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Research Article

EVALUATION OF ANTIOXIDANT AND ANTIULCER ACTIVITY OF METHANOLIC EXTRACT OF *GARDENIA GUMMIFERA* L. IN RATS

PRADEEP KUMAR SABBANI[1]*, PAVAN KUMAR CHITYALA[2], GOWRISHANKAR NL[1], NAVEEN KUMAR G[1], SHILPA K[1], TEJASWI CH[1]

[1]Department of Pharmacology, Swami Vivekananda Institute of Pharmaceutical Sciences, Vangapally, Nalgonda, Telangana, India. [2]Department of Pharmacology, Kakatiya University, Warangal, Telangana, India. Email: pradeep.pharma555@gmail.com

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ABSTRACT

Objective: The aim was to assess the effect of anti ulcer and anti oxidant activity of methanolic extract of Gardenia *Gummifera (MEGG)* whole plant in ulcer induced rats and *in-vitro* anti oxidants method respectively.

Methods: In the present study, the efficacy of an MEGG was evaluated against aspirin-induced ulcers in rats. *G. gummifera* at doses of 150 mg/kg and 300 mg/kg was administered orally once daily for 6 days.

Results: Results showed that extract treatments prevented ulcer area and gastric secretion in a dose-dependent manner. Administration of 2000 mg/kg extract did not show any toxicity in albino mice. In preliminary phytochemical studies identified the presence of flavonoids in the MEGG.

Conclusion: According to the results, it was concluded that the whole plant of *G. gummifera* has significant antiulcer activity due to the presence of potent antioxidants like flavonoids.

Keywords: Aspirin, Antiulcer, Hydrogen peroxide, *Gardenia gummifera.*

INTRODUCTION

Peptic ulcers, the most common gastrointestinal (GI) disorders, present as a crater in the lining of the GI tract mucosa due to acid, pepsin, bile acid, pancreatic enzyme, and bacteria. It is due to an imbalance between aggressive (acid and pepsin) and defensive (bicarbonates, mucin, PG, etc.) factors. Few recent reports indicated that prevention of GI disorders associated with *Helicobacter pylori* can be achieved by increasing the intake of natural antioxidants. Natural compounds are now-a-day's consider as a safe medication for the treatment of a number of diseases as it is a general notion that plant-based drugs are safer without any side effects.

A widespread search has been launched to identify new antiulcer therapies from natural sources. Herbs, medicinal plants, spices, vegetables, and crude drug substances are considered to be a potential source to control various diseases including gastric ulcer. In the scientific literature, a large number of medicinal plants having potential antioxidant with antiulcer activities have been reported.

Dikamali is the gum resin obtained from the leaf buds of *Gardenia gummifera* family Rubiaceae [1]. *G. gummifera* is geographically distributed in all districts of south India, Burma, Bangladesh, Konkan region, North Kanara, and Malabar Coast. *G. gummifera* is claimed to have a number of medicinal properties which include anthelmintic, antispasmodic, carminative, diaphoretic, expectorant, potentiation of pentobarbitone induced sleep, antiepileptic, peripheral and central analgesic, cardiotonic, antioxidant, and antihyperlipidemic. It is also claimed to be useful in dyspepsia, flatulence for cleaning foul ulcers and wounds, and to keep off flies from wounds in veterinary practice [2,3]. A number of flavonoids such as gardenin A, B, C, D, and E were isolated from Dikamali in the past [4,5].

METHODS

Plant material

Fresh parts of *G. gummifera* were collected from the Sri Venkateswara University, and it is authenticated by Dr. K. Madhava Chetty, Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. The specimen of herbarium is stored in S.V University, Tirupati (Reference no. 1052).

Preparation of extract

The whole plant were dried under shade and powdered and stored in an airtight container. For extraction, 250 g of dried powder was loosely packed in the thimble of Soxhlet apparatus and extracted with methanol for 18 hrs at 55°C. For oral administration, the extract was dissolved with 2% gum acacia.

Phytochemical screening

The methanolic extract of *G. gummifera* (MEGG) was evaluated for the presence of flavonoids, tannins, alkaloids, saponins, glycosides, and sterols/terpenes [6].

