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Gastro - Protective Effect of *Grewia optiva* (Dhamna) Leaf Extract on Ethanol Induced Gastric Ulcer in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author SA designed the project, performed experiment work and wrote first draft. Author MAT managed the material of manuscript and finalized it with technical assistance of author MSAA. All authors read and approved the final manuscript.

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ABSTRACT

In present examination, gastro defensive movement of *Grewia optiva* (GO) in rodents has been explored based on bioactive mixes (tannins, terpenoids, flavonoids, steroids and saponins) present in it. Ethanolic concentrate of *Grewia optiva* leaves was utilized in present study to research its gastro-defensive impact on ethanol actuated gastric ulcer in Albino rats. The investigation including negative, positive, drug, and reference controls.

Keywords: Albino rats; bioactive compounds; gastro-protective; Grewia optiva; ulcer.

1. INTRODUCTION

Gastric ulcer is one among the foremost serious diseases affecting many people within the world [1]. Gastric ulcer is produced by imbalance between defensive and aggressive mechanisms within the stomach [2]. protective mechanisms include mucus secretion, blood flow,

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prostaglandins, antioxidants while aggressive mechanisms involve acid-pepsin, and reactive oxygen species [3]. Pathophysiology of peptic ulceration includes infection with Helicobacter pylori, NSAIDS, advanced age, use of steroids, alcohol, tobacco, insufficient protection of mucosa , hyper secretory disorders, emotional stress and physical trauma [4].

Ulcer could also be described as circumscribed lesions that reach below the epithelium of mucosal membrane [5]. Sometimes erosions that are breaks within the mucosal membrane could also be mentioned as ulcer but these don't extend beneath the epithelium. Among the foremost conventional drugs employed for the treatment of gastritis are; antimicrobial agents, proton-pump inhibitors, antihistamine, prostaglandins, antacids and mucosal protective agents [6,7]. However use of these medicines can cause serious side effects like hypersensitivity, arrhythmias, gynecomastia, impotence and hematopoietic dysfunctions [8]. Use of medicinal plants in protection of induced peptic ulcer in laboratory animals was reported earlier [9]. The medicinal importance of this plant could also be attributed thanks to the presence of detected metabolites. Its antimicrobial and insecticidal activity has already been investigated [10].

The use of plant products and herbs for the treatment of peptic ulcer has also been reported earlier [11]. The treatment with medicinal plants is comparatively cheap and safe although given in high doses [12]. The Grewia comprises of about 150 species that include small trees and shrubs, cosmopolitan in tropical and subtropical regions. Among these about 10 species are identified in Pakistan. The name *Grewia* was given after the name of Nehemiah Grew who was one among the founders of plant physiology [13]. Other species of this genus have folk medicinal use for the remedy of diarrhea, malaria, typhoid, small pox, irritable condition of intestine and rheumatism [14]. It is a little tree and its phytochemical screening reveals the presence of tannins, terpenoids, flavonoids, steroids and saponins [15].

The medicinal importance of this plant could also be attributed to the presence of detected metabolites. Its antimicrobial and insecticidal activity has already been investigated [16]. In present study we'll investigate gastro protective activity of *Grewia optiva* on the idea of flavonoids present in it. Possible mechanism of action involves antioxidant behavior of flavonoids [17].

2. MATERIALS AND METHODS

Twenty-five rats (150-200 g each) were acclimatized for 7 days at Room temperature in animal house of University of Veterinary and Animal Sciences Lahore and given standard feed and water *ad libitum.*

2.1 Collection and Extraction of Plant Material

Grewia optiva was collected from botanical garden of Government College University Lahore.

2.2 Experimental Design

Acclimated rats were divided into 5 groups (A, B, C, D, E) including 5 rats in each group as follows: -

1. Negative Control Group (A)
2. Positive Control Group (B)
3. Positive treatment Group (C)
4. *Grewia optiva* extract Group (D), 250 mg dose.
5. *Grewia optiva* extract Group (E), 500 mg dose.

The animals were fasted for 18 hours before the commencement of experiment. Group A was given water while group D and E were pretreated with extract of *Grewia optiva* at a dose of 250 mg for every rat respectively. Group C was pretreated with sucralfate (medicine) at a dose of 400 mg/kg [18]. After one-hour peptic ulcer was induced by administration of ethanol at dose of 2 ml/rat in B, C, D and E. After an hour of ethanol administration, the rats were scarified by fuming with over dose of chloroform. Blood samples were taken then stomachs were aseptically removed. The stomach was cut along the greater curvature and therefore the contents were collected into small tubes containing 5ml phosphate buffer saline. These gastric tissues were homogenized then centrifuged at 4000 rpm for 20 minutes. The supernatant was separated and its volume was expressed as ml/100 g weight. This supernatant was used for the estimation of varied biochemical parameters [19].

