Hexane, Methanol, NaOH were of analytical grade and purchased from Merk, Qualigen and Loba chemicals.

Anti-ulcer Activity

Experimental Design for pyloric ligation method

Four groups, each comprising of six rats, were included in the antiulcer studies.

- 1) Group I (Normal control group): Rats were subjected to administration of 1ml normal saline orally for 7 days.
- 2) Group II (Pyloric ligation control group): Rats were subjected to pyloric ligation for induction of ulcer.
- 3) Group III (Ranitidine group): Rats were subjected to administration of ranitidine (50mg/kg, orally) for 7 days.
- 4) Group IV. (Extract treated group): Rats were subjected to administration of watermelon extract (300mg/kg, orally) for 7 days.

Procedure

The rats were subjected to pyloric ligation according to the method of Shay *et al.*¹⁶ (1954) with some modifications. In the pyloric ligation process the rats were fasted for 24 h before pylorus ligation with water *ad libitum*. Normal saline (1ml/rat, orally) was administered twice daily to all the animals during fasting period. Animals were anaesthetized using pentobarbitone (35 mg/kg body weight, i p) the abdomen was opened by midline incision below the abdomen. The pyloric portion of the stomach was slightly lifted out and ligated, avoiding damage to its blood supply. The stomach was placed back carefully and the abdominal muscular and skin layers were closed with sutures. Animals were sacrificed 4 h after pylorus ligation by cervical dislocation. The rat stomach was removed and opened along the greater curvature and then washed with serum physiological solution (0.9% w/v NaCl). Gastric content was subjected to measurement of gastric volume, free acidity and total acidity. Moreover, isolated tissue was employed for the measurement of ulcerative index and microscopic features.¹⁷

Experimental Design for Water-immersion oxidative stress induced model

Stress ulcers were induced by forced swimming in glass cylinders containing water up to the level of the xiphoid maintaining the temperature at 25^o C for 3 h. Animals were fasted for 24h prior to the experiment and divided into 5 groups with 6 animals in each group.¹⁸ The extract and the reference drug was given orally (p.o) by dissolving in 1% solution of carboxy methyl cellulose for 7 days and on the 8th day the experiment was stress was induced.

The experimental protocol is given below.

- 1) Group I (Normal control group): Rats were subjected to administration of 1ml normal saline orally for 7 days.
- 2) Group II (Swim stress group): Rats were subjected to forced swim stress for induction of ulcer.
- 3) Group III (Omeperazole group): Rats were subjected to administration of Omeperazole (20mg/kg, orally) for 7 days
- 4) Group IV (Extract treated group): Rats were subjected to administration of watermelon extract (300mg/kg, orally) for 7 days.

Anti-ulcer activity parameters

Measurement of ulcerative index

Ulcerative index was measured by method of Takagi *et al.* Briefly the stomach was opened and washed with running tap water. Then it was placed on a flat plate to count the ulcerative area. Standardization was made with a 10x10cm squared glass plate. Opened stomach, overlaid squared flat plate, exposing the mucous, showing the counting methodology of the injuries per square mm. The ulcer index was determined by using the formula,

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

Where, X = Total mucosal area / Total ulcerated area.

Estimation of Total gastric volume

The total volume of gastric content was measured in the measuring cylinder in case of pyloric ligation method.

Estimation of Free acidity

The gastric contents were centrifuged at 1000 rpm for 10 min. One ml of the supernatant liquid was pipette out and diluted to 10 ml with distilled water. The solution was titrated against 0.01N NaOH using Topfer's reagent (Dimethyl-amino-azo-benzene) as indicator, to the end point when the solution turned to orange colour. The volume of NaOH needed was taken as corresponding to the free acidity.

Estimation of total acidity

Titration was further continued by adding two drops of 1% solution of phenolphthalein till the solution gained the pink colour. The volume of NaOH required was noted and was taken as corresponding to the total acidity.

Acidity was expressed as

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{normality} \times 100 \text{ m Eq/L/100g}}{0.1}$$

RESULTS AND DISCUSSION

The Phytochemical screening of methanolic extract of *Citrullus lanatus* seeds reveals the presence of Carbohydrates, Triterpenoids, Phenolic compounds and steroids in the extract as shown in **Table 1**.

Table 1: Phytochemical screening of methanolic extract of *Citrullus lanatus* seeds

S. No.	Phytochemical Tests	Results
1.	Test for Alkaloids	-
2.	Test for Carbohydrates	+
3.	Test for Proteins and Amino Acids	+
4.	Test for Phytosterols	+
5.	Test for Phenolic compounds and Tannins	+
6.	Test for Saponins	-
7.	Test for Triterpenoids	+
8.	Test for Flavonoids	-
9.	Test for Anthraquinones	-
10.	Test for Coumarins	-

(-) represents the absence and (+) represents the presence for corresponding test.

