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EVALUATION OF ANTI-ULCER ACTIVITY OF ETHANOLIC EXTRACT OF *CYATHOCLINE PURPUREA*

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Abstract

Peptic ulcer disease is a serious gastrointestinal disorder that requires a well targeted therapeutic strategy. A number of drugs including proton pump inhibitors and H2 receptor antagonists are available for the treatment of peptic ulcer, but clinical evaluation of these drugs has shown incidence of relapses, side effects and drug interactions. The aim of this study to elucidate anti-ulcer activity of ethanolic extract of *Cyathocline purpurea*. The female albino rats were artificially induced ulcer by absolute ethanol (5 ml/kg). The animals were randomly assigned to different groups each consisting of six rats. About four groups were created. Groups III and IV received sucralfate and ethanolic extract of *Cyathocline purpurea* (Leaves) respectively one hour before the administration of ethanol once a day for all 15 days.The results showed that ethanolic extract of *Cyathocline purpurea* (EECP) has significant effect on protecting and treating gastric ulcer induced by ethanol. ethanolic extract of *Cyathocline purpurea* (EECP) significantly ($P<0.05$) reduced gastric lesions by 40.98% in ethanol induced ulcer models at 250 mg/kg when compared to sucralfate (100 mg/ kg) which reduced gastric lesions (60.41%). From the results it can be deduced that *Cyathocline purpurea* have effective anti- ulcer property.

Keywords: Anti -ulcer activity, *Cyathocline purpurea,* Medicinal plants, Sucralfate

Introduction

One of the most common diseases in the world, peptic ulcers affects four million individuals annually. The term "peptic ulcer" describes an acid peptic injury to the digestive tract that causes mucosal breakage to the submucosa. Pepsin, acid, and *Helicobacter pylori* are examples of offensive factors, while bicarbonates, prostaglandins, mucin, nitric oxide, and growth factors are examples of defensive factors. Additionally, it has been discovered that the peptic ulcer illness has a chronic remitting course and that there is an imperfect association between symptoms and ulcer occurrence. Primary peptic ulcers are frequently brought on by *Helicobacter pylori* infection. It is linked to 95% of duodenal ulcers and 70% of stomach ulcers. The use of alcohol, cocaine, tobacco, amphetamines, chronic nonsteroidal anti-inflammatory drug (NSAID) administration, fasting, Zollinger-Ellison syndrome, and cancer therapy with angiogenesis inhibitors are additional risk factors for developing peptic ulcer disease (Najam, 2011; Napolitano,2009)

Nature has been the source of medicinal agents since the beginning of man. Traditional medicine is currently well acknowledged and established as a viable profession. Traditional medicine plays a great role in providing primary health care worldwide. About 75–80% people in developing countries use traditional medicine because of better cultural acceptability, better compatibility with the human body, and lesser side effects. Large numbers of medicinal plants and their secondary metabolites have claimed antiulcer activity (Awaad *et al*., 2013).

One such plant *Cyathocline purpurea* (Buch-Ham ex D. Don.) Kuntze is an herb that can be found in rice fields in portions of northern and the peninsular India at a height of 1300 metres. It is traditionally used to cure a number of illnesses, including rheumatism, malaria, TB, and inflammatory and bleeding disorders. This plant's chemical components are said to have anti-inflammatory and antioxidant properties. There are very few phytochemicals, including Australian-sourced lactones that have been reported to have anticancer action. Additionally, it has been claimed that *Cyathocline purpurea* extracts have anti-oxidant, anti-microbial, anti-fungal, anti-helminthic, antiplaque, hypotensive, and insect repellent properties. Indian variety of *C. purpurea's* phytoconstituent, 6-hydroxy-4, 10-guaianadien-8, 12-olide, demonstrated plant growth regulatory activity (Tambewagh *et al*., 2017; Ma *et al*., 2009)

Materials & Method

Materials

The plants have been selected on the basis of its availability and folk use of the plant. The leaves of *Cyathocline purpurea* were collected from local area of Bhopal in the month of February, 2022. Drying of fresh plant parts was carried out in sun but under the shade. Dried leaves of *Cyathocline purpurea* were preserved in plastic bags, closed tightly and powdered as per the requirements.

