

EXPERIMENTAL EVALUATION OF THE ANTI-ULCER ACTIVITY OF GRAPE (*VITIS VINIFERA*) SEED EXTRACT IN WISTAR ALBINO RATSAnand M. Ingale^{*}, Vijaya Rajendran[§], Venkata BharatKumar Pinnelli^{§§}

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ABSTRACT

Background: Peptic ulcer disease is a common condition of present day life, and its incidence is markedly increasing. The available drugs for the treatment are associated with adverse effects. Hence, there is a need for newer and better treatment for the same. **Objectives:** To evaluate the anti-ulcer activity of ethanolic extract of *Vitis Vinifera* (seed) by Water Immersion stress induced gastric ulcer model in Wistar albino rats using two doses (100, 200mg/kg body weight.) **Methods:** The antiulcer activity was assessed by estimating gastric volume, pH, free and total acidity; ulcer number and its inhibition, gastric lesion, ulcer severity and ulcer index; and comparing it with the control groups. **Results:** The present study revealed that grape seed extract of 100mg/kg and 200mg/kg showed a significant ($p < 0.05$) reduction in the gastric volume, ulcer number, gastric lesion and severity. The ulcer index was 45.33 and 36.66 for 100 and 200mg/kg respectively. Ulcer inhibition for 100 and 200 mg/kg group was 32.79% and 44.86% when compared with the control group. Ulcer protection with 100 and 200mg/kg was 29.21% and 39.82% when compared with the control group. **Conclusion:** This present study indicates that *Vitis vinifera* seed extract have potential anti-ulcer activity in the model tested.

Keywords: *Vitis vinifera*, Anti-ulcer, Stress ulcer, Free acidity, Total acidity, Ulcer index

INTRODUCTION

Peptic ulcer disease (PUD) is a common disease affecting around 10-15% of population (Muchhandi A.A. et. al., 2011.) Its incidence is more because of increasing stress in day-to-day life (Ingale A.M. et.al., 2014.) PUD affects the area of the gastrointestinal tract with high concentrations of gastric acid as a result of the imbalance between the aggressive and the defensive factors that govern the synthesis of the gastric acid. (Kumar et.al., 2010.) Numerous classes of drugs which are manufactured are associated with many unwanted adverse effects. (Brunton L.L., et.al., 2012.) Therefore, there is an increasing need for the discovery of newer and safer treatment for the management of the condition. Herbal products are effective modality for the treatment of various health conditions including PUD. (Umashanker M., et.al., 2001.) Grape plant, which is a creeper, is amongst the commonly growing plant throughout the world including India. The different parts like fruit; leaves and roots have beneficial effects in health because of its high nutritional value which has a wide application in Ayurveda. The parts of the fruit like seeds, pulp and skin have deferring concentrations of the phytonutrients with many therapeutic applications. Grape seed is a waste product of winery. Grape Seed Extract (GSE) has high contents of phytonutrients that are finding place in various clinical conditions.

Exploration of numerous activities of grape seed is been undertaken throughout the world (Vaughan J.G., et.al., 1997.) GSE possess flavonoids, which are proven antioxidants and also possess antibacterial effects against *H. pylori* (Brown J.C. et.al., 2009) and MRSA (Al-Habib.A., et.al. 2010)], anti-inflammatory action (Wen-Guang L, et.al, 2001), cardiovascular and endothelial protection (Clifton P.M., et.al., 2004), antihepatotoxic action (Maheswari.U.M., et.al., 2004), adaptogenic and nootropic effects (Sreemantula. S., et.al., 2005), platelet function (Vitseva.O., et.al., 2005), apoptosis (Roy A.M. et.al.,2005), aromatase inhibitor (Kijima.I. et.al.,2006), vascular endothelial growth factor (VEGF) and Angiogenesis inhibition (Wen W. et.al., 2008), antiviral and antifungal properties. Most of the activities of GSE are attributed to their antioxidant activity. GSE is shown to have Antiulcer activity (Kim T.O., et.al., 2013), effect on ethanol (Cuevas V.M. et.al., 2011), stress induced gastric lesions and also the present study was undertaken to explore its possible antiulcer activity.

MATERIALS AND METHODS

The present study was carried out only after being approved by our Institutional Animal Ethical Committee. The Guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) were meticulously followed throughout our study.

