

## EXPERIMENTAL EVALUATION OF THE ANTI-ULCER ACTIVITY OF GRAPE (*VITIS VINIFERA*) SEED EXTRACT IN WISTAR ALBINO RATS AGAINST HYDROCHLORIC ACID – ETHANOL-INDUCED ULCER MODEL

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### ABSTRACT

**Objectives:** To evaluate the antiulcer activity of ethanolic extract of seeds of (grape) *Vitis vinifera* in Wistar albino rats.

**Methods:** The ethanolic extract of *V. vinifera* was investigated for its antiulcer activity in Wistar albino rats against hydrochloric acid – ethanol-induced ulcer model. The antiulcer activity was assessed by determining and comparing the gastric volume, pH, free, and total acidity; ulcer number and its inhibition, ulcer severity, and ulcer index.

**Results:** A significant antiulcer activity was observed. The grape seed 100 mg/kg group showed significant ( $p < 0.05$ ) reduction in ulcer number as compared to the control. The grape seed 200 mg/kg showed significant ( $p < 0.05$ ) reduction in ulcer number, free acidity, and gastric lesion as compared to the control.

**Conclusion:** This study indicates that *V. vinifera* seed extract has potential antiulcer activity in the model tested.

**Keywords:** *Vitis vinifera*, Anti-ulcer, Ethanol, Free acidity, Total acidity.

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### INTRODUCTION

Peptic ulcer disease, whose incidence is gradually increasing on account of the changing lifestyle and stress among people, is a common disease [1] of the present day life affecting numerous people worldwide with a male preponderance [2].

In spite of many drugs being available for the management of this common clinical condition, herbal products [3] are finding an important place in its therapy on account of their better safety and efficacy profile [4].

Grapes are among the most easily cultivated and available plants worldwide. Different parts of the grape plant have proven medical application [5] in Ayurveda. Grape seed, which is a waste product of winery, has also been proved to have various therapeutic applications.

Grape seed is a very rich source of antioxidants [6]. It possesses antibacterial effects against *Helicobacter pylori* [7] and methicillin-resistant *Staphylococcus aureus* [8]. It is proven to have anti-inflammatory action [9]; cardiovascular and endothelial protection activity [10]; anti-hepatotoxic action [11]; adaptogenic and nootropic effects [12]; platelet function [13]; apoptosis [14] and aromatase inhibitor action [15]; vascular endothelial growth factor and angiogenesis inhibition activity [16]; antiviral and antifungal properties. Most of the activities of grape seed extract (GSE) are attributed to its antioxidant activity. GSE is shown to have antiulcer activity [17], effect on ethanol [18], and stress-induced gastric lesions.

This study is hereby undertaken to evaluate the antiulcer activity of GSE against hydrochloric acid (HCl) - ethanol-induced gastric ulcer model in Wistar albino rats, which will determine the possible cytoprotective property of the extract.

Establishment of the antiulcer activity of GSE may provide a newer and economically better modality of the treatment for peptic ulcer, which may have a better safety and efficacy.

### METHODS

This study was conducted only after being approved by Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) on 30.08.2012. The CPCSEA Guidelines were meticulously and strictly followed throughout the study.

#### Procedure of GSE

Grape seeds were removed from the grapes and air dried for 1 week. 100 g of dried seeds were soaked in 300 ml of ethanol (95%) with occasional stirring for 24 hrs and filtered using a piece of filter cloth. The residue of grape seeds was soaked in 300 ml ethanol (95%), and the above procedure was repeated twice. The entire fluid was collected and concentrated at 500°C with vacuum in rotary evaporator (Fig. 1).

Fig. 1 shows the ethanolic extract of GSE employed in this study.

#### Animals

In-bred Wistar albino rats (*Rattus norvegicus*) of either sex weighing 150-250 g, acclimatized to the laboratory conditions for 2 weeks with a 12 hrs light and dark cycle, were used for this study. All experiments were performed during the same time of the day to avoid diurnal variations of gastric functions.

#### Drugs and chemicals

All the chemicals used in this evaluation were of high grade and purity.

