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ORIGINAL RESEARCH ARTICLE

Anti Ulcer Activity of Methanolic and Aqueous Extracts of leaves of *Gymnema sylverstre* in Rats

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ABSTRACT

Methanolic and aqueous extracts of *Gymnema sylverstre* leaves were investigated for gastric protective activity on ethanol and pylorus ligation induced ulcer models. The extract at a concentration of 100mg/kg, 200mg/kg, 400mg/kg produced a protective effect on ulcer-induced models and was comparable with the standard drug Sucralfate 400mg/kg (cytoprotective agent) and Ranitidine 50mg/kg (H_2-antagonist). The present study revealed that the methanolic extract of *Gymnema sylverstre* had ulcer protective activity comparable with standard drugs Sucralfate and Ranitidine.

Key words: Ulcer, Ethanol, pylorus ligation, Biochemical parameters, Methanolic extract.

INTRODUCTION

Peptic ulcer is one of the major gastro-intestinal disorders, which occur due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors.[1] Some other factors such as inadequate dietary habits, excessive ingestion of non-steroidal anti-inflammatory agents, stress, hereditary predisposition and infection by Helicobacter pylori, may be responsible for the development of peptic ulcer. Consequently, reduction of gastric acid production as well as re-inforcement of gastric mucosal production has been the major approaches for therapy of peptic ulcer disease. [2]

Gymnema sylverstre is an ethno medical plant used in different countries by diverse ancient cultures and tribal groups. It is one of the local herbs that is claimed to possess various physiological effects and it occupies an important place in the indigenous system of medicine. The leaves of *G. sylvestre* have been used for centuries in the traditional Indian system of Ayurvedic medicine. Gymnema has been used in India for the treatment of diabetes for over 2,000 years [3,4] the leaves were used for stomach ailments, constipation, water retention and liver disease. It is also used for many conditions including diabetes, digestion, urinary tract problems, obesity, hypoglycemia, allergies, anemia, cholesterol and hyperactivity. The leaves are also noted for lowering serum cholesterol and triglycerides. [5-9] Thus far, there is no data available on gastroprotective activity of *Gymnema sylverstre* leaf extracts.

In this study, Sucralfate (cytoprotective agent) and Ranitidine (H_2-antagonist) were used as the reference anti-ulcer drug. It is widely used as acid inhibitor agent for the treatment of disorders related to gastric acid secretion. The present study was undertaken to evaluate anti-ulcerogenic properties of methanol extract of *Gymnema sylverstre* leaf in rats.

MATERIALS AND METHODS

Collection of plant materials:

Gymnema Sylvestre was collected from School of science, Gujarat University, Ahmadabad. It was identified and authenticated by Dr. Himanshu A. Pandya, Associate Professor, School of science, Gujarat University, Ahmadabad.

Preparation of extract:

Dried leaves were coarsely powdered and packed into a soxhlet apparatus and subjected to hot continuous extraction by using solvent methanol. The extract was concentrated and used for ulcer model and toxicity study.

Experimental animals:

An albino wistar female rat of 180 – 250gm was used throughout the experiments. The animals were procured from TRC, Ghandhinagar. All the animals were housed in rectangular polypropylene cages (32×24×16 cm, four per cage) kept on racks

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built of slotted angles and the cages were provided with dust free paddy husk as a bedding material. The animals were housed in environmentally controlled conditions of temperature (22 ± 50 C), humidity (50 ± 5%) and photoperiod (12:12 h) light-dark cycle. They were maintained on a rodent pellet diet and fresh tap water ad libitum. The norms of Good Laboratory Practice (GLP) were followed for care of laboratory animals.

