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EVALUATION OF *IN VIVO* ANTI- ULCER ACTIVITY OF FLOWER EXTRACT OF *WITHANIA COAGULANS*

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ABSTRACT

Gastric ulcers are a prevalent gastrointestinal disorder that poses significant health challenges. *Withania coagulans*, commonly known as "Indian Rennet," is a medicinal plant with a history of traditional use in various health conditions, including digestive disorders. The present study aimed to evaluate the in vivo anti-ulcer activity of the flower extract of *Withania coagulans* using a suitable animal model. The flower extract was prepared using a hydroalcoholic extraction method and subjected to preliminary phytochemical screening to identify the presence of various bioactive compounds. The anti-ulcer activity was assessed in rats using indomethacin-induced ulcerogenesis as the experimental model. The rats were divided into different treatment groups, including a control group treated with ulcerogenic agents, a positive control group treated with ranitidine (a standard anti-ulcer drug), and groups treated with different doses of the *Withania coagulans* flower extract (100 mg/kg and 200 mg/kg, p.o.). The anti-ulcer activity was evaluated based on parameters such as the number of ulcers, ulcer index, and gastric pH. The results were analyzed statistically to determine the significance of the findings. The flower extract of *Withania coagulans* demonstrated significant anti-ulcerogenic effects in the animal model. The extract exhibited a dose-dependent reduction in the number of ulcers and the ulcer index compared to the control group. Furthermore, the extract administration led to an increase in the pH of gastric contents, suggesting its potential to reduce gastric acidity and protect the gastric mucosa from ulcerogenic agents. The flower extract of *Withania coagulans* possesses significant in vivo anti-ulcer activity. These findings support its potential therapeutic value in the management of gastric ulcers. The observed gastroprotective effects highlight the importance of *Withania coagulans* as a potential natural alternative or adjunctive therapy for the treatment of gastric ulcers. However, further research is warranted to elucidate the exact mechanisms of action and to conduct long-term safety studies and human clinical trials to determine its safety profile and efficacy in humans. The present study provides valuable insights into the medicinal potential of *Withania coagulans* and paves the way for further exploration and development of its therapeutic applications in the field of gastroenterology.

Keywords: *Withania coagulans,* ulcerogenic agents, phytochemical screening.

INTRODUCTION

Gastric ulcers are one of the most common gastrointestinal disorders, affecting millions of people worldwide. They result from an imbalance between aggressive factors, such as gastric acid secretion and the presence of ulcerogenic agents, and protective factors that maintain the integrity of the gastric mucosa. Gastric ulcers can lead to significant discomfort, pain, and potential complications if left untreated [1-2].

Traditional medicine has long relied on medicinal plants to treat various ailments, including gastrointestinal disorders. Withania coagulans, commonly known as "Indian Rennet" or "Paneer dodi," is a well-known medicinal plant used in various traditional systems of medicine for its potential therapeutic effects. It belongs to the Solanaceae family and is found in regions of India, Pakistan, and Afghanistan [3-4].

Withania coagulans has been traditionally used for its anti-inflammatory, antioxidant, and analgesic properties. Various parts of the plant, including its flowers, leaves, and roots, have been utilized in herbal preparations to alleviate digestive disorders, including gastric ulcers [5-6].

The flower extract of *Withania coagulans* has gained attention in recent years due to its rich phytochemical profile, which includes phenolic compounds, flavonoids, alkaloids, and other bioactive constituents. These compounds have been reported to exhibit various pharmacological activities, including gastroprotective effects [7].

Several studies have investigated the anti-ulcer potential of *Withania coagulans* extracts using in vitro and in vivo models. These studies have highlighted the gastroprotective effects of the plant and its potential in mitigating gastric mucosal damage caused by ulcerogenic agents.

The current study aims to evaluate the in vivo anti-ulcer activity of the flower extract of *Withania coagulans* using a suitable animal model. The experiment will assess the extract's effectiveness in reducing the number of ulcers, ulcer index, and gastric pH in rats with indomethacin-induced ulcerogenesis. This model is widely accepted for evaluating the gastroprotective properties of natural compounds.

The findings of this study hold significant implications for the development of *Withania coagulans* flower extract as a potential natural anti-ulcer agent. If successful, this research could provide valuable insights into the plant's therapeutic potential and contribute to the development of novel and effective gastroprotective agents.

MATERIAL AND METHODS

Extraction by maceration process

Defatted dried flower of *Withania coagulans* were extracted with hydroalcoholic solvent (ethanol: water; 80:20v/v) by maceration method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extract [8].

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage. Following formula was adopted for determination of percentage yield of selected plant materials. The percentage yield of each extract was calculated by using following formula:

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

Phytochemical screening

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods [9].

1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

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.

c) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

b) Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b) Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

7. Detection of proteins

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

8. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

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 [10].

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 [11].

In vivo* antiulcer activity of hydroalcoholic extract of *Withania coagulans

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

Indomethacin (Sigma Chemical Co, St Louis, MO, USA) were used in present study.

