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PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF HYDROALCOHOLIC EXTRACT OF *CALOTROPIS PROCERA* FOR ANTI-ULCER POTENTIAL

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ABSTRACT

The present study with Hydroalcoholic flower extract of *Calotropis procera* revealed that it has significant anti-ulcer activity. Usually, NSAIDs and corticosteroids are widely used in clinical practice as anti-inflammatory agents. With the exception of newer highly selective COX-2 inhibitors, NSAID's and corticosteroids produce significant gastric irritation resulting in gastritis and gastric ulceration, especially on long-term treatment. Present study revealed that Hydroalcoholic flower extract of *Calotropis procera* has ulcer protective properties. Previous studies showed its potent anti-inflammatory activity. Therefore, it can be consider as anideal substitute for conventional NSAIDs and glucocotricoids. Further studies have to be conducted to explain precisely the mechanism of action of this drug. Hydroalcoholic flower extract of *Calotropis procera* has an antiulcer effect. It increased healing of indomethacin induced ulcer.

Keywords: Anti-ulcer, *Calotropis procera*, Hydroalcoholic Extract, Phytochemical Analysis.

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. All over the globe, especially in South American countries, the use of medicinal plants has significantly supported primary health care.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. 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. 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. 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.

The currently used antiulcer drugs like H2–receptors blockers, proton pump inhibitors, anti-muscuranics 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. 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 Ayurvedha. Plant extracts some of the most attractive sources of new drugs show to produce promising and favorable reasons in the treatment of gastric ulcer further in the traditional medicine ayurvedha, several plants and herbs are advocated for the treatment of gastrointestinal disorders including gastric ulcers.

No scientific data is available in support of traditional uses of many plants including antiulcer activity of *Calotropis procera* Linn.

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

MATERIALS AND METHODS

Plant Material Collection

Flowers of *Calotropis procera* were collected from local area of Bhopal (M.P.) in the month of January, 2019.

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Extraction Procedure

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

Defatting of Plant Material

Powdered flowers of *Calotropis procera* 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 maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction by Maceration Process

50g. of dried plants material were exhaustively extracted with Hydroalcoholic solvent (80:20: ethanol: water) using maceration method. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts.

Determination of Percentage yield

Calculation of percentage yield

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

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

Phytochemical Screening

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

Detection of alkaloids:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

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.

Detection of glycosides:

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

In vivo Anti-ulcer activity of Hydroalcoholic flower extract of *Calotropis procera*

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.

Drugs & Chemicals

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

Toxicity study

Preliminary experiments were carried out on rats (n=6). Hydroalcoholic flower extract of *Calotropis procera* 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 Organisation for Economic Co-operation and Development (OECD) [(75)]. Animals were kept fasting providing only water, Hydroalcoholic flower extract of *Calotropis procera* 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: Hydroalcoholic flower extract of *Calotropis procera* (100mg/kg, p.o.)

Group –4: Hydroalcoholic flower extract of *Calotropis procera* (200mg/kg, p.o.)

The animals were fasted for 24 h prior to the experiment. Under anaesthesia, 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 Hydroalcoholic flower extract of *Calotropis procera* (100 m/kg p.o.) or high dose of Hydroalcoholic flower extract of *Calotropis procera* (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 [(76)]. 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.

Table 1: Result of percentage yield of Flowers of *Calotropis procera*

S. No.	Solvents	Percentage Yield
1.	Hydroalcoholic	8.12

Table 2: Result of Phytochemical screening of extracts of *Calotropis procera*.

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

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

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

Table 5: Total Phenolic and Total flavonoid content of Hydroalcoholic extract of *Calotropis procera*.

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

Table 6: Anti-ulcerogenic effect of Hydroalcoholic flower extract of *Calotropis procera* 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***
Hydroalcoholic flower extract of *Calotropis procera*(100 mg/kg, p.o.)	2.15 ± 8.0**
Hydroalcoholic flower extract of *Calotropis procera* (200 mg/kg, p.o.)	1.76 ±8.0***

Values are expressed as mean±S.E.M. (n = 6).

Table 7: Anti-ulcerogenic effect of Hydroalcoholic flower extract of *Lilium candidum* 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***
Hydroalcoholic flower extract *Lilium candidum* (100 mg/kg, p.o.)	5.20 ± 5.0*
Hydroalcoholic flower extract of *Lilium candidum* (200 mg/kg, p.o.)	6.10 ± 5.0***

Values are expressed as mean±S.E.M. (n = 6).