Grouping of animal: Each group contain 5 animals

Group 1: vehicle control
Group 2: standard 50mg/kg Ranitidine
Group 3: standard 400mg/ kg Sucralfate
Group 4: 100mg/ kg methanolic extract of *Gymnema sylverstre*
Group 5: 200 mg/ kg methanolic extract *Gymnema sylverstre*
Group 6: 400 mg/ kg methanolic extract *Gymnema sylverstre*

Acute oral toxicity study and selection of doses: A safe oral dose of extract was determined through the acute oral toxic test in rats as described by the Organization of Economic Co-Operation and Development (OECD) as per 423 guidelines [10]. The extract at different doses up to 2000 mg/kg, was prepared by dissolving the extract in distilled water and the concentration was adjusted in such a way that it did not exceed 1 ml/100 g of the rat. The extract was then administered (p.o.) and animals were observed for behavioural changes, any toxicity and mortality up to 48 h.

ANTI-ULCER STUDIES [15]:

1. Absolute alcohol-induced ulcer [12,14]:

Test extracts, Ranitidine and Sucralfate were administered orally 60 min before the oral administration of 1ml of absolute alcohol. Sixty minutes later, the animals were sacrificed and their stomachs excised and gastric contents were aspirated. Stomachs were removed and kept immersed in 10% formalin for 5 min. Each stomach was incised along the greater curvature and examined for linear haemorrhagic lesions in the glandular region.

Parameters evaluated for Ulcer: [11]

- **Determination of Ulcer Index**-The gastric mucosa was examined for ulcers by magnifying lens and the ulcer scored. According to its severity in comparison with the standard. Ulcer score was calculated as: 0 = normal, no ulcer; 1 = isolated haemorrhagic spot; 2 = dense haemorrhagic spot;3 = small ulcer; 4 = large ulcer; 5 = perforation.
- **Determination of Percentage protection index and healing index:**

  C-T/C ×100

  Where,

  C= ulcer index in control group;

  T= ulcer index in treated group.

2. Pyloric ligation induced ulcer model [13]:

Female albino Westar rats weighing around 180-250 gm were randomly divided into different groups and each group containing 6 animals. The different groups of animals received different treatment. Ranitidine (50 mg/kg) was used as standard anti-ulcer drug. On the 14th day, one hour after the drug administration, animals were fasted 18 h with free access to water prior the surgery. The animals were placed individually to avoid cannibalism and corophagy. After 18 hours pyloric ligation was carried out, then again animals were kept for 4 hrs then animals were sacrificed, stomach was isolated and opened along the greater curvature to count ulcer index. Gastric fluid was collected separately for measurement of total acidity.

Parameters evaluated for Ulcer:

- **Ulcer Score and Ulcer index:**Stomachs of animals were cut opened along the curvature, washed with saline and ulcer score was recorded as follows,

  0 - Normal colouration
  0.5 - Red colouration
  1.0 - spot ulcers
  1.5 - Haemorrhagic streaks < 3mm
  2.0 - Haemorrhagic streaks 3 to 5 mm
  3.0 - Haemorrhagic streaks > 5 mm
- Mean ulcer scores for each experimental group were calculated and expressed as ulcer index.

  No. lesions × ulcer score = Ulcer index
- **Measurement Total acidity from Gastric juice:** Total acidity of gastric juice was measured. Total acidity was determined using phenolphthalein indicator till a faint pink colour is obtained (pH 8.4). Unit of total acidity is the mL of 0.1 N NaOH required for titration of 100 mL of gastric juice and is expressed as milliequivalents per 100 mL of gastric juice. It was calculated by the formula:

  Acidity = (volume of NaoH × Normality × 100) / 0.1
- **Determination of pH:** An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and pH of solution was measured using pH meter.

Statistical analysis:

The data are reported as mean ± standard error of the mean (SEM) and were compared using one way analysis of variance (ANOVA), followed by the Dunnett's multiple comparison test using Graph Pad PRISM software ,and *p*- values < 0.05 was considered significant.