2.3 Ulcer Index Calculations

In this study, *Grewia optiva* was evaluated for its Gastro protective activity against ethanol induced gastric ulcer in rats. The ulcer index (UI) was

calculated on the basis of ulcer area $(mm)^2$ and classified as levels I, II and III related to ulcer areas < 1 $(mm)^2$, 1-3 $(mm)^2$ and > 3 $(mm)^2$ respectively. The ulcerative lesion index (ULI) and curative ratio (Table 1) were calculated using following formulas [20]:

1x (number of ulcer level I) +2x (number of ulcer level II) +3x (number of ulcer level III)

The curative ratio was determined by formula [21]:

100 – (ULI treated x 100/ULI control

Fig. 1. Branch of *Grewia optiva* Plant

2.4 Histopathology

Tissue samples from the rats in each group (A-E) of the experiment were fixed in 10% formalin for twenty-four hrs then dehydrated by washing in ascending grades of ethanol before clearing with xylene then embedded in paraffin for infiltration. The samples were sectioned with a microtome in 4-5 µm thickness, kept on glass slides and, stained with hematoxylin and Eosin (H and E) [22].

2.5 Determination of MDA in Serum and Tissue

Lipid per oxidative damage is involved after ethanol administration. It's measured as conc. of MDA (a secondary product of lipid per oxidation) in terms of n mol. there's a big increase within the conc. of MDA in positive control group as compared to normal group. However, level of MDA was decreased in rats pretreated with GO leaves extract and sucralfate [23]. This significant difference of Malondialdehyde level is analogous for both serum Malondialdehyde and tissue Malondialdehyde.

Statistical model

The data were expressed as mean ±S.E.M. Statistical analysis was performed with ANOVA followed by Post-Hoc Duncan's multiple range tests using the Statistical Package for the Social Sciences (SPSS) for Windows. P values <0.05 was considered statistically significant.

2.6 DETERMINATION OF TOTAL PROTEINS IN SERUM AND TISSUE

Tissue was homogenized by using phosphate buffer. Tissue and serum Catalase was determined according to the method as described in literature [24].

Similarly, total proteins in Serum was determined a follows.

1. Three test tubes were taken and marked as sample, total protein standard and blank.
2. Reagent (1 ml) was added in all three test tubes. 20µl of standard (6.0 g/dl) or sample was added in standard or sample test tubes, respectively.
3. Mixing and incubation was done for 10 minutes at room temperature. The optical density of serum and standard was taken against reagent blank within 30 minutes at wavelength of 546 nm.

Total proteins were calculated by using formula:

$$\text{Total protein Conc.} = \frac{\text{Abs of sample}}{\text{Abs of Standard}} \text{ X Concentration of standard.}$$

3. RESULTS AND DISCUSSION

Gastric ulcer may be a common global issue lately. In most of the cases the precise etiology of ulcer isn't known. However, the foremost accepted concept of its development is an imbalance between destructive factors and therefore the maintenance of the interior mucosal defensive mechanism. For re-establishment of this balance, different therapeutic agents, including plant extracts and medicines are used.

Table 1. Ulcer index and curative ratio (n=5)*

Groups	Ulcerative lesion index	Curative ratio
Negative control	0.0	-
Positive control	28.50±5.69[a]	-
Positive treatment control	12. 50±2.90[b]	56
GO 250 mg	4.25 ±1.31[c]	85
GO 500 mg	1.8 ±1.11[c]	98

**Values bearing different superscripts are significantly different (p < 0.05)*

Current study shows that *Grewia optiva* was evaluated for its Gastro protective activity against ethanol induced peptic ulcer in rats (Table 1). Oral administration of ethanol produced several ulcerative lesions consisted of elongated bands. These bands were parallel to the long axis of the stomach. *Grewia optiva* dose dependently reduced the amount and severity of ulcers induced by ethanol as shown in Fig. 2 (D and E). Results shown by the plant extract were comparable with the quality drug. Standard drug and GO also reduced ulcerative lesion index as shown in Table 1.