Animals

Male albino-Wistar rats, weighing 150-200 g were used in the present study. All the rats were kept at room temperature (22°C) in the animal house. The animals are maintained in the animal house of Sri Vasavi Institute of Pharmaceutical Sciences attached to the Department of Pharmacology. All the animals were housed and treated as per the internationally accepted ethical guidelines (Committee for the Purpose of Control and Supervision of Experiments on Animals) for the care of laboratory animals. All the experimental procedures were performed on animals after approval from the Institutional Animal Ethical Committee and in accordance with the recommendations for the proper care and use of laboratory animals.

Acute toxicity studies

Male and female Swiss mice were randomly divided into groups (n=10) that orally received saline solution (10 ml/kg) with *G. gummifera* methanolic extract at the same dose of 100, 200, 400, 800, 1000, 1200, 1400, 1800, and 2000 mg/kg p.o. [7]. After oral administration, the acute toxicity and behavioral parameters were described according to the methods of Pradeep Kumar [8]. The observations were performed at 30, 60, 120, 240, and 360 minutes after the oral treatments. For 14 days, the animals were weighed, and the number of deaths noted.

Antiulcer activity of *G. gummifera* methanolic extract

Aspirin-induced ulcers in rats

Animals were divided into 5 groups each containing 6 animals. Group-I received 2% gum acacia of 1 ml/kg, p.o. as normal control, Group-II received 2% gum acacia with aspirin 200 mg/kg, p.o. as diseased control, Group-III and Group-IV, respectively, received MEGG 150, 300 mg/kg of *G. gummifera* methanolic extract, Group-V received 50 mg/kg, p.o. of ranitidine as standard control and the treatment were administered, respectively, up to 6 days [9].

Determination of gastric secretion

On the 6th day, immediately after aspirin 200 mg/kg p.o. treatment pylorus ligation was performed under ether anesthesia on 36 hrs fasted rats [10]. 4 hrs after pylorus – Ligation, the animals were sacrificed by giving overdosage of ether. Stomach was excised and cut along the greater curvature, washed carefully with 5 ml of 0.9% NaCl and the ulcers were examined macroscopically for gastric erosions under a dissecting microscope (×10). The stomach was removed, and its gastric content drained into a graduated centrifuge tube and centrifuged at 3000 rpm for 10 minutes [11].

Total acid and free acid in the gastric secretion volume were determined in the supernatant by titration to pH 7.0 with 0.01 N NaOH (mEq/ml/4 hrs). The free acidity and total acidity were determined using the formula and values are expressed as mEq/L 100g [12].

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.01} \times 100 \text{ (mE/L/100 g)}$$

The ulcer index was calculated [13]. The percentage of protection availed to the animals through various treatments were calculated [14].

***In-vitro* antioxidant activity with hydrogen peroxide scavenging assay**

The ability of the *G. gummifera* extract to scavenge hydrogen peroxide was determined according to the method of Ruch *et al*., (1989) [15]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide.

% Scavenge H_2O_2 = ([Absorbance of control−Absorbance of standard]/ control) ×100

Statistical analysis

The results were analyzed by one-way analysis of variance. Significant differences were determined using the Dunnett's post-test (for three or more groups) or, for non-parametric results. All the results are expressed as mean±standard error mean (SEM). Significance was established when the probability value was <0.05.

RESULTS

Preliminary phytochemical screening

The qualitative chemical investigation, it is observed from the phytochemical study that flavonoids, glycosides, tannins, alkaloids, protein, phytosterols, terpenes, phenolic compounds, and triterpenoids are present in the extracts.

Acute toxicity study

Mild adverse effects and no mortality rate of the animals were observed during the period of 48 hrs study up to the dose 2 g/kg b.w.p.o. of the MEGG. The two random doses of 150 and 300 mg/kg b.w. were selected for the antiulcer studies.

Aspirin induced ulcer model

Oral administration of methanol extract of *G. gummifera* at doses of 150 and 300 mg/kg exhibited dose-dependent protection of 44.61 and 70.66 ($p<0.001$), respectively, compared to the ulcer control, proving the anti-ulcer activity (Fig. 1).

The effects of MEGG on acid parameters were less significant at 150 mg/kg dose. But methanol extract of *G. gummifera* showed significant ($p<0.001$) effect at 300 mg/kg dose compared to ulcer control animals. The volume of acid secretion, total and free acidity was decreased, and pH of the gastric juice was increased compared to ulcer control group (Table 1). But, in this gastric environment also able to induce ulcer, so it can be thought that the antisecretory activity might not be the main mechanism of action of these extracts. Macroscopical examination of stomachs ulcer area has shown less in the MEGG 300 mg/kg group when compared to the diseased control (Fig. 2).

