Current Trends in Biotechnology and Pharmacy
Vol. 16 (3) 357 - 364, july 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3.49

Evaluation of Anti-Ulcer Activity of Corchorus *Trilocularis* Linn Plant Extract

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Abstract

Herbal medicines are nowadays playing a significant role in the treatment of different ailments in human beings and animals. Bioactive molecules separated from natural sources assume a prevailing job in the development of a novel medication. The current study focused on evaluation of anti-ulcer activity of *Corchorus trilocularis* Linn plant extract. *Corchorus trilocularis* Linn belongs to Tiliaceae family and is rich in pharmacological properties. The plant extract was prepared using various solvents. The extract with high yield was selected for further studies. Various phytochemical components were evaluated for the selected extract. Anti-ulcer activity was evaluated using pylorus ligation, ethanol induced and aspirin induced ulcer models on albino rats using 250mg/kg and 500 mg/kg doses. Ethanolic extract showed highest yield of 11.28%w/w. Phytochemical screening showed the presence of glycosides, alkaloids, triterpenes, phytosterols, tannins, flavonoids and steroids. 70% ethanolic extract of *Corchorus trilocularis* Linn showed dose dependent decrease in the ulcer index levels. 500mg/kg of 70% ethanolic extract showed 56.46% of inhibition and 250mg/kg showed 51.02% inhibition of ulcer index. In ethanol induced gastric ulceration, 70% ethanolic extract of *Corchorus trilocularis* Linn showed 41.16% and 45.58% protection from ulcers at 250mg/kg and 500mg/kg doses respectively. In aspirin induced gastric ulceration in rats, the 70% ethanolic extract of *Corchorus trilocularis* Linn showed 18.70% and 46.99% protection from ulcers at 250mg/kg and 500mg/kg doses respectively. The acid secretory parameters like total acidity and volume of gastric secretions were also decreased. Thus the current study showed that *Corchorus trilocularis* Linn could be used as a potential anti-ulcer agent that could be beneficial for geriatrics and patients under frequent medication suffering with ulcers.

Keywords: Ulcer, Ethanol, Pylorus ligation and Aspirin.

Introduction

Tribal prescriptions are the material network in regard to old medicines. The reason for using medicinal plants as therapeutic agents is because they are the major sources of secondary metabolites (1). Bioactive molecules separated from natural merchandise assume a prevailing job in the improvement of novel medication leads for the treatment of sicknesses (2). Ulcers are sores on the lining of the stomach or small intestine. They may also appear on throat. Ulcer is a major health issue in people, especially for geriatrics (3). Ulcers are caused due to several reasons like *Helicobacter pylori* bacterial infection, excessive alcohol consumption or stress (4). They can be treated using

proton pump inhibitors or antibiotics or antacids or anti-depressant drugs. The agents which are being used in treatment of ulcers causes many side effects like hypersensitivity, kidney damage and arrhythmias (5). They also have many drug-drug interactions which limits their usage (6). As a result, focus has been shifted towards natural sources with low side effects.

Many natural remedies have also been in use for the treatment of ulcers (7). One of such plants is *Corchorus trilocularis.* It belongs to the family Tiliaceae. The plant leaves are salty. They have purgative, tonic and stimulant properties (8). The seeds taste bitter and consumed as medication for curing of fever, rheumatism and obstruction of the abdominal viscera. The entire plant is utilized in the curing diseases of the abdominal viscera by the rural populations in India. In addition anti-pyretic, anti-inflammatory and analgesic activities of the plant have been documented (9). The plant genus *Corchorus* has documented the protective activity for gastric ulceration and *in-vitro* anti-acidic properties (10).

Ulcers in wistar rats can be induced by many techniques. Among those, pylorus ligation technique, ethanol and NSAIDs induced ulcers are widely employed due to their ease of study (11). This present research is undertaken to analyze the gastro-protective and anti-acidic properties of *Corchorus trilocularis* Linn plant extract.

Materials and Methods

Plant material

A fresh entire plant of *Corchorus trilocularies* (Linn) were collected from Dharwad, Karnataka. The taxonomical identification of the plant and authentication was done by Prof. I.C. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli. The collected plant specimen was tagged and deposited in the college herbarium.

Preparation of extracts of Corchorus trilocularis

The entire plant of *Corchorus trilocularis* was shade dried, powdered and subjected to soxhlet extraction process after defatting by petroleum ether. The extraction was done using various solvents like chloroform, ethyl acetate, ethanol and water. At the end, the extracts were evaporated on water bath to obtain crude (12). After cooling, the condensed extracts were weighed and stored in air-tight containers at 4^0C till further investigation.