- Sucralfate powder (Fourrts India Ltd)
- Topfers reagent (Merck & Co.)
- Phenolphthalein indicator, sodium hydroxide (NaOH) (0.01 N) (S. D. Fine Chem. Ltd).

#### Procedure of HCl – ethanol model

The procedure was followed according to Saito *et al.* [19] healthy Wistar albino rats of either sex; weighing 150-200 g was included in this study. The rats were fasted (water allowed) for 1 day. They were divided into four groups of six rats each as follows:

- Group 1 (Control): Received 1 ml distilled water orally
- Group 2 (Standard): Treated with standard (100 mg/kg sucralfate) orally
- Group 3 (GSE 100): Treated with 100 mg/kg of GSE orally
- Group 4 (GSE 200): Treated with 200 mg/kg of GSE orally

The rats were fasted (water allowed) for 1 day. On the next day, respective test compounds (distilled water, GSE 100 mg/kg, GSE 200 mg/kg) were given. After 30 minutes of dosing, 1 ml 0.3M HCl and 60% ethanol was administered orally. 1 hr later, the rats were sacrificed by cervical dislocation.

The stomach was excised after tying both the ends of stomach. The gastric contents were drained into centrifuge tube and analyzed.

#### pH and gastric volume analysis

The contents of the stomach obtained were drained into the centrifuge tube and centrifuged at the speed of 1000 revolutions per minute for 20 minutes. The supernatant fluid obtained was collected and its volume was estimated. The pH of the content was noted using pH strips by matching the color obtained with that of the reference standard [20].

#### Free and total acidity analysis

The gastric content obtained was diluted to 10 ml by adding distilled water and analyzed for its free and total acidity by titrating it against 0.01 N NaOH solution using toppers reagent (dimethylaminoazobenzene in 95% alcohol) and phenolphthalein as indicators [21].

#### Ulcer number estimation

The gastric mucosa was observed macroscopically under  $\times 10$  magnifications and the number of superficial ulcers, deep ulcers, and perforations were noted [22].

#### Gastric lesion estimation

Gastric lesion of all possible ulcers was recorded by measuring its longest diameter (in mm) by means of divider and graph paper. Total gastric lesion in each rat was calculated [23].

#### Ulcer severity estimation

The ulcers in each rat were graded as:

- 0 - No ulcers,
- 1 - Superficial ulcers,
- 2 - Deep ulcers,
- 3 - Perforations.

Average severity score of each group was calculated [24].

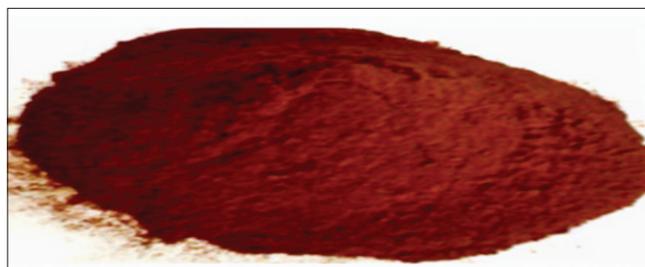


Fig. 1: Grape seed extract

#### Ulcer index estimation

Ulcer index in each group was calculated by the formula [25]:  $UI = UN + US + UP \times 10^{-1}$

Where, UI = Ulcer Index; UN = Average of number of ulcer per animal;

US = Average of severity score and UP = Percentage of animal with ulcer.

#### Statistical analysis

The data were expressed as mean  $\pm$  standard error of mean. Results were analyzed statistically by one-way analysis of variance followed by Dunnett's t-test using standard statistical Software Package of Social Science version 20. All the groups were compared with the control group. The difference was considered significant if  $p < 0.05$ .

#### RESULTS

In the HCl - ethanol model, the study with standard (sucralfate 100 mg/kg/p.o.) showed significant ( $p < 0.05$ ) reduction in free acidity (Table 1), ulcer number  $24.00 \pm 3.7$  (Fig. 3), and gastric lesion  $16.67 \pm 2.1$  (Fig. 4) as compared to the control. There was no significant change in the gastric volume, pH, total acidity, and bound acidity (Table 1).