***In-vitro* antioxidant activity with hydrogen peroxide scavenging assay**

It is observed that the methanolic extract has demonstrated a dose-dependent increase in the hydroxyl radical scavenging activity. Where the 25 µg of ascorbic acid (standard) has 85.1% of inhibition and MEGG at 20 µg shown 35.1%. However, the extracts at 100 µg have shown almost equivalent inhibition as compared to standard (Fig. 3).

DISCUSSION

Natural compounds are now-a-day's consider as a safe medication for the treatment of a number of diseases as it is a general notion that plant-based drugs are safer without any side effects. Few recent reports indicated that prevention of GI disorders associated with *H. pylori* can be achieved by increasing the intake of natural antioxidants; *G. gummifera* has been shown to be act as a potent antioxidant.

Aspirin is a cyclooxygenase inhibitor which suppresses gastroduodenal bicarbonate secretion, reduces endogenous prostaglandin biosynthesis and disrupts the mucosal barrier as well as mucosal blood flow in animals. It is also well-known that prostaglandins synthesized in large quantities by the GI mucosa can prevent experimentally induced ulcers by ulcerogens.

The ulcer protection of MEGG 150 and 300 mg/kg is 44.61% and 70.66%, respectively, ranitidine 50 mg/kg is 83.05%, respectively, which are statistically significant when compared with the disease control. The mean ulcer index values for animals pretreated with *G. gummifera* of Group-III and Group-IV are 16.67±0.61 and 8.83±0.30, respectively. When compared with Group-II (diseased control), the results are statistically significant ($p<0.001$). But when compared with Group-V (standard control) statistical significance is seen ($p>0.05$). *G. gummifera* has shown that it can reduce the gastric acid secretion levels and has a protective effect on the gastric mucosa in the aspirin-induced model.

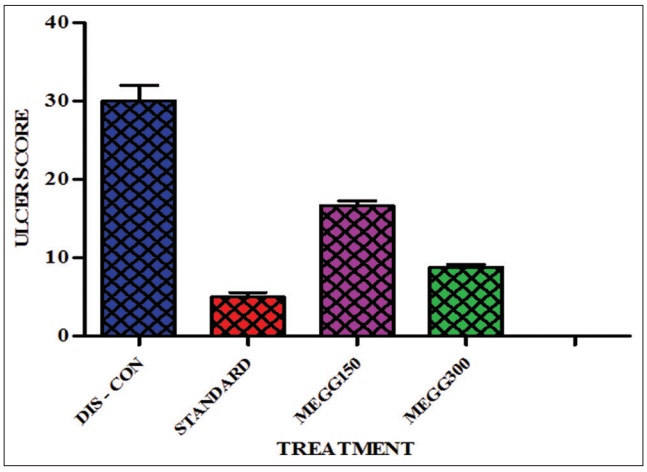

Fig. 1: Effect of methanolic extract of *Gardenia gummifera* root on ulcer index of aspirin-induced ulcers in rats

Table 1: Effect of MEGG on gastric secretion, total and free acidity using aspirin-induced ulcer

Group	Gastric volume (ml)	pH of gastric juice	Total acidity (m/Eq) 100 g	Free acidity (m/Eq) 100 g	Ulcer index (mm^2/rat)	Protection (%)
Normal control (2% acacia)	1.317±0.11	1.917±0.19	52.97±1.93	27.30±1.73	0.00±0.00	-
Diseased control (aspirin 200 mg/kg)	2.683±0.08	1.183±0.06	72.42±3.16	41.35±1.66	30.10±2.03	-
MEGG (150 mg/kg)	2.467±0.13**	1.85±0.09**	60.56±1.06*	35.88±2.14*	16.67±0.61*	44.61
MEGG (300 mg/kg)	1.767±0.18**	2.11±0.04**	51.84±1.19**	29.24±1.75**	8.83±0.30**	70.66
Standard control (ranitidine 50 mg/kg)	1.417±0.14**	2.817±0.11**	47.72±1.39**	26.70±0.99**	5.10±0.57**	83.05