Phytochemical analysis

Preliminary phytochemical screening was performed for the extracts of *Corchorus trilocularis* to detect the presence of various constituents responsible for the pharmacological activity like carbohydrates, glycosides, alkaloids, triterpenes, phytosterols, proteins, fixed oils, phenols and tannins, flavonoids, steroids, mucilage and saponins (13). The results were given in table 1.

Drugs and chemicals

Aspirin was commercially procured from Micro Labs Ltd., Bengaluru. Lansoprazole was commercially procured from Unichem Laboratories, Goa. Topfer's reagent was commercially procured from S.D Fine Chemicals, Mumbai. All other materials used were of analytic grade and procured commercially.

Experimental animals

Healthy adult albino rats (Wistar strain) weighing 150 to 200g were procured and housed in polypropylene cages, maintained under standardized condition i.e., 12:12 hour light/dark cycle at 25 ± 2^0C with paddy husk bedding. They were provided with standard pellet food and had free access to purified drinking water.

Evaluation of antiulcer activity

Pylorus ligation induced ulcer model

The selected animals (albino rats) were randomly categorized into 4 groups with 6 animals in each group. Group 1 is treated as control and received only normal saline. Group 2 received the standard Lansoprazole at a dose

of 8 mg/kg i.p. Group 3 received 70% of ethanolic extract of *Corchorus trilocularis* at a dose of 250 mg/kg p.o. Group 4 received 70% of ethanolic extract of *Corchorus trilocularis* at a dose of 500 mg/kg p.o. The individual caged albino rats were subjected to 24 fasting hours and care was taken to avoid coprophagy. 30 minutes prior to pyloric ligation, ethanolic extract of *Corchorus trilocularis* or standard drug were administered and abdomen was sutured. Animals were sacrificed to the end of 6 hours by giving anaesthesia after ligation. The animal stomachs were surgically collected and dissected. The gastric fluids of the stomach were squeezed into collecting tubes followed by centrifugation (1000 rpm for 10 min) and gastric fluid volume was recorded. Employing a pH meter, the gastric juice pH was recorded. Total and free acidity of the collected contents was analyzed. The animal stomach were washed and examined for the presence of ulcers in the glandular portion employing the hand lens (10X) and the numbers of ulcers per stomach were documented and the ulcer severity is microscopically scored. For each animal, mean ulcer score was expressed as ulcer index (14). The results were given in table 2 and 3. The % of protection was calculated using the formula;

$$\% \text{ Protection} = 100 - u_t/u_c \times 100$$

Where, u_c is ulcer index of control group and u_t is ulcer index of treated group.

Determination of total acidity and free acidity

From the collected gastric juice, 1 ml was pipetted into a 100ml conical flask along with 2 to 3 drops of Topfer's reagent. The solution is titrated with 0.01N sodium hydroxide till the solution turns to yellowish orange in color followed by disappearance of red color. Alkali volume run down was recorded which corresponds to free acidity. The titration is continued by adding 2 to 3 drops of phenolphthalein till red tinge recurs. Volume of alkali run down is recorded which is related to total acidity (15). The results were given in table 3. The acidity was calculated with the following formula.

$$\text{Acidity} = \frac{(\text{Volume of NaOH consumed} \times \text{Normality of NaOH} \times \text{Eq.wt})}{(\text{W (g)} \times 1000)} \times 100$$

Ethanol induced ulcer model

The selected animals (albino rats) were randomly categorized into 4 groups with 6 rats in each group. Group 1 is treated as Control in which the animals are administered with 1 ml/200g body weight of 95% alcohol p.o. Group 2 animals were administered with Lansoprazole at a dose of 8 mg/kg i.p. Group 3 received 70% of ethanolic extract of *Corchorus trilocularis* at a dose of 250 mg/kg p.o. Group 4 received 70% of ethanolic extract of *Corchorus trilocularis* at a dose of 500 mg/kg p.o. All the animals selected for the study were subjected to fasting prior to 24hours of study with free access to water only. After 30 min of the dose administration, the animals were given 1ml/200g of 95% ethanol p.o. The animals were carefully sacrificed after 1 hour of ethanol administration (16). The animal stomachs were carefully isolated and dissected to cut open along the greater curvature pinned on the soft board. The number of ulcers per stomach was recorded using a 10X hand lens. The ulcer severity was scored microscopically and percentage protection was calculated. The results were given in table 4.