The grape seed 100 mg/kg group showed significant ( $p < 0.05$ ) reduction in ulcer number  $30.00 \pm 3.23$  (Fig. 3) when compared with the control group. There was no significant change in the gastric volume, pH, free acidity, total acidity, bound acidity (Table 1), and gastric lesion  $23.00 \pm 4.69$  (Fig. 4).

The grape seed 200 mg/kg showed significant ( $p < 0.05$ ) reduction in free acidity (Table 1) ulcer number  $27.33 \pm 2.97$  (Fig. 3) and gastric lesion  $18.00 \pm 2.96$  (Fig. 4) when compared with the control group. There was no significant change in the gastric volume, pH, total acidity, and bound acidity (Table 1).

The ulcer inhibition was 42.39%, 27.98% and 34.67%, respectively, with the standard, 100 mg/kg group and 200 mg/kg group (Fig. 3).

The ulcer protection was 25%, 10.4% and 18.06%, respectively, with the standard, 100 mg/kg group and 200 mg/kg group (Fig. 6).

The gastric mucosa of the rats in the model tested is shown in Fig. 2.

#### DISCUSSION

In this model, ethanol, which is an alcohol, produced its action by having a direct erosive irritant effect on the gastric mucosa. It causes significant production of oxygen free radicals leading to increased lipid peroxidation, causing damage to the cell and cell membrane [26]. Ethanol may also cause depletion of gastric mucus content, damaged mucosal blood flow and mucosal cell injury causing an imbalance between the aggressive and defensive factors governing the gastric mucosa resulting in increased vascular permeability, edema formation, and epithelial lifting. Ethanol is metabolized in the body releasing superoxide anion and hydroperoxy free radicals which are involved in the mechanism of acute and chronic ulceration in the gastric mucosa [27].

HCl also brings about its effect by having a direct irritant property on the gastric mucosa by causing necrotic lesions on the gastric mucosa [19].

Table 1: Effect of the ethanolic extract of GSE on gastric content parameters in the HCl - ethanol ulcer model

	Control (1 ml)	Standard (mg/kg/p.o.)	GSE 100 (mg/kg/p.o.)	GSE 200 (mg/kg/p.o.)
Gastric volume (in ml/kg)	0.16 $\pm$ 0.00	0.10 $\pm$ 0.01	0.18 $\pm$ 0.01	0.24 $\pm$ 0.04
pH	1.00 $\pm$ 0.0	1.5 $\pm$ 0.22	1.00 $\pm$ 0.00	1.16 $\pm$ 0.07
Free acidity (in mEq/L/100 g)	28.33 $\pm$ 1.17	18.83 $\pm$ 3.14*	26.66 $\pm$ 0.84	20.00 $\pm$ 2.26*
Total acidity (in mEq/L/100 g)	48.33 $\pm$ 2.15	37.00 $\pm$ 4.06	54.16 $\pm$ 3.82	51.33 $\pm$ 2.66

All the values are expressed as mean  $\pm$  SEM; n=6. Statistical significance: \* $p < 0.05$ . One-way ANOVA followed by Dunnett's test. SEM: Standard error of mean, GSE: Ethanolic extract of grape seed extract, ANOVA: Analysis of variance, HCl: Hydrochloric acid

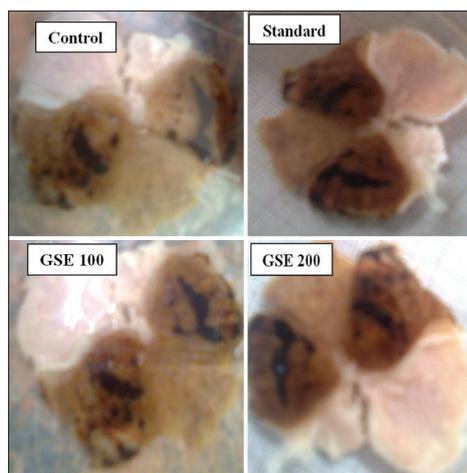


Fig. 2: Stomach mucosa of rats of various groups in the hydrochloric acid - ethanol model