All values are expressed as mean±SEM; (n=6) animals in each group. ***p<0.001, **p<0.01, diseased control group was compared with normal control group. Ranitidine and extract treated groups were compared with diseased control group. MEGG: Methanolic extract of *Gardenia gummifera*

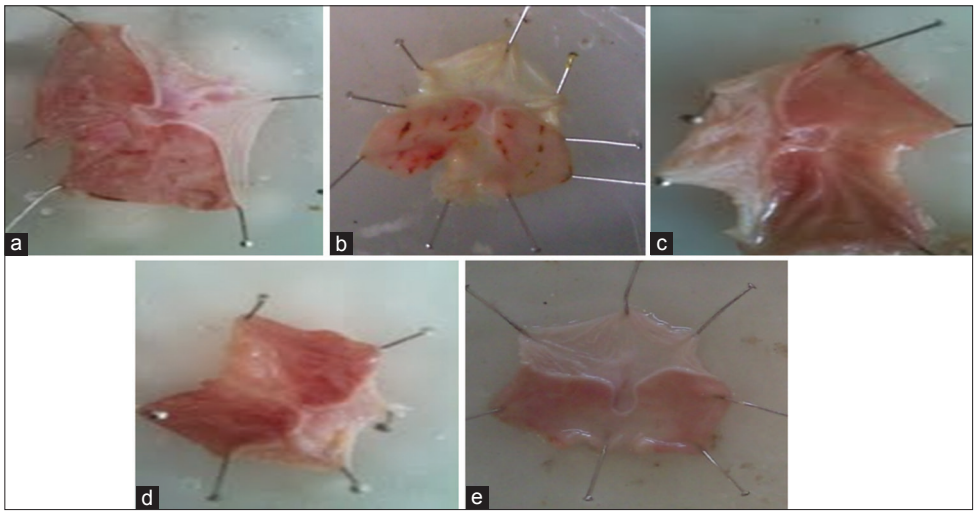

Fig. 2: Effect of methanolic extract of *Gardenia gummifera* (MEGG) on aspirin-induced ulcers, (a) Normal control, (b) diseased control, (c) MEGG 150 mg/kg, (d) MEGG 300 mg/kg, (e) standard control (ranitidine 50 mg/kg)

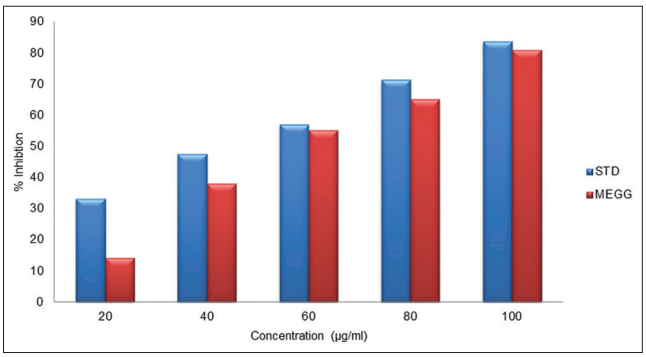

Fig. 3: *In-vitro* antioxidant activity of methanolic extract of *Gardenia gummifera* by H_2O_2 scavenging activity

There is increasing importance of antioxidants in all the fields of research. Especially in the treatment of peptic ulcer treatment and non-steroidal anti-inflammatory drugs (NSAIDs) therapy, there can be a path breaking improvement in the therapy. The expressive antioxidant action of extract, in addition to the already well-known benefits to health, may be contributing to its mechanism of antiulcer effect. Regular NSAIDs can be given along with antioxidants like *G. gummifera* as a conjugate therapy.

CONCLUSION

The results obtained in this study suggest that the MEGG possesses antiulcer activity and *in-vitro* antioxidant effects possibly due to the induction of antioxidant enzymes and promotion of the scavenging of reactive free radicals. The inhibition of ulcer lesion in animals may also be due to the antioxidant activity of the extract. The antiulcer activity of the MEGG may be due to its predominant activity on mucosal defensive factors rather than offensive factors. Therefore, due to both, antioxidant and cytoprotective action, the methanol extract of *G. gummifera* exhibited antiulcer properties. Further research is required to isolate the active phytoconstituents present in the extract and experimentation on the healing action of drug on chronic ulcer as well as the molecular mechanism of action.

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