Figure 1: Graph of estimation of total flavonoids content

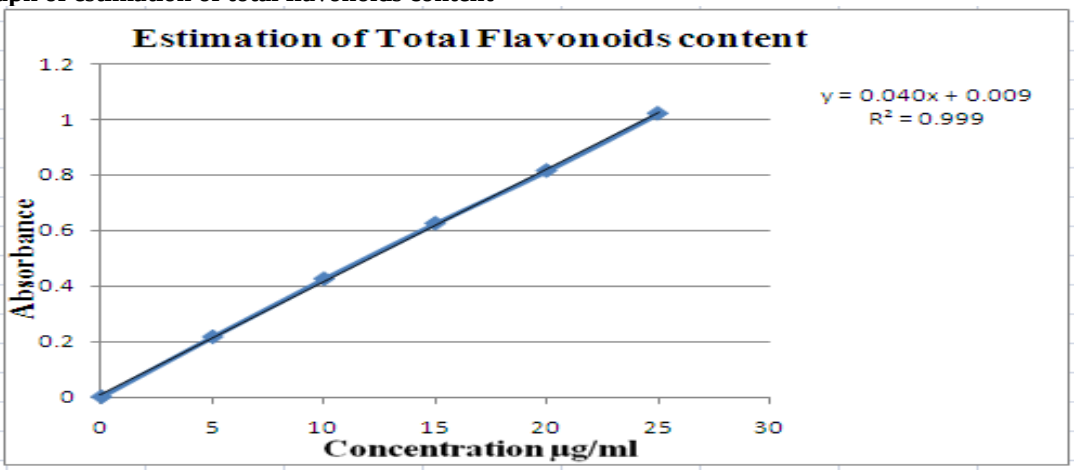


Figure 2: Graph of Estimation of Total Phenolic content.

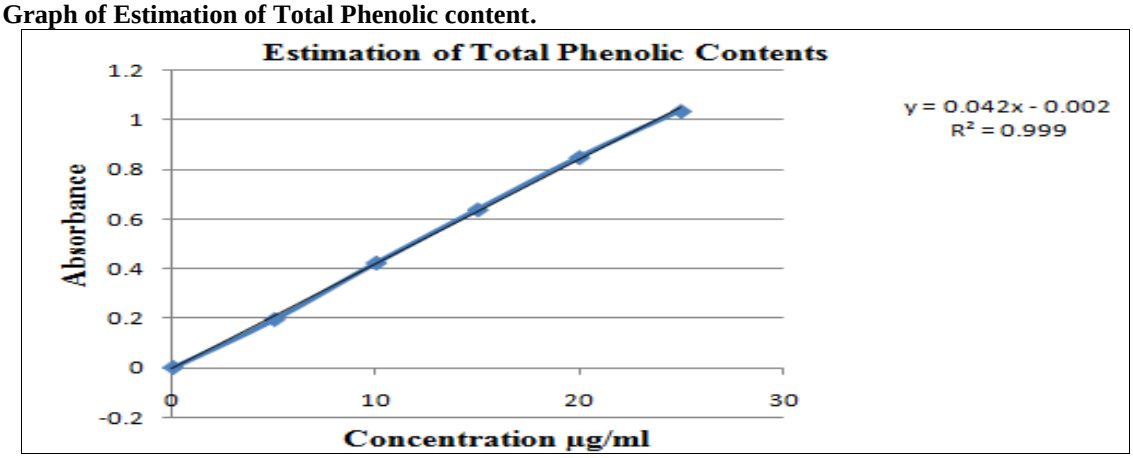


Figure 3: Anti-ulcerogenic effect of Hydroalcoholic flower extract of *Lilium candidum* against ulcerogenic agents in rats (PH)