Procedure - GSE

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

Animals

In-bred Wistar albino rats (*Ratus Norvegicus*) of either sex weighing 150 to 250 grams, acclimatized to the laboratory conditions for two weeks, were used for the study. They were housed in clean polypropylene cages, in a well-ventilated room at controlled temperature (26-28°C) with a 12 hour light and dark cycle. 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.

- Omeprazole powder [Spansules formulations Ltd.]
- Toppers reagent [Merck & Co.]
- Phenolphthalein indicator, Sodium hydroxide (0.01N) [S. D. Fine Chem. Ltd]

Pharmacological Screening

Anti-ulcer activity by Water immersion Stress method (Shenoy A.M. et.al., 2011)

Healthy Wistar albino rats were divided into four groups of six rats each.

Group 1 (Control): received 1 ml distilled water orally

Group 2 (Standard): treated with 20mg/kg Omeprazole orally

Group 3 (GSE 100): treated with 100mg/kg of Grape Seed Extract orally

Group 4 (GSE 200): treated with 200mg/kg of Grape Seed Extract orally

Rats were fasted for 1 day (water also withdrawn). On the next day, Distilled water / Omeprazole 20mg/kg / GSE 100mg/kg / GSE 200mg/kg were given orally according to the groups mentioned above, and the rats were subjected to swimming in Standard glass cylinder having dimensions of height-45cms and diameter-25cm containing 35cms of water for 3 hours, after which they were sacrificed by cervical dislocation.

The stomach was excised. Its contents were analyzed for its pH, volume, free and total acidity. The stomach was opened up through the greater curvature and its mucosa was observed for the number of ulcers, their severity, gastric lesion and ulcer index. (Bickel M. et.al., 2002.)

PARAMETERS ANALYSED

i. Estimation of pH and gastric volume:

The gastric content was centrifuged at 1000 revolutions per minute for 20 minutes and its volume was estimated. The pH was noted by means of pH strips.

ii. Estimation of Free and total acidity

The gastric content was diluted to 10ml by adding distilled water (Firdous S.M. et.al.,2012) and analyzed for its free and total acidity by titrating it against 0.01 N NaOH solution using Toppers reagent (di-methyl amino azobenzene in 95 % alcohol) and phenolphthalein as indicators and noting the colour change. (Firdous S.M. et.al.,2012.)

iii. Estimation of Ulcer number

The gastric mucosa was observed macroscopically under 10x magnifications for the number of superficial ulcers, deep ulcers and perforations. (Bickel M. et.al., 2002.)

iv. Estimation of Gastric lesion

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. (Malairajan P, et.al.,2008)

v. Estimation of Ulcer severity

The ulcers were graded (Mittal R. et.al., 2009) as 0 - no ulcers; 1 - superficial ulcers; 2 - deep ulcers; 3 – perforations. Average severity score was calculated.

vi. Estimation of Ulcer index

Ulcer index in each group was calculated as (Mittal R. et.al., 2009) $UI = UN + US + UP \cdot 10^{-1}$. 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

Results were analyzed statistically by *One-way ANOVA (Analysis Of Variance) followed by Dunnett's t Test* using standard statistical software package of social science (SPSS) version 20 where all the groups were compared with the control group in each model, with p value set at 0.05.

RESULTS

Grape seed 100mg/kg group caused significant reduction $p < 0.05$ in the gastric volume mean \pm SEM - 0.18 ± 0.03 , ulcer number 55.66 ± 7.53 , gastric lesion 29.33 ± 3.44 and severity 2.16 ± 0.16 when compared with the control group. [Table-1, 2]. Grape seed 200mg/kg group caused significant reduction $p < 0.05$ in the gastric volume 0.13 ± 0.01 , free acidity 19.83 ± 1.49 and total acidity 38.5 ± 2.07 acidity, ulcer number 45.66 ± 2.61 , severity 2.00 ± 0.0 and gastric lesion 23.16 ± 2.88 as compared to the control group. [Table-1, 2].

