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Phytochemical and pharmacological evaluation of hydroalcoholic extract of *Lilium candidum* for anti-ulcer potential

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Abstract

The present study with the hydroalcoholic flower extract of *Lilium candidum (HELC)* revealed that it has significant anti-ulcer activity. Usually, NSAIDs and corticosteroids are widely used in clinical practice as anti-inflammatory agents. Except for newer highly selective COX-2 inhibitors, NSAIDs and corticosteroids produce significant gastric irritation resulting in gastritis and gastric ulceration, especially on long-term treatment. The present study revealed that the HELC has ulcer-protective properties. Previous studies showed its potent anti-inflammatory activity. Therefore, it can be considered as an ideal substitute for conventional NSAIDs and glucocorticoids. Further studies have to be conducted to explain precisely the mechanism of action of this drug. It increased the healing of the indomethacin-induced ulcer.

Keywords: Anti-ulcer, *Lilium candidum*, Hydroalcoholic Extract, Phytochemical Analysis.

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1. Introduction

Plants used for treating diseases are as old as the human species. Popular observations on the use and efficacy of medicinal plants significantly contribute to the disclosure of their therapeutic properties, so that they are frequently prescribed, even if their chemical constituents are not always completely known [1-3]. All over the globe, especially in South American countries, the use of medicinal plants has significantly supported primary health care [4].

Ulcers are an open sore of the skin or mucus membrane characterized by sloughing of inflamed dead tissue. A gastric ulcer would give epigastria pain during the meal, as gastric acid production is increased as food enters the stomach [5-6]. Symptoms of duodenal ulcers would initially be relieved by a meal, as the pyloric sphincter closes to concentrate the stomach contents; therefore, acid is not reaching the duodenum [7]. Peptic ulcer disease (PUD) is an illness that affects a considerable number of people worldwide. It develops when there is an imbalance between the ‘‘aggressive’’ and ‘‘protective’’ factors at the luminal surface of the epithelial cells [8-9]. Aggressive factors include *Helicobacter pylori*, HCl, pepsins, non-steroidal anti-inflammatory drugs (NSAIDs), bile acids, ischemia, hypoxia, smoking and alcohol. While defensive factors include bicarbonate, mucus layer, mucosal blood flows, PGs and growth factors [10].

The currently used antiulcer drugs like H2–receptors blockers, proton pump inhibitors, anti-muscarinic produce adverse reactions such as hypersensitivity, arrhythmia, impotence and haemopoietic changes with is a possibility of increased rate of ulcer recurrence within one year after cessation of the treatment [11-12]. Because of the above-mentioned demerits reported with the current antiulcer therapy there is a need for the search of newer therapeutic antiulcer agents from plant sources from the alternative therapy Ayurveda [13]. Plant extracts some of the most

attractive sources of new drugs shown to produce promising and favourable results in the treatment of gastric ulcers. Further, in the traditional medicine Ayurveda, several plants and herbs are advocated for the treatment of gastrointestinal disorders, including gastric ulcers [14-15].

No scientific data is available in support of traditional uses of many plants, including antiulcer activity of *Lilium candidum* Linn.

Hence the present study was planned to evaluate antiulcer activity of hydro-alcoholic extracts of *Lilium candidum* Linn in pylorus ligation, stress, ethanol and aspirin induced gastric ulcer model in experimental animal rats.

2. Materials and Methods

2.1 Plant Material Collection

Flowers of *Lilium candidum* were collected from local area of Bhopal (M.P.) in the month of January, 2024.

2.2 Extraction Procedure

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs.

2.3 Defatting of Plant Material

Powdered flowers of *Lilium candidum* were shade-dried at room temperature. The shade-dried plants material was coarsely powdered and preserved in an air-tight bottle for further use, subjected to extraction with petroleum ether using the maceration method. The extraction was continued till the defatting of the material had taken place [16].

2.3 Extraction by Maceration Process

50 g of dried plant material were exhaustively extracted with hydroalcoholic solvent (80:20: ethanol: water) using the maceration method. The extract was evaporated above its boiling point. Finally, the percentage yields of the dried extracts were calculated[17].

2.4 Determination of Percentage Yield

Calculation of percentage yield

The percentage yield of each extract was calculated by using the formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

2.5 Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods.

2.5.1 Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered.

2.5.2 Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

2.5.3 Detection of glycosides

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

2.6 *In vivo* Anti-ulcer activity of Hydroalcoholic flower extract of *Lilium candidum* (HELC)

2.61 Experimental Animals: -

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

2.6.2 Drugs & Chemicals

Ranitidine (Sigma Lab, Mumbai) were used in present study.

2.6.3 Toxicity study

Preliminary experiments were carried out on rats (n=6). HELC were administered orally in different doses to find out the range of doses which cause zero and 100 % mortality of animals. Acute oral toxicity was conducted according to the method of Organization for Economic Co-operation and Development (OECD). Animals were kept fasting providing only water, HELC were given p. o. in doses of 500, 1000 and 2000 mg/kg/p.o. administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-ulcer effect.