RESULTS AND DISSCUSSION

In the present study the Antiulcer activity of *leaves* of *Gymnema sylverstre* were carried out in ethanol and pylorus ligation induced ulcer models.The selection of doses of test drug is based upon oral toxicity studies. The detailed results of methanolic extract of *Gymnema sylverstre leaves* at a dose of 100mg/kg, 200 mg/kg and 400 mg/kg in ethanol and pylorus ligation induced ulcer model is tabulated in (**Table 1 & 2**) and (**Figure 1&2**). Oral administration of 75% ethanol produced severe haemorrhagic lesions in glandular mucosa consisting of elongated bands, usually parallel to the long axis of the stomach. The control rats had an U.I. of 6.16±1.34. In animals pretreated with *G. Sylverstre* extract at doses of 100,200 and 400 mg/kg, a significant inhibition of ethanol mucosal injury was detected, showing an U.I. of 6.46±1.55, 1.37±0.84 and 1.04±0.42, respectively. The dose of 400 mg/kg showed an inhibition of lesion formation (83.12%) greater than that of sucralfate (66.23%), which also significantly inhibited mucosal damage. By contrast, no reduction in ethanol-induced lesions was observed following the ranitidine administration. In order to determine whether the *G. Sylverstre* extract protected the gastric mucosa by a local or systemic effect, its cytoprotective activity against ethanol-induced gastric lesions was tested by intraperitoneal administration at a dose of 100 mg/kg. The control value of U.I. was 11.8±0.8. In animals pre-treated with the extract the ethanol-induced gastric damage was aggravated and the U.I. increased significantly to 15.5±1.1 (P<0.05).

Gastric secretion was evaluated as gastric juice volume, acid output, and pepsin output for 4 h after pylorus ligation. When administered immediately after ligation, *G. Sylverstre* extract (100, 200, and 400 mg/kg; intraduodenally) dose-dependently decreased the gastric juice volume, acid secretion, as well as pepsin secretion. This decrease reached statistical significance at doses of 100, 200 and 4oo mg/kg for the volume, and at 400 mg/kg for acid and pepsin secretions. Ranitidine (50 mg/kg) also caused a marked decrease in each secretory parameter, while sucralfate (400 mg:kg) did not show any modification on gastric secretion. Mucus output did not show significant changes in response to any of the tested substances, except sucralfate.

Table 1: Effect of Gymnema *Sylverstre* on ethanol induced ulcer on Rats

Treatment	No. of animals	Ulcer index	Inhibition (%)	No. of Ulcers	Ulcerated animals
Control (Vehicle)	5	6.16±1.43	-	5.00±1.83	5/5
Ranitidine 50 mg/kg	5	5.97±1.34	3.08	5.10±1.21	5/5
Sucralfate 400 mg/kg	5	2.08±0.45[b]	66.23	0.83±0.24[b]	4/5
Extract 1 100 mg/kg	5	6.46±1.55	-4.87	4.67±1.96	2/5
Extract 1 200 mg/kg	5	1.37±0.84[b]	77.60	0.67±0.67	1/5
Extract 1 400 mg/kg	5	1.04±0.42[b]	83.12	0.17±0.17	0/5

[a] *Each value represents the mean ± SEM*
[b] *Significantly different from control P < 0.05*

Table 2: Effect of *Gymnema Sylverstre* on Pylorus ligation induced ulcer on Rats

Treatment	Vol (ml/4 hr)	Acid output (µEq+/4 hr)	Pepsin output (mg/4 hr)	Pepsin conc. (mg/ml)
Control (Vehicle)	3.44±0.52	282.39±56.42	14.44±2.97	5.45±0.99
Ranitidine 50 mg/kg	1.70±o.34[b]	54.71±23.34[b]	3.17±1.46[b]	.25±1.27
Sucralfate 400 mg/kg	2.92±0.48	220.91±53.09	11.84±2.11	4.44±0.95
Extract 1 100 mg/kg	2.95±0.62	273.24±88.47	8.31±2.74	3.51±0.54
Extract 1 200 mg/kg	1.86±0.32[b]	146.41±36.78	7.02±1.54	4.39±0.70
Extract 1 400 mg/kg	1.06±0.21[b]	75.42±24.61[b]	4.78±0.95	4.89±0.52