Additionally, ethanol administration induced a big increase in ulcer index in reference to negative control group. Conversely groups treated with sucralfate, 250 mg/kg and 500 mg/kg GO showed significant decrease in ulcer index compared with positive treatment group. (Fig. 3 D & E).

An antioxidant enzyme catalase (CAT) conc. was decreased after ethanol administration in positive control comparison to negative control (Table 3). There was a big increase in CAT levels in groups pretreated with GO methanolic leaves extract and sucralfate as compared to positive control (Table 3). Thus statistical analysis indicated that the quality and experimental drug treatment resulted in increased level of catalase thus having antiulcer property. This significant difference of catalase level is analogous for both serum catalase and tissue catalase (Table 3).

Groups pretreated with methanolic leaves extract of GO. and sucralfate it was restored to normal level as shown in Table 4.

Photo graphs of microscopic examination of all the slides were recorded alongside histopathological study as shown in Fig. 4. Microscopic damage (gastric ulcer) was compared with control group (Fig. 4, A-B) in appearance of perforations, in epithelium of mucosa, dilated blood vessels and also perforations within the Muscularis. Moreover, animals received GO leaves extract at 250 and 500 mg was ready to prevent the damage caused by ethanol (Fig. 4 D and E respectively).

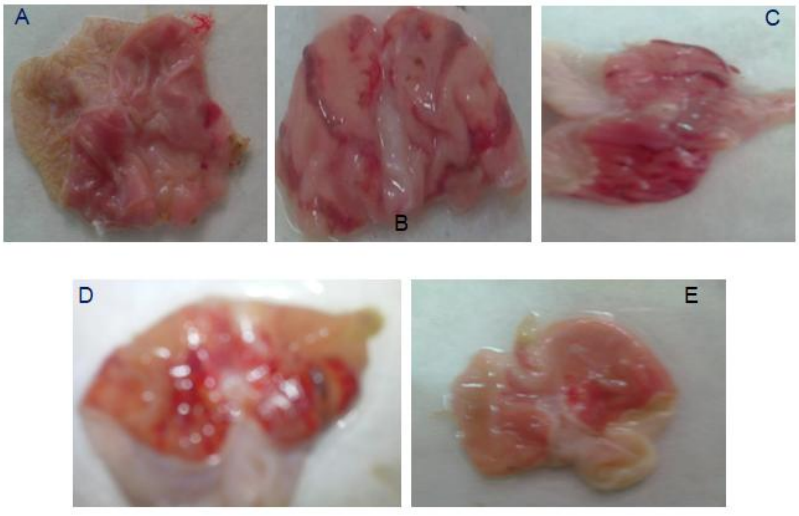

Fig. 2. The glandular portion of stomach- (A) Negative control, (B) Positive control, (C) Positive treatment group, (D) GO treated group 250 mg, (E) GO treated group 500 mg

Table 2. Conc. of MDA in serum and tissue (n=5)*

Groups	Conc. in serum (n mol)	Conc. in tissue (n mol)
Negative control	0.02±0.01[a]	0.02±0.01[a]
Positive control	0.25±0.03[b]	0.10±0.03[b]
Positive treatment control	0.05±0.02[a]	0.03±0.01[a]
GO. 250mg	0.03±0.01[a]	0.05±0.02[a]
GO. 500mg	0.04±0.01[a]	0.02±0.01[a]