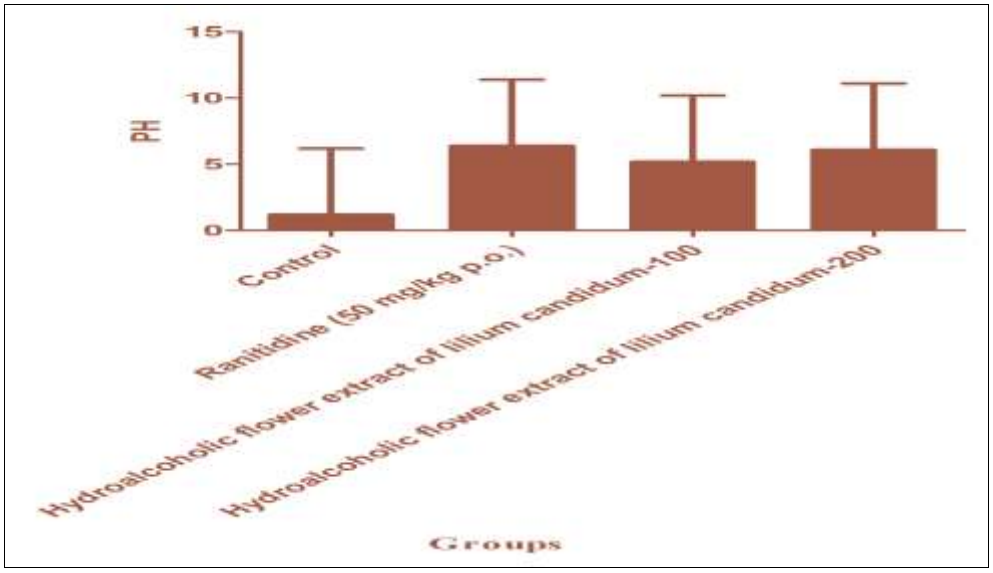
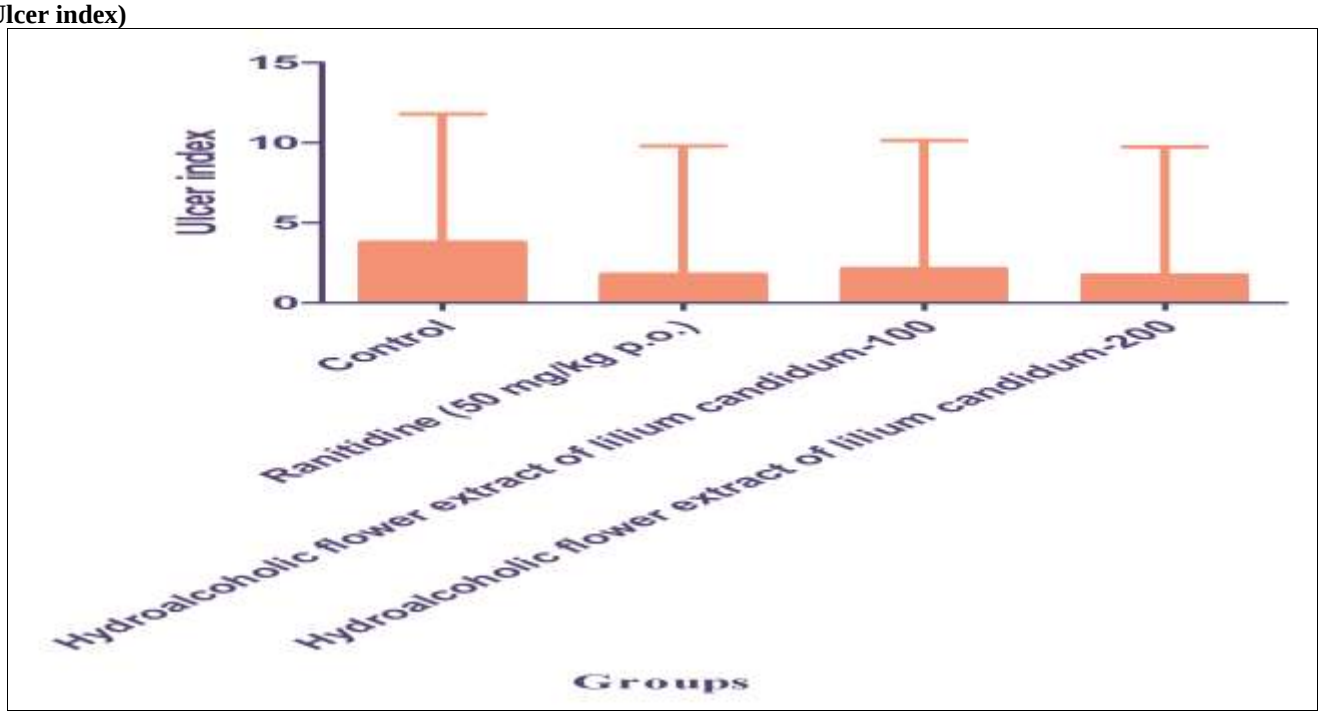


Figure 4: Anti-ulcerogenic effect of Hydroalcoholic flower extract of *Lilium candidum* against ulcerogenic agents in rats (Ulcer index)



RESULTS AND DISCUSSION

Result of percentage yield of extract

The yield of extracts obtained from sample using Hydroalcoholic solvent is depicted in the table 1.1

Phytochemical analysis

The phytochemical analysis of hydroalcoholic extract of flowers of *Calotropis procera* was analysed (Table-1.2) for the compounds such as alkaloids, flavanoids, 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.

Results of estimation of total flavonoid and total phenol content of *Calotropis procera*

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 metabilites of of hydroalcoholic extract of *Calotropis procera*. The total phenolic and flavonoid contents were analyzed and presented in table 1.3-1.5.

Total flavonoids content estimation (TFC)

Total flavonoids 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.

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

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).

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).

DISCUSSION

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

CONCLUSION

Present study revealed that Hydroalcoholic flower extract of *Lilium candidum* has ulcer protective properties. Therefore, it can be consider as anideal substitute for conventional NSAIDs and glucocotricoids. Further studies have to be conducted to explain precisely the mechanism

of action of this drug. Hydroalcoholic flower extract of *Lilium candidum* has an antiulcer effect. It increased healing of indomethacin induced ulcer.

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