Animals

Healthy adult Wistar albino rats of either sex were selected randomly for the study. The rats were obtained from College of Veterinary Sciences and Animal Husbandry, Mhow, Indore. Rats of 12–16 weeks, weighing 160–200 g, were used for the experiment. Each rat was housed in a plastic box cage under standard conditions at 19–25°C and was kept under 12/12 h light/dark cycle. The rats were allowed free access to standard pellet feed and water ad libitum. The study was carried out according to the CPCSEA and Organization of Economic Co-operation and Development (OECD) guidelines. Approval from Institutional animal ethical committee (IAEC) was also obtained.

Methods

Extraction process

70.56 grams shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. Defatted powdered of *Cyathocline purpurea* has been extracted with ethanol solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Mukherjee,2007).

Determination of percentage yield

The yield of the collected plant extracts was measured in grams after extraction, and then converted into percentage.

Phytochemical screening

Phytochemical analyses were performed according to the normal protocols for extract. Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

Quantitative estimation of bioactive compounds

Total phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 10-50µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm (Mishra *et al*., 2017)

Acute toxicity test

Acute toxicity study was carried out using the limit test dose of 2000 mg/kg as described by OECD 425 guideline. Three female albino rats were fasted for 24 hours but allowed free access to water. A limit dose of 2000 mg/kg of ethanolic extract of *Cyathocline purpurea* (Leaves) was administered sequentially and animals were observed individually for behavioral profile (alertness, restlessness, irritability, and fearfulness), autonomic profiles (defecation and urination), neurologic profile (spontaneous activity, reactivity, touch response, pain response, and gait), physical states such as lacrimation, loss of appetite, tremors, hair erection, salivation, diarrhea, and for morbidity or mortality, after dosing

continuously for 2 hours, periodically during the first 24 hours (with special attention given during the first 4 hours) and daily thereafter, for a total of 14 days.

Grouping and dosing of animals

Animals were randomly assigned to different groups each consisting of six rats. All treatments were given orally 1 hour before the experiment by oral gavage. Doses were determined based on the acute toxicity studies as per OECD guidelines (OECD)

Ethanol induced gastric ulcer model and dosing

Rats were fasted for 48 hours before administration of absolute ethanol (5 ml/kg) for the induction of gastric ulcer model (Park *et al*., 2015) The dosing was performed as follows;

Group I (Negative control): received 2 ml of distilled water or normal saline once a day for fifteen days

Group II (Positive control); received 90% ethanol (5 ml/kg) only once a day of for fifteen days (Abebaw *et al*., 2017)

Group III (Standard drug control); received sucralfate (100 mg/kg) once a day for fifteen days

Group IV (Extract treated); received ethanolic extract of *Cyathocline purpurea* (Leaves) (250 mg/kg) once a day for fifteen days.

Groups III and **IV** received sucralfate and ethanolic extract of *Cyathocline purpurea* (Leaves) respectively one hour before the administration of ethanol once a day for all 15 days (Somani *et al*., 2008)

Determination of pH

Gastric juice obtained from the stomach of rats were directly projected for testing of pH with litmus paper (More *et al*., 1983)

Determination of Total Acidity

Gastric juice was diluted with 1 ml of distilled water and then taken into a conical flask and then two to three drops of phenolphthalein indicator was added and titrated against 0.01N NaOH until a permanent pink color was seen. The volume of 0.01N NaOH used was noted. The total acidity was expressed as mEq/L and calculated by the given formula:

Macroscopic Evaluation

The stomach was opened and rinsed with distilled water to remove gastric contents and examined by a magnifying glass to assess for the induction of ulcers. Scoring of ulcer was made as follows (Dashputre and Naikwade, 2011):

Normal colored stomach (0)

Red coloration (0.5)

Spot ulcer (1)

Hemorrhagic streak (1.5)

Deep ulcers (2)

Perforation (3)

Determination of Ulcer Index

The Ulcer index (UI) was calculated by,

$UI = (Un + Us + Up) \times 10^{-1}$

Whereas; Un = average number of ulcers per animal, Us = average number of severity of scores

Up = percentage of animals with ulcers and % protection = (C-T/C) X 100

Where C = ulcer index in control group, T = ulcer index in treated group (Ozbakiş & Gürsan,2005)

Statistical Analysis

Data was expressed as mean ± standard error of mean and statistically evaluated using one-way analysis of variance, followed by Tukey's multiple comparison tests. $P<0.05$ was considered as significant.

