IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
May 2020 Vol.:18, Issue:2

Evaluation of *Ceratophyllum demersum* Linn. Whole Plant for Gastric Antiulcer Activity in Experimental Rats

IJPPR
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ISSN 2349-7203
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Sunil S. Karale[1*], Suhas S. Awati[2], Abhijeet S. Kulkarni[1]

1. Ashokrao Mane Institute of Pharmaceutical Sciences & Research, Save. India.

2. Dr. Shivajirao Kadam College of Pharmacy, Kasabe Digraj. India.

Submission: 23 April 2020
Accepted: 01 May 2020
Published: 30 May 2020

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: *Ceratophyllum demersum* Linn, gastric antiulcer activity, pylorus ligation, aspirin induced, water immersion induced stress

ABSTRACT

Aim: To evaluate the methanolic and aqueous extracts of *Ceratophyllum demersum* Linn. whole plant for gastric antiulcer activity using experimental models in rats. **Methods:** Acute oral toxicity studies were performed according as per the OECD 423 guidelines. Antiulcer activity of methanolic and aqueous extracts of *Ceratophyllum demersum* was studied at a dose of 250 mg/kg and 500 mg/kg body weight. Plant was evaluated using pylorus ligation, aspirin induced and water immersion induced stress ulcer in rats and the parameters were taken into account during the experiment on each rat for pylorus ligation model were: gastric acid volume, pH, free acidity, total acidity, ulcer index, mucus content, percentage inhibition of ulcer and for aspirin induced and stress induced ulcer models the parameters were: ulcer index, mucus content and percentage inhibition of ulcer. Glycosides, flavonoids, alkaloids, steroids and tannins have been reported to be present in whole plant of *Ceratophyllum demersum*. **Results:** The study indicates that, both the extracts at a dose of 500 mg/kg showed significant increase in pH and mucus content and decrease in gastric acid volume, free acidity, total acidity and ulcer index. **Conclusion:** It can be concluded that *Ceratophyllum demersum* possesses antiulcer activity, which support the use of whole plant in traditional medicine to treat the ulcer conditions.

INTRODUCTION

India has a rich tradition of plant-based knowledge on healthcare. The concept of developing drugs from plants in indigenous medical system is much older, while in some cases direct links between a local and biomedical use exists, in other cases the relationship is much more complex [1]. However, plants are the most important source for the new drug development due to the resurgence of the interest in the use of herbal preparation [2].

Ulcer is a well defined loss of tissue from the surface of an organ [3]. Peptic ulcer is one of the major gastrointestinal disorders, which occur due to an imbalance between offensive (acid and pepsin) and defensive (bicarbonate, mucin, prostaglandin, nitric oxide) factors. Consequently, reduction of gastric acid production as well as reinforcement of gastric mucosal production has been the major approaches for therapy of peptic ulcer disease. As a result, more and more drugs, both herbal and synthetic are coming up offering newer and better options for treatment of peptic ulcer[4].

The current medical treatment of peptic ulcer is generally based on the inhibition of gastric acid secretion by H_2 blockers, proton pump inhibitors and antimuscarinics, as well as on the acid independent therapy (Cytoprotective action) provided by sucralfate and bismuth. In the case of *Helicobacter pylori* infection, antibiotics are used. Obviously, drugs endowed with antisecretory activity coupled with gastroprotective effect could represent a promising approach for successful treatment of peptic gastric ulcer because of potential complementary effects of therapeutic modalities acting via different mechanisms[5]. At the same time, each of these drugs confers simpler to several side effects like arrhythmias, impotence, gynaecomastia, enterochromaffin-like cell (ELC) hyperplasia and haemopoeitic changes [4].

Ceratophyllum demersum Linn. is an aquatic, rootless, perennial plant of Ceratophyllaceae family, about 8 inches to 3 feet long, densely leaved, green in colour. Leaves are long which are spreaded in water and forming a net-like, inter-jointly, gradually a dense coverage on water surface. The whole plant has been traditionally used in the treatment of ulcer, diarrhoea, dysentery, wounds, fever, burning sensation, haemorrhoids or piles, intrinsic hemorrhage, hyperdipsia, epistaxis, hematemesis [6-8].

However, the antiulcer activity of *Ceratophyllum demersum* has not been scientifically investigated. Hence, we undertook a study for pharmacological evaluation of *Ceratophyllum demersum* for its traditionally claimed antiulcer property.