The Triterpenoids and the phenolic compounds present in the extract may be responsible for the anti-ulcer activity of the *Citrullus lanatus* seeds. The phenolic compounds and the triterpenoids have many pharmacological activities including antioxidant, anti-inflammatory, anti-bacterial etc. some phenolic and triterpenoids which have anti-ulcer activity are also been published earlier.¹⁸ Recently, phytomedicines and nutraceuticals from food sources have become attractive sources of natural drugs.¹⁹⁻²⁰

In the present study the *Citrullus lanatus* seeds were evaluated for the anti-ulcer activity. The activity was checked by two methods pyloric ligation and water immersion stress induced ulcer methods. The pyloric ligation method involves the anti-secretory mechanism and water immersion stress induced ulcer involves the proton pump inhibitory activity.^{10, 18}

In the pyloric ligation method the animals were pretreated with methanolic extract of *Citrullus lanatus* seeds at the dose of 300 mg/kg for 7 days. It has been proposed that the ulcer formation in pyloric ligation method is developed due to accumulation of gastric acid and pepsin, which leads to auto-digestion of gastric mucosa.^{21, 22}

The pyloric ligation has caused the accumulation of gastric secretions 2.95 ml in the disease control group. The free and total acidity estimated were 65.16±2.151 mEq/l and 107.66±2.044 mEq/l respectively. Pretreatment with the seed extract at the dose of 300 mg/kg has reduced the volume of gastric secretions (1.37 ml), free acidity and total acidity were also significantly reduced

(28.00 ± 0.516 and 68.33 ± 1.838 respectively) which were comparable to the standard drug Ranitidine (50 mg/kg) used, thus

showing the anti-secretory mechanism of the plant. The results are compiled in the **Table 2**.

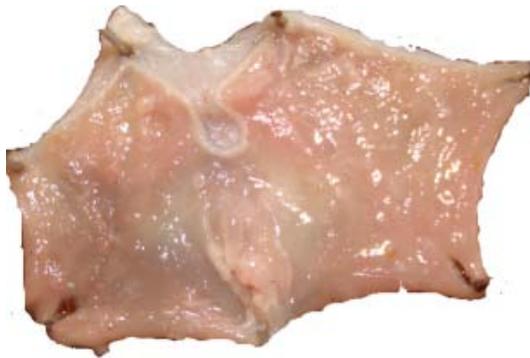


Fig. 1: Normal control group



Fig. 2: Pyloric ligation control group



Fig. 3: Ranitidine group



Fig. 4: Extract treated group

Table 2: Effect of methanolic extract of *Citrullus lanatus* seeds on Gastric volume, free acidity, and total acidity in pyloric ligated rats

Treatment	Dose (mg/kg)	Gastric Volume (ml/100g)	Free Acidity (mEq/l)	Total Acidity (mEq/l)
Normal	1 ml NS	1.18 ± 0.06	23.16 ± 0.94	57.66 ± 2.06
Disease	Pyloric ligation	2.95 ± 0.07	65.16 ± 2.15	107.66 ± 2.04
Ranitidine	50	1.08 ± 0.08^a	25.66 ± 1.45^a	61.83 ± 1.89^a
MECL	300	1.37 ± 0.04^a	28.00 ± 0.52^a	68.33 ± 1.84^a

Data are represented as mean \pm S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test. $^aP < 0.05$ as compared to disease group ($n = 6$ in each group)

Citrullus lanatus extract administered in dose of 300 mg/kg orally caused a decrease in ulcer index in both pyloric ligated and water immersion stress induced rats. It showed 57.33% and 63.38% ulcer protection compared to disease control group, which was

comparable to the standard drugs Ranitidine (50 mg/kg) and Omeprazole (20 mg/kg) in pyloric ligation and Water immersion stress model respectively. The results were given in **Table 3** and **Table 4**.

Table 3: Effect of methanolic extract of *Citrullus lanatus* seeds on ulcers induced by pyloric ligation method

Treatment	Dose (mg/kg)	Ulcer Index	% Inhibition
Normal	1 ml NS	0 ± 0.0	-
Disease	Pyloric ligation	5.18 ± 0.28	-
Ranitidine	50	1.84 ± 0.018^a	64.47
MECL 300	300	2.21 ± 0.022^a	57.33

Data are represented as mean \pm S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison tests. $^aP < 0.05$ as compared to disease group ($n = 6$ in each group)

Table 4: Effect of methanolic extract of *Citrullus lanatus* seeds on ulcers induced by water immersion stress model

Treatment	Dose (mg/kg)	Ulcer Index	% Inhibition
Normal	1 ml NS	0	-
Disease	Swim Stress	5.68 ± 0.31	-
Omeprazole	20 mg/kg	1.67 ± 0.03^a	70.59
MECL 300	300 mg/kg	2.08 ± 0.02^a	63.38

Data are represented as mean \pm S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison tests. $^aP < 0.05$ as compared to disease group ($n = 6$ in each group)



Fig. 5: Normal control group



Fig. 6: Swim stress group



Fig. 7: Omeperazole group



Fig. 8: Extract treated group

Ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors like gastric volume, free acidity and total acidity.^{23, 24} Water immersion stress is one of the best methods of stress in rats to induce ulcer. The model provides both emotional as well as physiological stress to the animals. Which affect gastrointestinal defense and increased accumulation of acid due to influx of H⁺ into the lumen of the stomach by parietal cell plasma membrane bound H⁺, K⁺-ATPase leading to auto digestion of the gastric mucosa and generation of free radicals which further increase the ulcers in the body.^{10, 25} As the methanolic extract has shown the significant anti-ulcer activity, it indicates the proton pump inhibitory activity of the *Citrullus lanatus* seeds.

From the present investigation it may be concluded that the methanolic extract of the *Citrullus lanatus* seeds has anti-secretory as well as proton pump inhibitory activity. So it has a good anti-ulcer therapeutic potential and can be used as an anti-ulcer remedy.

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