Results

Percentage yield determination

Table 1: % Yield of ethanolic extract of *Cyathocline purpurea*

S. No.	Extracts	% Yield (w/w)
1.	Pet. ether	0.95%
2.	Ethanolic	8.46%

Results of phytochemical screening

Table 2: Phytochemical screening of extract of *Cyathocline purpurea*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendroff's Test	-ve
	Hager's Test	-ve
2.	**Glycosides**	
	Modified Borntrager's Test	-ve
	Legal's Test	+ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	-ve
4.	**Phenol**	
	Ferric chloride test	+ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Molisch's Test	-ve
	Benedict's Test	-ve
	Fehling's Test	-ve
7.	**Saponins**	
	Froth Test	-ve
	Foam Test	+ve
8.	**Diterpenes**	
	Copper acetate test	-ve
9.	**Tannins**	
	Gelatin Test	+ve

Table 3: Estimation of total phenolic and flavonoids content of *Cyathocline purpurea*

S. No.	Ethanolic extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	***Cyathocline purpurea***	0.732	0.614

Table 4: Effect of EECP and standard drug on gastric acid pH in ethanol induced ulcer in rats

Group	Drug	Dose	pH (Mean±SEM)
I	Normal Control	Normal saline	3.1 ± 0.0
II	Ulcer Control	Normal saline	2.26± 0.25*
III	Sucralfate	100 mg/kg p.o.	4.9 ± 0.4**
IV	EECP	250 mg/kg p.o.	4.22 ±0.25**

Table 5: Effect of EECP and standard drug on total acidity in ethanol induced ulcer in rats

Group	Drug	Dose	Total Acidity (Mean±SEM)
I	Normal Control	Normal saline	50.21 ± 0.47
II	Ulcer Control	Normal saline	73.41 ± 1.04*
III	Sucralfate	100 mg/kg p.o.	53.21 ±1.35**
IV	EECP	250 mg/kg p.o.	61.43 ± 0.47**

Table 6: % age gastric ulcer protection in standard drug control (group III) and extract treated group of rats (group IV)

Group	Drug	Dose	Ulcer Index (UI)	Percentage gastric ulcer protection
I	Normal Control	Normal saline	0	-
II	Ulcer Control	Normal saline	13.32 ± 0.036	0%
III	Sucralfate	100 mg/kg p.o.	5.27 ± 0.595	60.41%
IV	EECP	250 mg/kg p.o.	7.76 ± 0.007	41.68%

Table 7: Macroscopic evaluation of gastric mucosa treated with standard and EECP.

Group	Drug	Dose	Macroscopic Evaluation
I	Normal Control	Normal saline	0.0 ±0.0
II	Ulcer Control	Normal saline	1.5 ± 0.2
III	Sucralfate	100 mg/kg p.o.	0.5 ± 0*
IV	EECP	250 mg/kg p.o.	1.1 ± 0.2*

Table 8: Ulcer index in different groups of animals in ethanol -induced ulcer in rats

Group	Drug	Dose	Un	Us	Up	UI
I	Normal Control	Normal saline	0	0	0	0
II	Ulcer Control	Normal saline	31.2	3.03	100	13.32 ± 0.36
III	Sucralfate	100 mg/kg p.o.	12.4	1.35	40	5.27 ± 0.59
IV	EECP	250 mg/kg p.o.	16.8	1.88	60	7.76 ± 0.007

Conclusion

In conclusion, the results of the present study strongly indicated the gastro protective effects of ethanloic extract of *Cyathocline purpurea* (EECP) in ethanol induced gastric ulcer. The ameliorating effect of ethanloic extract of *Cyathocline purpurea* (EECP) against gastric ulcer might be assigned to the observed anti-oxidative and anti-inflammatory properties of flavonoids present in *Cyathocline purpurea*. It is well known that flavonoids show anti-ulcer and anti-inflammatory activities and most of the flavonoids are strong antioxidants. However further studies are warranted to examine the gastro protective efficacy of *Cyathocline purpurea* in a clinical setup.

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