MATERIALS AND METHODS

Plant material

The fresh whole plants of *Ceratophyllum demersum* were collected from Kolhapur district of Maharashtra state, India. The specimens were identified and authenticated by Dr. Harsha Hegde, research officer, Regional Medical Research Centre, Indian Council of Medical Research, Belgaum. A voucher specimen of the plant (RMRC-465) was deposited at the herbarium of Regional Medical Research Centre, Indian Council of Medical Research, Belgaum.

Preparation of extract

The collected whole plants were washed under running tape water, dried under shade and coarse powdered in mechanical grinder. The dried powder (55 gm) was extracted in a Soxhlet extractor with methanol and a total of 50 cycles were run to obtain thick slurry. 50 gm of powder was macerated successively with 500 ml of distilled water for three days with intermitted stirring and then it was filtered and concentrated to get think slurry. This slurry was then vacuum evaporated to yield solid extract. The dried extracts were stored in a well-closed, airtight and light resistant borosil glass container.

Preliminary phytochemical screening

In order to determine the presence of alkaloids, glycosides, flavonoids, tannins, steroids, saponins a preliminary phytochemical study with plant extracts was performed [9-10].

Animals used

Female Swiss albino mice, weighing between 20-25 gm and Male Wistar albino rats, weighing 150-200 g were used for toxicity studies and antiulcer activity, respectively. Animals were acclimatized in the laboratory for 7 days before experimentation and housed in groups of four per cage at temperature 25±1^{0}C with 12:12 hours light: dark cycle was maintained. Animals were provided with standard rodent pellets diet (Gold Mohur food and feeds Ltd., Vikhroli (East), Mumbai) and water *ad libitum*. The experiment was carried out according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA No.25/1/99-AWD) and the study was approved by institutional animal ethical committee.

Preparation of doses

For antiulcer activity, the methanolic extract was suspended in 1% w/v solution of Carboxyl methylcellulose (CMC) in distilled water and aqueous extract was dissolved in distilled water.

Acute toxicity studies

The acute oral toxicity studies were performed according to OECD (Organization for Economic Control and Development) 423 guidelines on female Swiss albino mice by Acute Toxic Class Method [11]. Animals were fasted for 4 h with free access of water only. The methanolic and aqueous extracts were administered orally at a dose of 5 mg/kg initially and mortality if any was observed for 3 days. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then higher (50, 300, 2000 and 5000 mg/kg) doses of both extracts were employed for further toxicity studies.

Drugs and Chemicals

Ranitidine (Cadila Pharma Ltd.), aspirin (USV Pharma Ltd.) and methanol (Sd fine-chem Ltd.) were used during the experimental protocol.

Pyloric ligated rat model

The pyloric ligation induced gastric ulceration method was carried out according to the method of Shay *et al*. Male Wistar albino rats, weighing 150-200 g were housed in individual cages and fasted for 24 h prior to pyloric ligation with free access of water. Before the initiation of experiment, care was taken to avoid coprophagy. The animals were divided into six groups of six rats in each.

Group I - Served as control, received 1% CMC.

Group II - Served as positive control, received ranitidine (50 mg/kg) orally.

Group III & IV - Served as test groups, treated orally with methanolic extract 250 & 500 mg/kg, respectively.

Group V & VI - Served as test groups, treated orally with aqueous extract 250 & 500 mg/kg, respectively.

Extracts, ranitidine and vehicle were administered orally, 1 h before the pylorus ligation. The pylorus of each rat was ligated under light ether anaesthesia by abdominal incision and closed after surgery. The rats were sacrificed 6 h later, their stomach was removed and the gastric contents were collected. The gastric juice was centrifuged at 3000 rpm for 30 min and gastric volume, pH, free acidity and total acidity were estimated. The stomach was cut opened along the greater curvature and pinned on a soft board for evaluating ulcer index and mucus content [2, 12].

The percentage inhibition of ulcer was calculated as:

$$\% \text{ inhibition of ulcer} = \frac{\text{Mean ulcer index of control} - \text{Mean ulcer index of test}}{\text{Mean ulcer index of control}} \times 100$$

Scoring for ulcer index: 0 – Normal coloured stomach, 0.5 – red colouration, 1 – spot ulcers, 1.5 – hemorrhagic streaks, 2 – ulcers ≥3 but ≤ 5, 3 – Ulcers >5.