Table 1: Effect of GSE On Gastric Content Parameters In Water Immersion Stress Model In Rats

Group	Control (Distilled water)	Standard (Omeprazole -20mg/kg/p.o.)	GSE 100 (Grape seed extract – 100mg/kg/ p.o.)	GSE 200 (Grape seed extract - 200mg/kg/ p.o.)
Gastric volume (in ml/kg)	0.41 \pm 0.04	0.07 \pm 0.00*	0.18\pm 0.03*	0.13\pm 0.01*
pH	1.00 \pm 0.00	1.16 \pm 0.21*	1.00 \pm 0.00	1.00 \pm 0.00
Free acidity (in mEq/lt/100gm)	28.33 \pm 1.22	17.5\pm 1.54*	24.16 \pm 1.16	19.83\pm 1.49*
Total acidity (in mEq/lt/100gm)	48.16 \pm 1.04	35.83\pm 2.07*	41.83 \pm 1.81	38.5\pm 2.07*
Bound acidity (in mEq/lt/100gm)	19.83 \pm 1.24	18.33 \pm 1.40	17.66 \pm 2.57	18.66 \pm 1.28

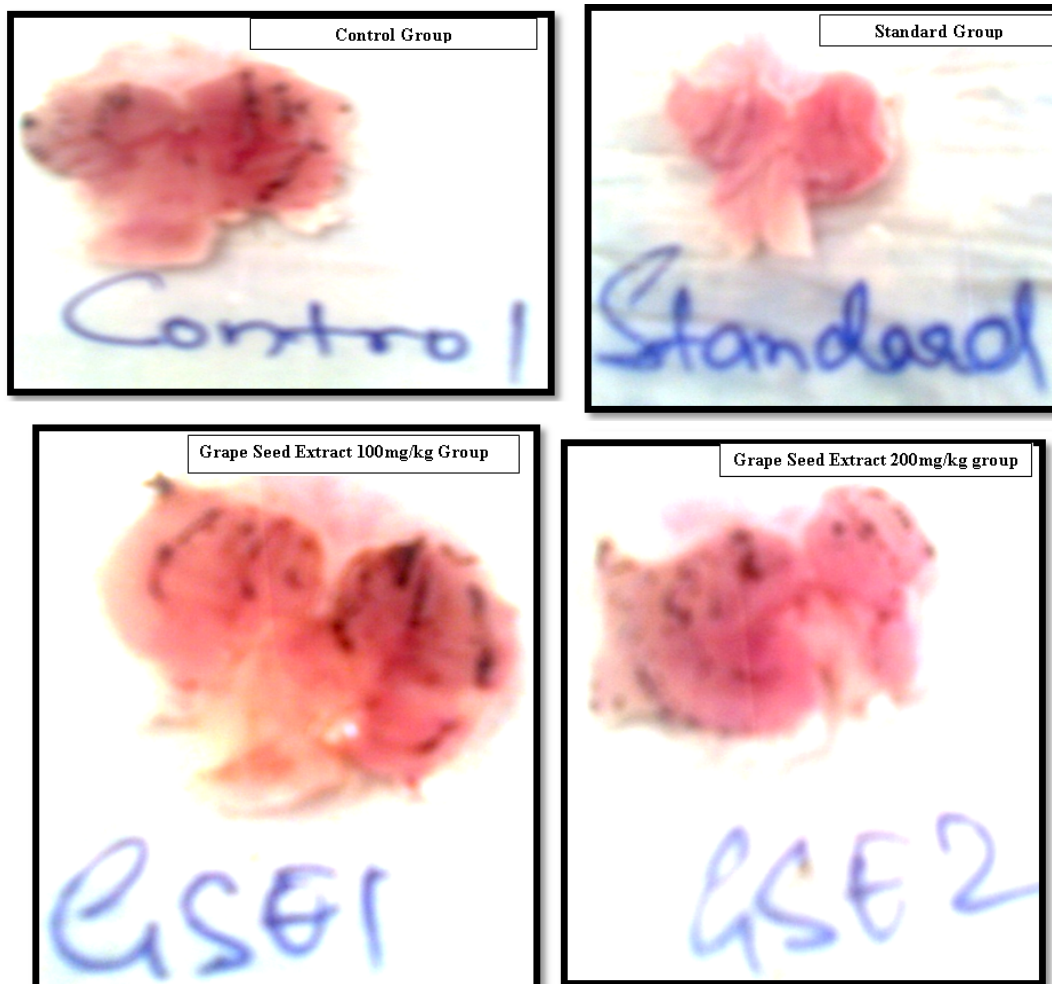
Values are expressed as Mean \pm SEM, n=6, * $p < 0.05$