Experimental designs

Aspirin-induced gastric ulcer

Group –1: Control
Group –2: Ranitidine (Standard)
Group –3: HELC (100mg/kg, p.o.)
Group –4: HELC (200mg/kg, p.o.)

The animals were fasted for 24 h before the experiment. Under anesthesia, ulcers were induced by applying aspirin (500 mg/kg. p.o.) over the anterior serosal surface of the stomach for 60 seconds. The animals were treated with Ranitidine (50 mg/kg, p.o.), low dose of HELC (100 m/kg p.o.) or high dose of HELC (100 m/kg p.o.) [once daily, for 5 days after the induction of ulcer, while the control group received only the vehicle. The rats were sacrificed on the 5th day, the stomachs removed and cut open along the greater curvature. The ulcer index was determined using the formula:

Ulcer index = 10/X

Where X = Total mucosal area/Total ulcerated area.

Based on their intensity, the ulcers were given scores as follows:

0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis, 3 = perforated or penetrated ulcer.

3. Results and Discussion

3.1 Result of the percentage yield of the extract

The yield of extracts obtained from the sample using the Hydroalcoholic solvent is depicted in Table 1

Table 1: Result of Percentage yield of Flowers of *Lilium candidum*

S. No.	Solvents	Percentage Yield
1.	Hydroalcoholic	8.12

3.2 Phytochemical analysis

The phytochemical analysis of hydroalcoholic extract of flowers of *Lilium candidum* was analysed (Table-2) for the compounds such as alkaloids, flavonoids, and glycosides, carbohydrates, saponins, phenols, proteins and amino acids and diterpenes. The preliminary phytochemical analysis revealed the presence of four compounds i.e. alkaloids, flavonoids, phenolics, saponins, and absence of glycosides, diterpenes, carbohydrate, proteins and amino acids. Various tests have been performed to find out the phytochemical constituents mentioned above [18].

Table 2: Result of Phytochemical Screening of Extracts of *Lilium candidum*

S. No.	Constituents	Hydroalcoholic Extract
1.	Alkaloids	+
2.	Glycosides	-
3.	Flavonoids	+
4.	Saponins	+
5.	Phenolics	+
6.	Amino Acids	-
7.	Carbohydrate	-
8.	Proteins	-
9.	Diterpenes	-

3.3 Results of estimation of total flavonoid and total phenol content of *Lilium candidum*

Natural antioxidants derived from plants, chiefly phenolics, are of considerable interest as dietary supplements or food preservatives. Hence, an attempt was made to quantify some secondary metabolites of hydroalcoholic extract of *Lilium candidum*. The total phenolic and flavonoid contents were analyzed and presented in Table 3-5.

3.4 Total flavonoid content estimation (TFC)

Total flavonoid content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: **Y=0.040X + 0.009, R^2=0.999**, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table 3: Preparation of Calibration Curve of Quercetin

S. No.	Concentration (µg/ml)	Absorbance
1	5	0.216
2	10	0.425
3	15	0.625
4	20	0.815
5	25	1.021

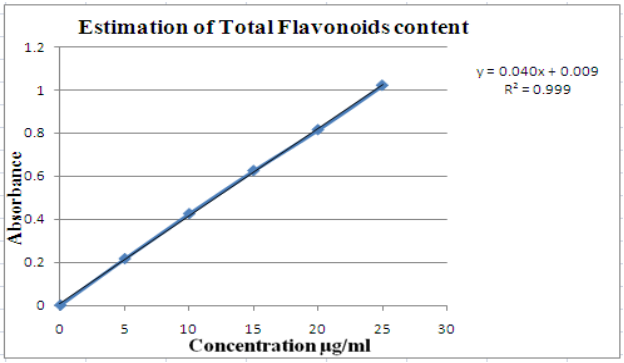

Figure 1: Graph of Estimation of Total Flavonoids Content

3.5 Total Phenolic content estimation (TPC)

The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **Y = 0.042X+0.002, R^2= 0.999,** where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Calibration Curve of Gallic acid

Table 4: Preparation of Calibration Curve of Gallic acid

S. No.	Concentration	Absorbance
1	5	0.194
2	10	0.422
3	15	0.637
4	20	0.848
5	25	1.035

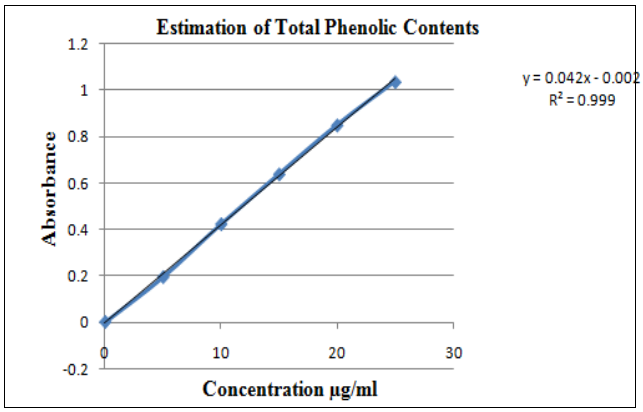

Figure 2: Graph of Estimation of Total Phenolic content

Table 5: Total Phenolic and Total flavonoid content of Hydroalcoholic extract of *Lilium candidum*