**Values bearing different superscripts are significantly different ($p < 0.05$)*

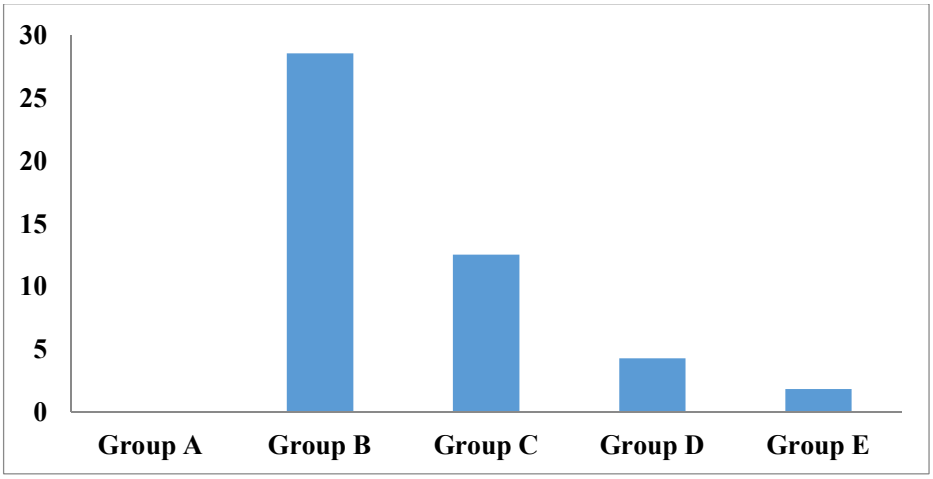

Fig. 3. Ulcer index of *Grewia optiva* against ethanol induced ulceration

Table 3. Concentration of catalase in serum and tissue*

Groups	Conc. In serum	Conc. In tissue
Negative control	69.60±14.02[a]	114.20±12.64[a]
Positive control	21.40±2.56[b]	13.10±2.78[c]
Positive treatment control	138.90±19.03[c]	58.80±9.67[b]
GO. 250mg	35.80±6.3[b]	116.40±10.01[a]
GO. 500mg	121.60±8.37[c]	66.60±13.33[b]

**Values bearing different superscripts are significantly different ($p < 0.05$)*

Table 4. Concentration of total protein in serum (n=5)*

Groups	Total protein (g/dl)
Negative control	8.18±0.31[a]
Positive control	6.6±0.15[b]
Positive treatment control	10.32±0.56[c]
GO. 250mg	7.06±0.25[a]
GO. 500mg	8.22±0.27[a]

**Values bearing different superscripts are significantly different ($p<0.05$)*

The glandular portion of stomach was examined for lesions as shown in Fig. 2.

Their mechanism of action involves inhibition of acid secretions or to reinforce the mucosal defense mechanisms by increasing mucus production, stabilizing the surface epithelial cells increasing prostaglandin synthesis [25]. Treatment with anti-ulcer drugs has various side effects; therefore, interest has been shifted towards naturally produced agents as an alternate mode of treatment. Thanks to the increasing interest in natural products, various plants are investigated on the idea of their traditional use as antiulcer agents. In present study, gastro protective activity of *Grewia optiva* was evaluated.

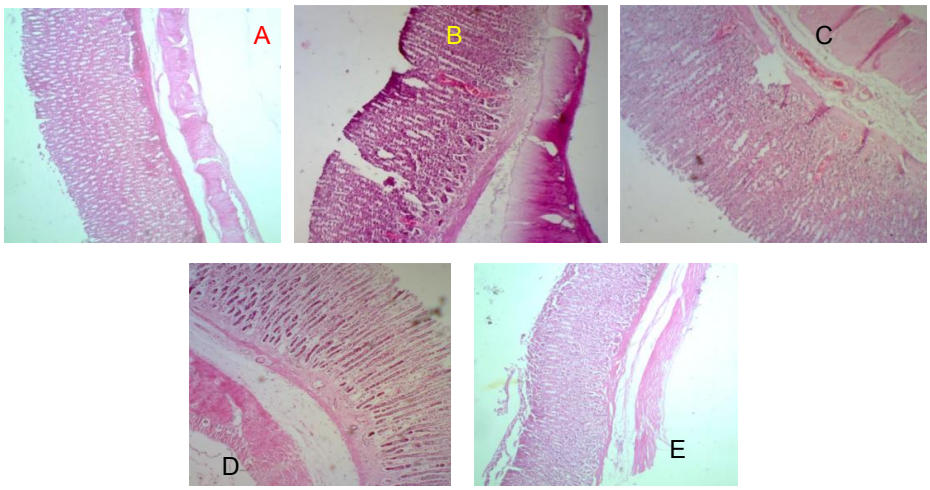

Fig. 4. Histopathological study; (A) Negative control (B) Positive control (C) Positive treatment group (D) GO 250 mg (E) GO 500 mg