Determination of free and total acidity

One ml of gastric juice was diluted to 10 ml with distilled water. The solution was titrated against 0.01 N sodium hydroxide solution using Topfer's reagent (dimethyl-amino-azo-benzene) as an indicator. The solution was titrated to the endpoint when the solution turns to orange colour and the volume of NaOH which corresponds to the free acidity was noted. Then solution was further titrated till it regains pink colour. The total volume of NaOH which corresponds to the total acidity was noted [13].

Acidity (mEq/l/100 g) can be expressed as:

$$\text{Acidity} = \text{Vol. of NaOH} \times \text{Normality} \times 100 / 0.1 \text{ mEq/l/100 g}$$

Determination of gastric mucus content

Adherent gastric mucus was determined by the method of *Corn et al.* The stomach of each animal was removed, opened along the greater curvature and rinsed in cold saline. The glandular part of the stomach was excised, weighted and immersed for 2 h in 10 ml of 0.01%

w/v Alcian blue in 0.16 M sucrose solution. The excess dye was removed by rinsing twice in 0.25 M sucrose solution (15 min each). The mucus bound dye was extracted by immersing the gastric tissue in 0.5 M $MgCl_2$ solution which was intermittently shaken for 1 min at 30 min intervals during a 2 h period. The blue extract was shaken with diethyl ether. The emulsion was then centrifuged at 3600 rpm for 10 min and the optical density of the aqueous phase was measured spectrometrically at 600 nm. The results are expressed as absorbance per gram of tissue (A/g of tissue)[14].

Aspirin induced ulcer in rats

Gastric ulcerations were induced experimentally in male Wistar rats according to the method of *Asano et al.* Rats weighing 150-200 g were housed in individual cages and fasted for 24 h prior to experimentation with free access of water. Before the start of experimentation, care was taken to avoid coprophagy. The animals were divided into six groups of six rats in each.

Group I - Served as control, received 1% CMC +Aspirin (500 mg/kg)

Group II - Served as positive control, received ranitidine (50 mg/kg), orally

Group III & IV - Served as test groups, treated orally with methanolic extract 250 & 500 mg/kg, respectively.

Group V & VI - Served as test groups, treated orally with aqueous extract 250 & 500 mg/kg, respectively.

Extracts, ranitidine and vehicle were administered 1 h before the administration of 500 mg/kg per oral of aspirin. The animals were sacrificed 4 h after aspirin dosing, stomach was removed and observed for percent protection. The mucus content of the stomach was determined [2].

Water immersion induced stress ulcer in rats

Male Wistar albino rats, weighing 150-200 g were housed in an individual cages and fasted 24 h prior to experimentation with free access to water and care was taken to avoid coprophagy. The animals were divided into six groups of six rats in each.

Group I - Served as control, received 1% CMC.

Group II - Served as positive control, received ranitidine (50 mg/kg), orally.

Group III & IV - Served as test groups, treated orally with methanolic extract 250 & 500 mg/kg, respectively.

Group V & VI - Served as test groups, treated orally with aqueous extract 250 & 500 mg/kg, respectively.

After the drug treatment, animals were forced to swim inside the vertical cylinder (height 30 cm, diameter 15 cm) containing water up to 15 cm height, maintained at 23^0C for 3 h. After three hour they were removed from the cylinder and stomach of each animal was removed and observed for percent protection. The mucus content of the stomach was determined [15-16].

Statistical analysis

The groups were compared using one-way analysis of variance (ANOVA) followed by Dunnett's test and $P < 0.05$ was considered as significant.

RESULTS

Preliminary phytochemical screening

The percentage yield of methanolic and aqueous extracts was found to be 15.90% w/w and 18.44% w/w, respectively. The methanolic and aqueous extracts showed presence of glycosides, flavonoids, alkaloids, steroids and tannins.

Acute toxicity studies

All the doses of (5, 50, 300, 2000 and 5000 mg/kg) of methanolic and aqueous extracts employed for acute oral toxicity studies were found to be non-toxic. Both the extracts did not produce any mortality even at the highest dose (5000 mg/kg). Hence, $1/10^{th}$ and $1/20^{th}$ of the highest safer dose, i.e. 500 and 250 mg/kg body weight were selected for evaluation of antiulcer activity.

Pylorus ligation-induced gastric ulceration

Plants extracts are one of the most attractive sources of new drugs and have shown promising result for the treatment of gastric ulcer in several experimental models for evaluating antiulcer drugs.