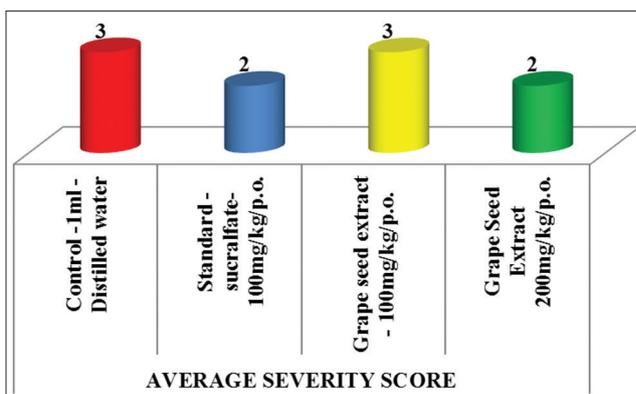


Fig. 5: Effect of the ethanolic extract of grape seed extract on average severity score in the hydrochloric acid - ethanol ulcer model. n=6 in all groups

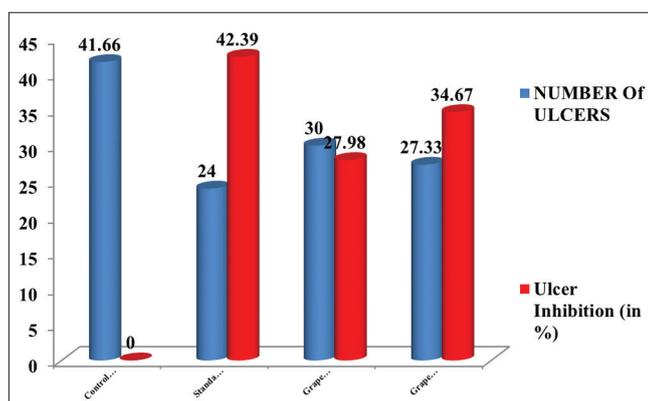


Fig. 3: Effect of the ethanolic extract of grape seed extract on number of ulcers and ulcer inhibition in the hydrochloric acid - ethanol ulcer model. n=6 in all groups

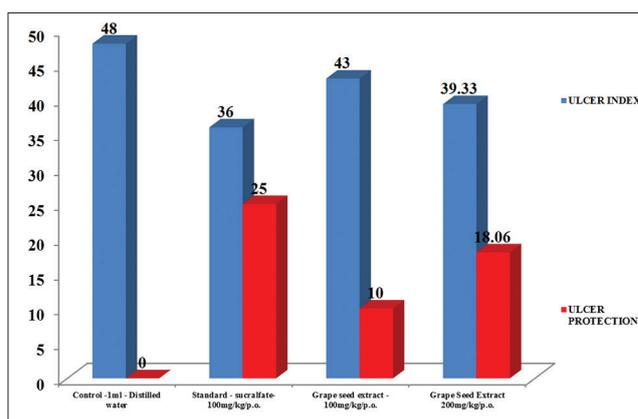


Fig. 6: Effect of the ethanolic extract of grape seed extract on ulcer index and ulcer protection in the hydrochloric acid - ethanol ulcer model. n=6 in all groups

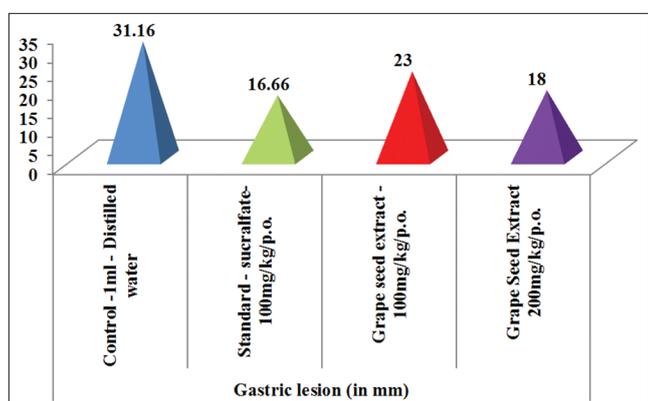


Fig. 4: Effect of the ethanolic extract of grape seed extract on gastric lesion in the hydrochloric acid - ethanol ulcer model. n=6 in all groups

Administration of HCl and ethanol produced ulcerative lesions in the gastric mucosa on account of their synergistic actions.