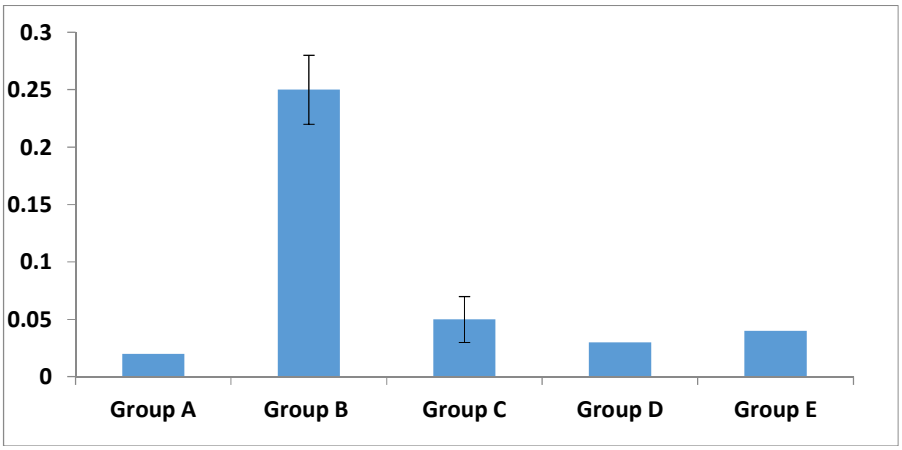

Fig. 5. Malondialdehyde (MDA) in tissue

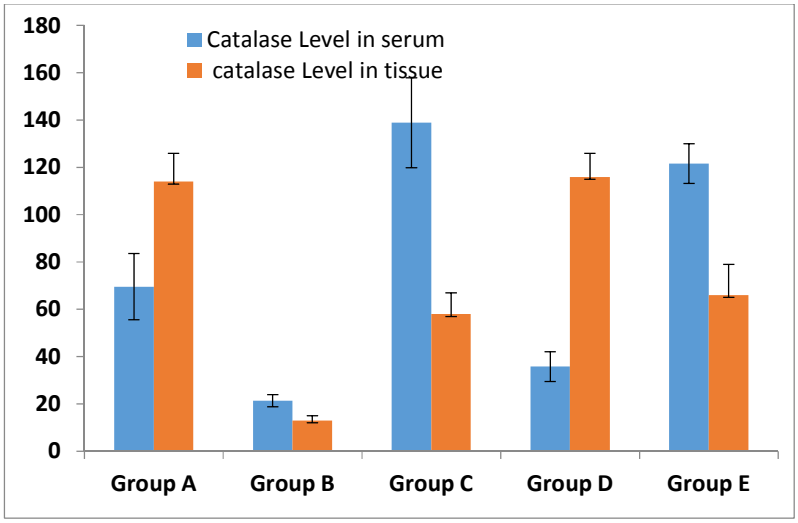

Fig. 6. Level of catalase in serum and tissue

The results indicated the dose-dependent protection against the necrotizing effects of ethanol on gastric mucosa. *Grewia optiva* at dose of 500 mg/kg, seemed to be significantly best as compared to plain drug sucralfate. During experimental process of ulcer induction, there wasn't an equal degree of ulceration altogether treated samples. it's going to flow from to improper dose of ethanol ingested by rats during handling, however it produced comparable results with literature. Different assessment parameters including ulcer index, histological evaluation, CAT and MDA conc. in serum and tissue and total protein in serum were studied. Histopathology results of our study showed that there was a superb repairing within the epithelium of gastric mucosa, no significant perforations and breaks were seen in groups pretreated with *GO* leaves extract as compared to positive control. Effect of preserving mucosal integrity was more pronounced than that of sucralfate. Lipid per oxidation measured in terms of MDA was increased in positive control group. Action of *GO* seemed to significantly drop ($p < 0.05$) the extent of Malondialdehyde in serum and tissues as compared to ethanol which is a crucial oxidative marker.

Protective effect for peptic ulcer of *GO* was increased when given at doses of 250 mg and 500 mg/kg but in tissues it had been not within the similar manner because it showed greater value at dose of 250 mg/kg as compared to 500 mg/kg. this might flow from to improper handling of tissue samples of rats treated with *GO* leaves extract. Pretreatment with *GO* at doses 250 mg/kg and 500 mg/kg seemed to significantly increased ($p < 0.05$) the extent of total proteins and in serum tissues as compared to ethanol group during which it had been dropped this is often not always true, as level of protein may vary in serum or tissue. So as a whole *Grewia optiva* exhibited good results like many other medicinal plants.

4. CONCLUSION

In conclusion, results of our study showed that methanolic extract of GO presents gastro protective activity, as evidenced by the parameters of ethanol induced ulcer models. Preventive effect of plant extract is possibly due to synthesis of mucus, a crucial gastro protective factor partly due to the stimulation of PG synthesis that play fundamental role in gastric mucosal protection. Preparations obtained from GO might be used for the formulation of a phyto pharmaceutical which will be used for the treatment of peptic ulcer. It may be a good alternative therapeutic option for the treatment of ethanol-induced gastric ulcer. Biochemical parameters are in strong support with it. However, further pharmacological investigations are required to demonstrate the precise mechanism of action.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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