S. No.	Solvents→ Bioactive compound↓	Hydroalcoholic extract
Flowers of *Lilium candidum*		
1.	Total Phenol (Gallic acid equivalent (GAE) mg/100mg)	1.039
2.	Total flavonoid (Quercetin equivalent (QE) mg/100mg)	0.941

Table 1.6: Anti-ulcerogenic effect of HELC against ulcerogenic agents in rats (Ulcer index)

Treatment and dose	Aspirin
Control	3.80 ± 8.0
Ranitidine (50 mg/kg, p.o.)	1.80 ± 8.0***
HELC (100 mg/kg, p.o.)	2.15 ± 8.0**
HELC (200 mg/kg, p.o.)	1.76 ±8.0***

Values are expressed as mean±S.E.M. (n = 6).
Percent inhibition calculated as compared to control group. ***P < 0.001, ** P < 0.01, * P < 0.05 (One-way ANOVA followed by Tukey's post hoc test).

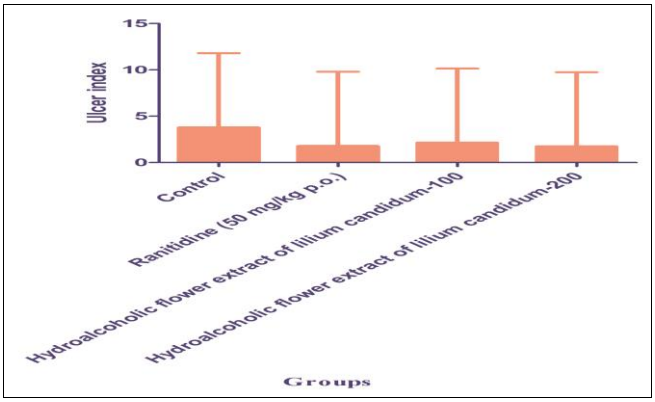

Figure 7.3: Anti-ulcerogenic effect of HELC against ulcerogenic agents in rats (Ulcer index)

Table 1.7: Anti-ulcerogenic effect of HELC against ulcerogenic agents in rats (PH)

Treatment and dose	Aspirin
Control	1.20 ± 5.0
Ranitidine (50 mg/kg, p.o.)	6.40 ± 5.0***
HELC (100 mg/kg, p.o.)	5.20 ± 5.0*
HELC (200 mg/kg, p.o.)	6.10 ± 5.0***

Values are expressed as mean±S.E.M. (n = 6).
Percent inhibition calculated as compared to control group. ***P < 0.001, ** P < 0.01, * P < 0.05 (One-way ANOVA followed by Tukey's post hoc test).

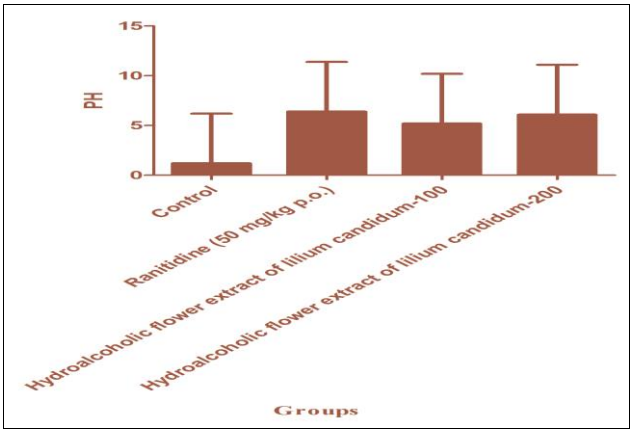

Figure 1.4: Anti-ulcerogenic effect of HELC against ulcerogenic agents in rats (PH)

4. Discussion

The present study investigated the effect of HELC on the ulcers. HELC showed effect on the healing of gastric ulcers induced by aspirin. HELC showed significant protection against aspirin-induced gastric ulcer in all dose levels. There is a dose-dependent increase in anti-ulcer effect of HELC. HELC was effective in reducing the ulcer area and the ulcer score.

5. Conclusion

The present study revealed that the HELC has ulcer-protective properties. Therefore, it can be considered as an ideal substitute for conventional NSAIDs and glucocorticoids. Further studies have to be conducted to explain precisely the mechanism of action of this drug. HELC has an antiulcer effect. It increased healing of indomethacin induced ulcer.

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