Aspirin induced ulcer model

Ulcers can also be induced with the over administration of Aspirin at higher doses. Prior to the execution of the experiment the selected animals (albino rats) were randomly categorized into 3 groups with 6 animals in each group and are subjected to 36 hours of fasting with free access to water. Group 1 is considered as control and treated with aspirin at a dose of 30 mg/kg, p.o. Group 2 received standard Lansoprazole at a dose of 8mg/kg, p.o along with aspirin at a dose of 30 mg/kg, p.o. Group 3 received 70% of ethanolic extract of *Corchorus trilocularis* at a dose of 250 mg/kg p.o along with aspirin at a dose of 30 mg/kg, p.o. Group 4 received 70% of ethanolic extract of *Corchorus trilocularis* at a dose of 500 mg/kg p.o along with aspirin at a dose of 30 mg/kg, p.o. The animals were carefully sacrificed after 6 hours of aspirin administration and stomachs were carefully isolated

and dissected to cut open along the greater curvature pinned on the soft board. The number of ulcers per stomach was recorded using a 10X hand lens (17). The ulcer severity was scored microscopically and percentage protection was calculated. The results were given in table 5.

Statistical analysis

Graph Pad Prism 5.0 software was used for the analysis of the results obtained. The mean value is accompanied by the standard error of mean (mean ± SD).

Results and Discussion

The present study was performed to prepare extracts of *Corchorus trilocularis* and to evaluate its anti-ulcer property. Among all the extracts prepared, the extract derived using ethanol showed highest yield of 11.28% w/w. The extract was blackish green in colour with characteristic plant odour. This extract was selected for further evaluation process.

Phytochemical screening

The phytochemical investigation of ethanolic extract of *Corchorus trilocularis* revealed the presence of glycosides, alkaloids, triterpenes, phytosterols, phenols and tannins, flavonoids, mucilage and steroids which promises their huge pharmacological abilities. The results were indicated in table 1.

Past studies indicated that the phytochemicals which are present in the ethanolic extracts of *Corchorus trilocularis* have been responsible for the antiulcer activity. Especially, the phenols and flavonoids tend to have anti-ulcer effect due to their cyto-protective, anti-secretory, anti-oxidant, anti-inflammatory and anti-*H.pylori* effects (18). They also promote stress defense mechanisms, synthesis of anti-oxidant enzymes, prostaglandin synthesis and wound healing (19). Tannins protect the mucosal layer and prevent damage due to chemicals (20).

Table 1: Phytochemical Screening of Ethanolic Extract of *Corchorus trilocularis*

Phytochemical Constituents	**Ethanolic Extract of *Corchorus trilocularis***
Carbohydrates	–
Glycosides	+
Alkaloids	+
Triterpenes	+
Phytosterols	+
Proteins	–
Fixed oils	–
Tannins	+
Flavonoids	+
Saponins	–
Mucilage	+
Steroids	+

+ indicates Presence; – indicates Absence

Evaluation of antiulcer activity

Pylorus ligation induced ulcer model

Ligation of pylorus region in stomach

Table 2: Effect of Ethanolic Extract of Corchorus trilocularis on Ulcer Index in Pylorus Ligation induced Gastric Ulceration in Rats

Groups	Treatment	Dose	Ulcer Index (Mean ± SEM)	% Protection
I	Control	--	4.41±0.37	--
II	Lansoprazole Standard	8 mg/kg	0.916±0.472***	79.22
III	70% Ethanolic extract of Corchorus trilocularis	250 mg/kg	2.16±0.40***	51.02
IV	70% Ethanolic extract of Corchorus trilocularis	500 mg/kg	1.92±0.23***	56.46

Mean ± S.E.M. (N=6); *P<0.05, **P<0.001 and *** P<0.001 Vs. Control

leads to accumulation of gastric acid and pepsin activation which leads to ulcer formation. It also enhances the synthesis of prostaglandins, the factors responsible for ulcers (21). Histamine, an inflammatory mediator also forms due to pylorus ligation (22). In case of pylorus ligation induced gastric ulceration in rats, there is marked decrease in levels of mean ulcer index. 70% ethanolic extract of *Corchorus trilocularis* (Linn) showed dose dependent decrease in the ulcer index levels. The results were given in table 2.

A significant decrease in total acidity and increase in gastric pH has been observed in rats treated with *Corchorus trilocularis.* This is due to the presence of phytochemicals in *Corchorus trilocularis* with anti-ulcer potency. The results were indicated in table 3.