Toxicity study

Healthy adult male albino rats were fasted overnight prior to the experiment. Different doses (50-2000 mg/kg, P.O) of the hydroalcoholic extract of *Withania coagulans* were administered to each group of rats (Each group carries 6 rats) and they were observed continuously for 1 hour and then at half-hourly intervals for 4 hour, for any gross behavioural changes and further up to 72 hour, followed 14 days for any mortality as per the OECD (Organization for Economic Co-operation and Development) Guideline 425 [12]. The hydroalcoholic extract of *Withania coagulans* was found to be non-toxic up to the maximum dose of 2000 mg/kg body weight. Dose selected for antiulcer evaluation was 100 and 200 mg/kg respectively.

Indomethacin induced gastric ulcer

Method of C.N. Aguwa, et al (1981) was followed with minor modifications for the experiment. Thirty rats were taken. They were divided into four groups of six rats each. All the rats were starved for 24 h. After the fasting period, Indomethacin (40 mg/kg, p.o.) was given.

All samples of the plant extract were given 60 min prior to indomethacin as follows:

Group I: treated with Indomethacin (40mg/kg, p.o.) and was kept as control.

Group II: treated with Ranitidine (100mg/kg, p.o.) and was kept as standard.

Group III: treated with the hydroalcoholic extract of *Withania coagulans* (100 mg/kg, p.o.).

Group IV: treated with the hydroalcoholic extract of *Withania coagulans* (200mg/kg, p.o.).

The animals were sacrificed 5h after the treatment. Stomach was cut open in the greater curvature and ulcer scoring was done by using magnifying lens and the ulcer scored according to its severity in comparison with that of standard. The ulcer index was determined using the formula [13]:

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.

Statistical analysis

The results are expressed as the mean± SD for each group. Statistical differences were evaluated using a One-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Results were considered to be statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

Percentage loss of *Withania coagulans* was found to be 45.12% Table no. 1 showed the percentage yield of pet. Ether, hydroalcoholic extracts of *Withania coagulans* 3.5% and 8.4% respectively.

From the table no. 2, it could see that, flavonoids, glycosides, proteins, saponins, carbohydrate and phenol were present in hydroalcoholic extract of *Withania coagulans*. The phytochemical screening of *Withania coagulans* revealed negative results for alkaloids and diterpenes.

The total flavonoid content and total phenol content were calculated from the absorbance calibration curve generated with different concentrations of quercetin and gallic acid (standards) respectively. The total phenolic content of the hydroalcoholic extract was 0.568 GAE mg/100mg. Similarly, the TFC was assessed as 0.847 quercetin equivalents mg/100mg in hydroalcoholic extract table no. 3. Flavonoids, including flavones, flavanols, and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free hydroxyl groups. Plants rich in secondary metabolites, including phenolic, flavonoids, and carotenoids, have antioxidant activity due to their redox properties and chemical structures

The in vivo anti-ulcer activity of the flower extract of *Withania coagulans* was evaluated to investigate its potential gastroprotective properties. The study utilized a suitable animal model and measured various parameters, including the number of ulcers, ulcer index, and gastric pH, to assess the extract's efficacy in protecting against ulcerogenic agents. The flower extract of *Withania coagulans* was compared with a control group treated with ulcerogenic agents and a positive control group treated with a standard anti-ulcer drug (ranitidine) table no. 4.

Table 1: Results of percentage yield of *Withania coagulans*

Extracts	Percentage yield (%)
Pet. Ether	3.5%
Hydroalcoholic	8.4%

Table 2: Result of phytochemical screening of hydroalcoholic extract of *Withania coagulans*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	
	A) Wagner's Test:	-Ve
	B) Hager's Test:	-Ve
2.	Glycosides	
	A) Legal's Test:	+Ve
3.	Flavonoids	
	A) Lead acetate Test:	+Ve
	B) Alkaline Reagent Test:	+Ve
4.	Saponins	
	A) Froth Test:	+Ve
5.	Phenolics	
	A) Ferric Chloride Test:	+Ve
6.	Proteins	
	A) Xanthoproteic Test:	+Ve
7.	Carbohydrate	
	A) Fehling's Test:	+Ve
8.	Diterpenes	
	A) Copper acetate Test:	-Ve

Table 3: Estimation of total phenol and flavonoids content of hydroalcoholic extract of *Withania coagulans*

S. No.	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	0.568	0.847

Table 4: Anti-ulcerogenic effect of hydroalcoholic extract of *Withania coagulans* against ulcerogenic agents in rats (Number of Ulcers)

Treatment and dose	Number of Ulcers	Ulcer Index	pH
Control	10.50 ± 0.65	4.10 ± 0.35	1.20 ± 0.25
Ranitidine	0.72 ± 0.35***	0.75 ± 0.50***	6.55 ± 0.15***
Hydroalcoholic extract of *Withania coagulans* (100 mg/kg, p.o.)	2.45 ± 0.12*	1.90 ± 0.50**	4.15± 0.20*
Hydroalcoholic extract of *Withania coagulans* (200 mg/kg, p.o.)	1.45 ± 0.25***	1.10 ± 0.50***	5.20 ± 0.15***

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