Table 2: Effect of GSE On Gastric Mucosal Parameters In Water Immersion Stress Model In Rats

Treatment	Dose (mg/ kg)	Number of Ulcers		Gastric lesion (in mm)	Ulcer index		Average Severity Score
		Total	Inhibition (in %)		Ulcer index	Ulcer Protection	
Control (Distilled Water)	1 ml	82.83 \pm 7.69	0.0	45.33 \pm 7.16	95.83	0.0	3
Standard (Omeprazole)	20	33.33 \pm 1.17*	59.76	17.00 \pm 2.76*	45	53.04	1.67
Grape Seed Extract	100	55.66 \pm 7.53*	32.79	29.33 \pm 3.44*	67.84	29.21	2.17
Grape Seed Extract	200	45.66 \pm 2.61*	44.86	23.16 \pm 2.88*	57.67	39.82	2

Values are expressed as mean \pm SEM. n=6. * $p < 0.05$ as compared to control

The ulcer index determined was 93.49 for the control group, 43.83 for the standard group, 45.33 for grape seed 100mg/kg group and 36.66 for grape seed 200mg/kg group. Ulcer inhibition with standard group was 59.76%, grape seed 100mg/kg group was 32.79 % and with grape seed 200mg/kg group was 44.86 % when compared with the control group. Ulcer protection with standard group was 53.04%, with grape seed 100mg/kg group was 29.21 % and with grape seed 200mg/kg group was 39.82 % when compared with the control group.



Picture - 1: Stomach mucosa of Rat

DISCUSSION

Water immersion stress is one of the best models for stress induced ulcer in animals as it provides both emotional stress as well as physiological stress to the animal. As stress is the main factor encountered in present day life, giving rise to numerous consequences, the model was chosen for the antiulcer evaluation. The method has the advantages of being easily reproducible and involvement of psychological stress that is an important aspect correlating to humans. This model has a central role in the ulcer genesis. It screens antiulcer agents acting through central mechanism. The ulcers are formed in this model as a result of increase in the gastric acid secretion and a decrease in the mucosal microcirculation and mucosal content. (Bickel M. et.al., 2002.). Our study showed that the both concentrations of grape seed extract significantly reduced the gastric volume proving the anti-secretory activity of the grape seed extract. Grape seed proanthocyanidins, that are antioxidants, cause reduction in the gastric secretion by acting on the gastric mucosa and inhibiting the generation of reactive oxygen species that initiate the oxidative stress in the gastric lumen.

There was no significant increase in the pH of any groups as the pH was noted by means of pH strips.

Free and total acidity was significantly decreased in the grape seed 200mg/kg group. Change in the volume of the gastric contents and reduction of their pH consequently reduces the free and total acidity that could be attributable to the antioxidant activity of the polyphenols protecting the gastric mucosa by blocking the oxidation process.

Number of ulcers was significantly decreased in the grape seed 100mg/kg group and grape seed 200mg/kg group. As a result of decrease in the gastric volume, increase in the pH, and decrease in the free and total acidity, there was consequently a decrease in the number of ulcers formed in the test groups. The action was profound with grape seed 200mg/kg group that possessed higher polyphenol content. Proanthocyanidines are powerful antioxidants, and hence may have contributed to their activity. Gastroprotective nature of the chemicals may have added onto the activity. Gastric lesion was significantly reduced in the grape seed 100mg/kg group and also grape seed 200mg/kg group. Gastric lesions reflect the area of the ulcers. As the number of ulcers is reduced, the gastric lesion is also reduced. There was significant decrease in the severity of the ulcers in all groups with higher concentration of the extract showing better effect on the severity.

CONCLUSION

The present study done with the ethanolic extract of GSE using the water immersion stress induced rodent models of antiulcer evaluation shows that the ethanolic extract of GSE had potential antiulcer activity. The activity of the extract was seen in all the parameters comparable to the standard drug signifying that it acts by proton pump inhibition. Grape seed extract of 200mg/kg dose possesses a better effect on all the parameters analyzed than that of 100mg/kg dose.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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