In pylorus ligation rat model, methanolic extract of *Ceratophyllum demersum* (MECD) at a dose of 500 mg/kg body weight showed significantly ($p < 0.01$) decrease in volume of gastric acid secretion, free acidity, total acidity, ulcer index and aqueous extract of *Ceratophyllum demersum* (AECD) at a dose of 500 mg/kg body weight showed significant decrease in free acidity, total acidity, ulcer index ($p < 0.01$) and volume of gastric acid secretion ($p < 0.01$) when compared to control. Both the extracts, at a dose of 500 mg/kg showed significant increase in pH and mucus content ($p < 0.01$) and at a dose of 250 mg/kg showed significant decrease in ulcer index and increase in mucus content ($p < 0.01$). As expected, Ranitidine at a dose of 50 mg/kg body weight showed significant decrease in volume of gastric secretion, free acidity, total acidity, ulcer index and increase in pH and mucus content ($p < 0.01$). MECD and AECD, at the doses of 250 mg/kg inhibited ulceration by 41.18% and 35.29% and at a dose of 500 mg/kg inhibited ulceration by 60.29% and 42.65%, respectively. Ranitidine inhibited ulceration by 74.99 % [Table No.1].

Aspirin induced gastric ulceration:

Animals treated with aspirin at a dose of 500 mg/kg orally, developed ulcers in the glandular portion of the stomach. MECD and AECD (500 mg/kg b.w.) reduced gastric ulcers as evidenced by a significant reduction in the ulcer index and increase in the mucus content ($p < 0.01$) when compared to control group. Ranitidine (50 mg/kg) significantly reduced ulcer index and increase in mucus content ($p < 0.01$). MECD and AECD at a dose of 250 mg/kg showed protection index of 43.52% and 33.32% and at a dose of 500 mg/kg 63.33% and 60%, respectively. Ranitidine showed protection index 66.66% [Table No.2].

Stress induced gastric ulceration:

Severe hemorrhagic gastric – glandular mucosal ulcers were observed in stress induced controlled animals. MECD and AECD showed significant reduction in gastric index and increase in mucus content ($p < 0.01$) when compared with control group. Ranitidine (50 mg/kg) significantly reduced ulcer index and increase in mucus content ($p < 0.01$). MECD and AECD at a dose of 250 mg/kg showed protection index of 34.79% and 21.75% and at a dose of 500 mg/kg showed protection index 56.31% and 47.83% respectively, ranitidine showed protection index 60.87% [Table No.3].

DISCUSSION

There are several factors that may induce ulcer in human beings, such as: stress, chronic use of anti-inflammatory drugs and continuous alcohol ingestion. Although in most cases the etiology of ulcer is unknown, it is accepted that it is result of an imbalance between aggressive factors and maintenance of the mucosal integrity through the endogenous defence mechanism. The candidate for an effective drug against peptic ulcer should basically act either by reducing the aggressive factors on gastrointestinal mucosa or by increasing mucosal resistance against them [17].

Pylorus ligation-induced ulcer model was used to study the effect of extracts on gastric secretion. The ligation of the pylorus end of the stomach causes accumulation of gastric acid in the stomach that produces ulcers in the stomach. Agents that decrease gastric acid secretion and /or increase mucus secretion are effective in protecting the ulcers induced by this model. The methanolic and aqueous extracts of *Ceratophyllum demersum* produced reduction in gastric acid secretion proving their antisecretory effect [18].

Nonsteroidal anti-inflammatory drugs (NSAIDS), like aspirin and indomethacin, are known to induce ulcers during the course of anti-inflammatory therapy, by inhibiting prostaglandin synthesis through the cycloxygenase pathway. In the stomach, prostaglandins play a vital protective role, stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and maintaining the mucosal cell turnover and repair. Thus, the suppression of prostaglandin synthesis by NSAIDS results in increased susceptibility to mucosal injury and gastric ulceration. It was observed that, both the extracts of *Ceratophyllum demersum* displayed significant reduction of the mucosal damage in the aspirin induced rat model [17].

Gastric stress ulceration is probably mediated by the release of histamine. It not only enhances the gastric secretion, often called the aggressive factor but also causes disturbances of the gastric microcirculation and abnormal motility and reduces mucus production, known as the defensive factor. Moreover, stress induced ulcer in animal models may be partially or entirely prevented by vagotomy, since increased vagal activity has been suggested as the main factor in stress induced ulceration. Both the extracts of *Ceratophyllum demersum* inhibited the production of stress-induced ulcers. This finding indicates that, both the extracts may enhance gastric mucosal defence factors [17].

