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## Evaluation of antiulcer activity of organic grapes produced by microbial fertigation

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### ABSTRACT

The investigation is aimed to evaluate the antiulcer activity of organic grapes produced by microbial fertigation and foliar Panchagavya. The gastric lesion was induced by oral administration of HCl - ethanol, and by pyloric ligation. The methanolic extract of grapes was administered at a dose of 100, and 200 mg/kg orally for five days before ulcer induction. Ranitidine was used as a reference standard. The antiulcer activity was assessed by determining and comparing the ulcer index in the test group with that of the standard drug-treated group. Gastric volume, total acid and free acid were estimated in the pylorus-ligated rats. The methanolic fruit extract (200 mg/Kg) showed maximum inhibition of gastric acid, free acidity and total acidity. The ulcer index of grape juice treated animals was found to be significantly less in all the models compared to standard drug-treated cases. The methanolic extract of *Vitis vinifera*(EVV) recorded higher ulcer inhibition rate against HCl – Ethanol-induced gastric lesion when compared with control and reported effective anti-secretory and antiulcer activity against pyloric ligation induced gastric ulcer in rats. Ulcer induced by aspirin plus ligated rats, pretreated with EVV showed a reduction in gastric acid secretion, free acidity, total acidity, ulcer score and increase in pH and ulcer inhibition rate when compared to control. The results showed the significant efficacy of fertigated grapes in antiulcer activity. The results were statistically analyzed by one-way ANOVA test and were found to be significant at  $p < 0.05$ .



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### INTRODUCTION

Peptic ulcers are the deep and painful lesions, that affects the entire path of the gastrointestinal tract, which causes inflammation, bleeding of the

mucosal lining and severe abdominal pain (Bharathi DP *et al.*, 2010 & Panneerselvam S *et al.*, 2011). The major causes of this chronic disease include alcohol consumption, smoking, usage of non-steroidal drugs, *H.pylori* infection (Milosavljevic T *et al.*, 2011), accounting mainly due to modern lifestyle changes. Current management of peptic ulcer is achieved by antacids, proton pump inhibitors, histamine receptor antagonists and anticholinergics (Sarojkumaret *al.*, 2016).

Day to day global demand of consumers for organic remedies is on elevation, due to the increasing concern about the harmful and undesirable consequences of allopathic medicines. With the development of medicine and technology, grapes (*Vitis vinifera*) have been found to possess several health-promoting qualities, which has led to a large

amount of research and increased popularity for this fruit. Various chemical fertilizers used in grapes cultivation have adverse effects on human health. The chemical residues on the grapes, owing to the improper and increased use of chemical pesticides prevents the consumers from purchasing grapes. Organic farming in recent years is gaining impetus due to the realization of the inherent advantages it confers in sustaining crop production and its positive effects on human health (Lokanath and Parameshwarappa, 2006). Previous studies have reported antiulcer activity of grape seed extract (Saito M, 1998; AnandM, 2016). However, there is no scientific evidence on the antiulcer activity of organic grapefruit extract so far. Hence, the present investigation is aimed at studying the antiulcer activity of organic grapes, produced by microbial fertigation and foliar panchagavya in albino rats.

## MATERIALS AND METHODS

### Test animal preparatory work

Swiss albino rats (Wistar strain) weighing 120 – 200 gm, procured from King's Institute, Guindy, Chennai, India. All the rats were housed in standard plastic cages with stainless steel over lids and wheat straw as bedding material. Animals were given a week's time for acclimatizing lab conditions before the commencement of the experiment. Rats were fed with standard rat feed Hindustan Lever Ltd, India. The CPCSEA ethical committee approved the study protocol.

### Preparation of the *Vitis vinifera* (Grape) Fruit Extract

Fresh grapes were harvested and rinsed with cold water. The grapes were then de-stemmed and mixed with dry ice to form frozen stemless grapes. The frozen grape mixture was stored in a moisture-proof container at < 32° F and used for the animal toxicology studies. The powdered fruit material was then subjected to successive extraction by hot percolation method using petroleum ether, chloroform and methanol solvents in a Soxhlet extractor. The different extracts obtained were evaporated at 45°C to get a semi-solid mass. The percentage yield of alcoholic extract was found to be 37.50% and the methanolic extract was used for further studies Acute toxicity studies (OECD 2006 guidelines) (Mukaherjee PK, 2002).

### LD<sub>50</sub> DETERMINATION

Acute toxicity studies were carried out using the acute toxic class method as per guidelines 425 toxicity for the *Vitis vinifera* (Grape) extract. The study was carried out by administering

5 mg/kg, of methanolic extract of *Vitis vinifera*(EVV) to the test group and the control group, received only the vehicle. The groups were observed for mortality and behavioural changes for 48 hours.

### Effect of methanolic extract of grapes on aspirin plus pylorus ligated rats

The effect of methanolic extract of *Vitis vinifera* was pretreated orally to all animals in test groups T 1(100 mg/Kg) & T 2 (200 mg/Kg) for 5 days. On the day 6, rats were weighed and fasted for 24-36 hours before pyloric ligation. Under anesthesia, the pyloric end of the stomach was ligated and the stomach was replaced carefully and interrupted sutures closed the abdominal wall. After 4 hours of pyloric ligation, animals were sacrificed and the stomach was dissected out. The gastric contents were drained into calibrated centrifugal tubes. The parameters such as volume and pH of gastric content, free acidity, total acidity, Ulcer score and ulcer Inhibition (%) will be evaluated using the gastric content and exposed stomach (Shay *et al.*, 1945).