This study showed a decrease in the gastric volume in the standard groups and its increase in the grape seed 100 group and grape seed 200 group when compared with the control group, but they were not significant. The increase in the gastric content production could be due to the compensatory mechanisms acting in the mucosa to overcome

the acidic environment in the stomach. As the rise was not significant, the result cannot be authenticated and could be a result of biological variations among individuals. The data can be better confirmed by seeing the effect on gastric volume on a larger study population.

As the pH was noted using pH strips colorimetrically by comparing with the reference standard, the accurate recording of the pH could not be done. The pH could have been accurately estimated using pH meter.

The results of free and total acidity point out that higher concentration of the extract possessing higher proanthocyanidin content [28] exert better effect in decreasing the acidity. The antioxidant activity of the extract abolishes the genesis of oxidative stress, causing reversal of oxidative activities and having a beneficial role in the gastric mucosa.

The number of ulcers was significantly decreased in the grape seed 100 mg/kg group and 200 mg/kg group when compared with the control group. As a result of change in the gastric volume and increase in the acidity, the ulcer number was consequently reduced. Antioxidant activity [6] of the extract reverses the oxidative stress and inhibits reactive oxygen species to have a protective role on the gastric mucosa. The percentage decrease in the number of ulcers with grape seed 100 mg/kg group was 27.98% and with grape seed 200 mg/kg group was 34.67% when compared with the control group.

The gastric lesion was significantly reduced in the grape seed 200 mg/kg group on account of higher antioxidant activity [29] exerted by the high proanthocyanidins content possessing antioxidant actions.

A significant decrease in the ulcer severity was seen in grape seed 200 mg/kg group that possesses higher polyphenol content.

The study of the effect on gastric lesions by GSE by employing ethanol was done by in 2005 [30]. They evaluated the gastric defense mechanism of grapefruit seed extract against ethanol and stress-induced gastric lesions in male Wistar rats by inducing gastric mucosal lesions by 100% ethanol or 3.5 hrs of water immersion stress. Their study showed a significant fall in the gastric blood flow and superoxide dismutase activity and rise in the mucosal malondialdehyde (MDA) content. Pretreatment with GSE caused a dose dependent attenuation of gastric lesions and, the dose reducing these lesions by 50% was 25 and 36 mg/kg respectively. The study showed GSE exerting a potent gastroprotective activity through an increase in endogenous prostaglandin generation, suppression of lipid peroxidation, and hyperemia. The results obtained in this study do not correlate to the study quoted above. The reasons for this can be the higher concentration (100%) of ethanol being used, and also the different parameters analyzed in their study.

In another study done in 2011 [31], the effects of GSE, vitamin C (VC), and vitamin E (VE) on ethanol and aspirin-induced ulcers were evaluated. Oral administration of GSE, VC, and VE at 25 and 250 mg/kg prevented gastric mucosal ulceration and reduced the increase of gastric MDA elicited by these aggressive agents. GSE 25 and 250 mg/kg produced greater reduction of ethanol than aspirin-induced ulcers. GSE prevented ethanol-induced gastric ulcers more effectively than VC or VE, while its protection against aspirin ulcers was comparable for all treatments. GSE produced the greatest reductions of gastric MDA in both models. The gastroprotective effect of GSE is proportional to its ability of reducing lipid peroxidation in the gastric mucosa. The results obtained in this study do not correlate to the above-quoted study on account of the different species of rats used, and also the different dose employed in their study. Levels of MDA were analyzed, which was not done in this study.

## CONCLUSION

This study concludes that the ethanolic extract of GSE has antiulcer activity in the model employed. It has an antiulcer activity comparable to that of the standard drug.

GSE of 200 mg/kg dose possesses a better effect on all the parameters analyzed than that of 100 mg/kg dose. There was no significant change in the parameters except the number of ulcers, gastric lesion, and free acidity in the HCl – ethanol model.

The results can be more accurately assessed by either involving a larger sample size or using a higher concentration of the extract.

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