Table 3: Effect of Ethanolic Extract of *Corchorus trilocularis* on Volume of Gastric Juice, Acidity and Gastric pH by Pylorus Ligation induced Gastric Ulceration in Rats

Groups	Treatment	Volume of Gastric Juice (ml)	Free Acidity (mEq/L/100g)	Total Acidity (mEq/L/100g)	Gastric pH
I	Control	9.11±0.30	125.5±0.635	134.91±5.96	2.68±0.030
II	Standard (Lansoprazole 8 mg/kg)	3.0±0.634***	34.5±0.444***	55.35±4.64***	6.58±0.195***
III	70% Ethanolic extract of *Corchorus trilocularis* (250 mg/kg)	3.63±0.65***	67.3±0.434***	76.56±3.60***	3.683±0.079***
IV	70% Ethanolic extract of *Corchorus trilocularis* (500 mg/kg)	3.36±0.44***	60.6±1.209***	66.35±8.93***	3.90±0.046***

*Mean ± S.E.M. (N=6); *** P<0.001 Vs. Control*

Ethanol induced ulcer model

Ethanol is an agent which induces ulcer in similar to the condition of excess secretion of hydrochloric acid. It indirectly damages the sensitive gastric mucosal layer through lipid peroxidation (23). It also increases the ulcer formation and reduces mucous formation. It also causes increased production of free radicals that leads to decreased prostaglandin levels (24). This ultimately results in reduction of gastric protection and increases gastric acid secretion. In case of ethanol induced gastric ulceration in rats, *Corchorus trilocularis* showed a marked decrease in levels of mean ulcer index. 70% ethanolic extract of *Corchorus trilocularis* showed dose dependent decrease in the ulcer index levels and increase in protection from ulcers. This is attributed to the presence of flavonoids which protects the stomach by producing antioxidants (25). The results were indicated in table 4.

Conclusion

From the above results, it was concluded that the ethanolic extract of *Corchorus trilocularis* showed promising anti-ulcer effect. Further work is needed to be done on the isolation of specific phytochemical component that

Table 4: Effect of Ethanolic Extract of *Corchorus trilocularis* on Ulcer Index in Ethanol induced Gastric Ulceration in Rats

Groups	Treatment	Dose	Ulcer Index (Mean ± SEM)	% Protection
I	Control	--	5.66±0.802	--
II	Standard (Lansoprazole 8mg/kg)	8mg/kg	1.416±0.3***	75.08
III	70% Ethanolic extract of *Corchorus trilocularis*	250mg/kg	3.33±0.27**	41.16
IV	70% Ethanolic extract of *Corchorus trilocularis*	500mg/kg	3.08±0.35**	45.58

Mean ± S.E.M. (N=6); **P<0.001 and *** P<0.001 Vs. Control

Aspirin induced ulcer model

Aspirin is one of the powerful drugs which inhibit biosynthesis of PGE2. PGE2 is generally needed for production of bicarbonates and mucous in stomach which protects the stomach from ulcers (26). When aspirin is administered, it leads to production of ulcers and damage to gastric mucous membrane. It also produces reactive oxygen species which causes ulcers (27). In case of aspirin induced gastric ulceration in rats, *Corchorus trilocularis* showed a marked decrease in levels of mean ulcer index. 70% ethanolic extract of *Corchorus trilocularis* showed dose dependent decrease in the ulcer index levels and increase in protection from ulcers. This is also due to the presence of significant phytochemical constituents of *Corchorus trilocularis*. The results were given in table 5.

Table 5: Effect of Ethanolic Extract of Corchorus trilocularis on Ulcer Index in Aspirin induced Gastric Ulceration in Rats

Groups	**Treatment**	**Dose**	**Ulcer Index (Mean ± SEM)**	**% Protection**
I	Control	--	2.66±0.42	--
II	Standard (Lansoprazole 8mg/kg)	8mg/kg	0.916±0.30***	65.78
III	70% Ethanolic extract of *Corchorus trilocularis*	250mg/kg	2.16±0.24	18.70
IV	70% Ethanolic extract of *Corchorus trilocularis*	500mg/kg	1.41±0.15	46.99

Mean ± S.E.M. (N=6); *** P<0.001 Vs. Control

From the above results, it was concluded that the ethanolic extract of Corchorus trilocularis showed promising anti-ulcer effect. Further work is needed to be done on the isolation of specific phytochemical component that is responsible for anti-ulcer activity.

Acknowledgement

The authors are thankful to the management of S.C.S. College of Pharmacy, Harapanahalli and Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram for their sheer support during the work.

Conflict of interest

Authors declare no conflict of interest.

Ethical statement

The guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India were followed and prior approval was soughted from Institutional Animal Ethics Committee (IAEC) for conducting the study (IPS/COP/IAEC/02).

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