[a] *Each value represents the mean ± SEM in all six animal*
[b] *Significantly different from control P < 0.05*

Fig 1: Ulcer index and % inhibition of Gymnema *Sylverstre* on ethanol induced ulcer on Rats

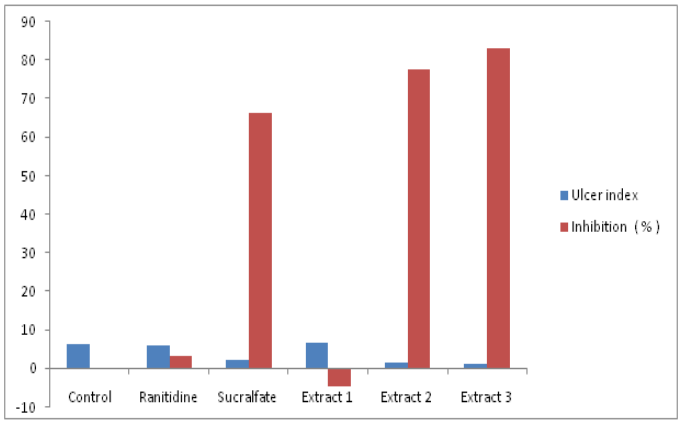
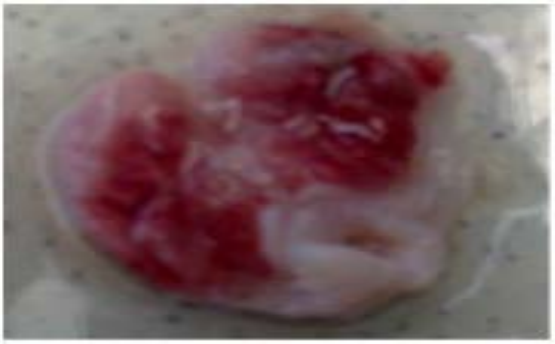
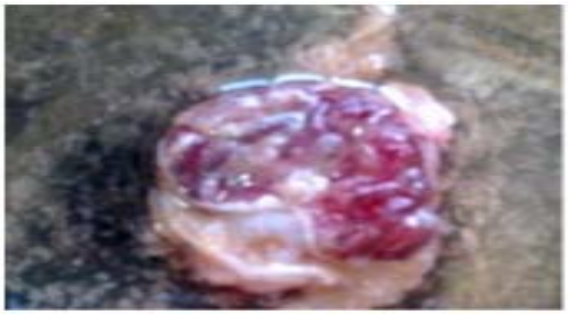


Fig 2 (a-e) : Photos of Ulcer
a. Disease Controlled

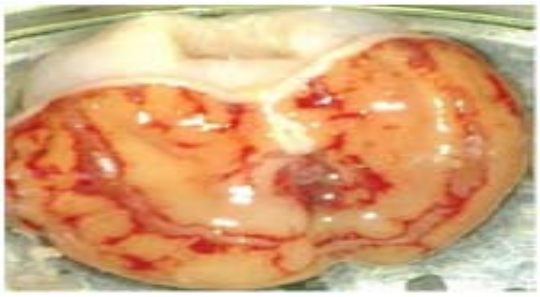


b. Rantidine 50mg/kg

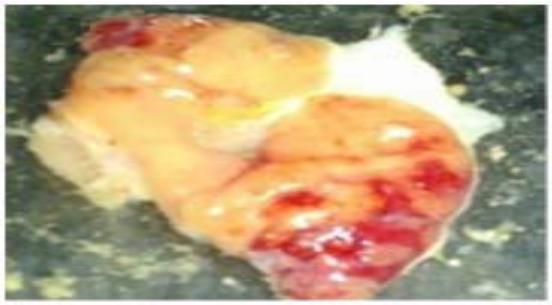
c. Extract 1



d. Extract-II



e. Extract -III



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