### Effect of methanolic extract of *Vitisvinifera* against HCl – Ethanol-induced gastric Lesion in rats

The methanolic extract of *Vitis vinifera* was administered orally to all animals in group Test Group T 1(100 mg/Kg) & T 2 (200 mg/Kg) for 5 days. On the day 6, rats were weighed and allowed to starve for 24 hours with free access to drinking water. The test drug (HCl/ethanol mixture (1.5 ml of 0.15 N HCl in 70% ethanol) was administered through oral route 1 hour before the necrotizing agents (1 ml of absolute alcohol). The absolute alcohol was administered through the oral route. After 1 hour of alcohol administration, animals were sacrificed and the isolated stomach was evaluated for a total number of lesions and % of Ulcer inhibition (Anadan *et al.*, 1998).

### Statistical analysis of the data

The statistical analysis was carried out using SPSS 17 Statistical software package. The experimental data were statistically expressed as mean ± standard error mean – SEM (Gomez and Gomez, 1984). One-way ANOVA was applied to study the effect of microbial fertigation on antiulcer activity of organic grapes and was expressed to be significant at p<0.05 level.

## RESULTS AND DISCUSSION

In acute toxicity study, grapes produced by microbial fertigation did not show any toxicity signs following a single dose of 2000 mg/kg did not show any toxicity signs, suggesting that the fruits are practically nontoxic after an acute exposure in

**Table 1: Effect of methanolic extract of *Vitis vinifera* against HCl –methanol-induced gastric lesion in mice**

Treated	Gastric lesion	Ulcer inhibition (%)
Control	21.55±2.809	-
Sucralfate	1.017 ± 0.512	91.85
T1 – EVV 100 mg/kg	1.8± 4.46	82.71
T2 – EVV 200mg/kg	1.5 ± 3.54	86.56

Values are expressed as mean ± SEM, n= 6; Control - 1% SMC (Sodium salt of Carboxymethylcellulose); Standard -Sucralfate 100 mg/ Kg body weight; T 1 - EVV 1 - 100 mg/Kg body weight of methanolic extract of *Vitis vinifera*; T 2 - EVV 2 - 200 mg/Kg body weight of methanolic extract of *Vitis vinifera*

**Table 2: Effect of methanolic extract of *Vitis vinifera* on gastric secretion, acidity, pH and ulcer score in aspirin plus pylorus ligated rats**

Treated	Volume of gastric acid secretion (ml/100g)	Free acidity (mEq/l/100g)	Total acidity (mEq/l/100g)	pH	Ulcer score	Ulcer inhibition (%)
Vehicle control	2.633 ± 0.642	225.00± 6.124	555.00± 7.55	2.200± 0.163	3.600 ±0.200	-
Standard	1.217 ± 0.172	141.75± 11.475	452.50± 21.736	3.067± 0.166	0.146±0.136	94.37
T 1	0.953±0.073	134.00± 10.882	542.50± 9.714	2.13± 0.210	1.567±0.207*	51.61
T 2	1.003±0.083	137.00± 10.782	522.50± 11.124	2.43± 0.250	1.267±0.301	57.69

Values are expressed as mean ± SEM., n= 6; \*Significantly different from control at p< 0.05 level. Control - Vehicle control, Standard - Ranitidine 50 mg/Kg body weight, T 1 - EVV1 - 100 mg/Kg body weight of a methanolic extract of *Vitis vinifera* T 2 - EVV2 - 200 mg/Kg body weight of a methanolic extract of *Vitis vinifera*

rats. Administration of methanolic extract did not show any mortality and behavioural changes.

The methanolic extract of *Vitis vinifera* (EVV) recorded higher ulcer inhibition rate against HCl – Ethanol-induced gastric lesion when compared with control. The results of the current investigation showed that the test extracts (EVV) had an effective anti-secretory and antiulcer activity against pyloric ligation induced gastric ulcer in rats (Table 1). The test extract T2 – EVV(200mg/Kg) showed about 86.56% ulcer inhibition followed by 82.71% inhibition by T1-EVV (100mg/Kg) when compared with the standard which recorded 91.85% of ulcer inhibition.

Ulcer induced by aspirin plus ligated rats, pretreated with EVV showed a reduction in gastric acid secretion, free acidity, total acidity, ulcer score and increase in pH and ulcer inhibition rate. The test extract EVV-T2 showed a significant decrease in acidity when compared with EVV-T1 and standard (Table 2). Thus the extracts show protection against characteristic lesions produced by ethanol administration and the effect may be due to the reduction in gastric acid secretion. Further studies are needed for exact mechanism of

action organic grape extract on gastric acid secretion.

## CONCLUSION

The toxicological study of the organic grapes produced by microbial fertigation is proved non-toxic and safe to consume and also exerts a protective effect against gastric ulcer and acidity. It could be recommended as a dietary supplement with any system of medicine in treating ulcers eliminating the side effects of allopathic medicines.

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