Several plants containing tannins have been reported to possess anti-ulcerogenic activity. Besides tannins, flavonoids are known as naturally occurring compounds having gastroprotective effects. Several mechanisms have been proposed to explain their biological effects; including increase of mucosal prostaglandin content, decrease of histamine secretion from mast cells, inhibition of acid secretion and inhibition of *H. pylori* growth. Several plants containing high amount of saponins have been shown to possess anti-ulcer activity in several experimental ulcer models. The protective activities of these saponins may be due to the activation of mucous membrane protective factors, inhibition of gastric secretion volume and acid secretion.[19] Eventually, tannin, saponins and flavonoid content of *Ceratophyllum demersum* could contribute to its gastroprotective effect.

CONCLUSION

From the above discussion, it may be concluded that administration of methanolic and aqueous extracts may prevent ulcers, probably by inhibiting gastric acid secretion and increasing mucus content, which scientifically support the use of whole plant in traditional medicine to treat ulcer condition.

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TABLES:

Table No. 1: Effect of *Ceratophyllum demersum* on Pyloric ligated rat model

Parameters	Control	Standard	MECD250	MECD500	AECD250	AECD500
Gastric volume (ml)	10.42 ± 0.468	5.808 ± 0.435**	9.040 ± 0.403	7.670 ± 0.436**	9.258 ± 0.627	8.195 ± 0.473*
pH	2.658 ± 0.154	3.965 ± 0.188**	2.633 ± 0.210	3.585 ± 0.145**	2.612 ± 0.163	3.567 ± 0.171**
Free acidity (mEq/l/100g)	42.67 ± 1.892	22.33 ± 1.764**	38.50 ± 0.764	24 ± 1.238**	38.17 ± 1.778**	28.67 ± 1.726**
Total acidity (mEq/l/100g)	72.17 ± 6.690	45.83 ± 5.160**	69.83 ± 2.120	49.50 ± 2.790**	74.17 ± 5.212	50.83 ± 2.455**
Ulcer index	5.667 ± 0.441	1.417 ± 0.239**	3.333 ± 0.247**	2.250 ± 0.382**	3.667 ± 0.307**	3.250 ± 0.382**
Mucus content	0.111 ± 0.005	0.259 ± 0.011**	0.171 ± 0.002**	0.199 ± 0.008**	0.158 ± 0.005**	0.181 ± 0.004**
Percentage inhibition	-	74.99	41.18	60.29	35.29	42.65

Data are expressed as the Mean ± S.E.M., n = 6 in each group. * $p < 0.05$, ** $p < 0.01$ when compared to control group (one way ANOVA followed by Dunnett's test).

MECD: Methanolic extract of *Ceratophyllum demersum.*

AECD: Aqueous extract of *Ceratophyllum demersum.*

Table No. 2: Effect of *Ceratophyllum demersum* on Aspirin induced ulcer in rats

Parameters	Negative Control	Standard	MECD250	MECD500	AECD250	AECD500
Ulcer index	2.500 ± 0.289	0.833 ± 0.279**	1.667 ± 0.422	0.917 ± 0.239**	1.667 ± 0.380	1 ± 0.224**
Mucus Content	0.141 ± 0.003	0.304 ± 0.013**	0.172 ± 0.010	0.263 ± 0.015**	0.160 ± 0.006	0.254 ± 0.013**
Percentage inhibition	-	66.66	43.32	63.33	33.32	60

Data are expressed as the mean ± S.E.M., n = 6 in each group. ** $p < 0.01$ when compared to negative control group (one way ANOVA followed by Dunnett's test).

Table No. 3: Effect of *Ceratophyllum demersum* on Water immersion stress-induced ulcer in rats

Parameters	Control	Standard	MECD250	MECD500	AECD250	AECD500
Ulcer index	1.917 ± 0.154	0.750 ± 0.214**	1.250 ± 0.214	0.833 ± 0.247**	1.500 ± 0.289	1 ± 0.183**
Mucus Content	0.153 ± 0.009	0.343 ± 0.022**	0.198 ± 0.010	0.302 ± 0.011**	0.174 ± 0.007	0.262 ± 0.016**
Percentage inhibition	-	60.87	34.79	56.53	21.75	47.83

Data are expressed as the mean ± S.E.M., n = 6 in each group. ** $p < 0.01$ when compared to control group (one way ANOVA followed by Dunnett's test).