

Investigation of Gastroprotective Potential of Grape Seed Proanthocyanidin Extract in Experimental Models of Gastric Ulcer, in Wistar Rats

Vivek Bhardwaj*, Rajat Bhardwaj, B.V. Krishna Reddy and Pyare Lal Sharma

Department of Pharmacology, I.S.F College of Pharmacy, Moga, Punjab, India

*Corresponding author: Bhardwaj V, Department of Pharmacology, I.S.F College of Pharmacy, Moga, Punjab, India, Tel: 01636324201; E-mail: 9vivekbhardwaj9@gmail.com

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Abstract

Aim and Objective: To investigate the anti-ulcer activity of grape seed proanthocyanidin extract (GSPE) in various experimental models of gastric ulcer, in wistar rats.

Materials and Methods: Rats were assigned to 5 groups in each model; Normal control, Vehicle control (5% CMC treated), GSPE (50 mg/kg, p.o. 4 days), GSPE (100 mg/kg, p.o. 4 days), Famotidine (40 mg/kg, p.o) or Omeprazole (50 mg/kg, p.o.). Three animal models used in study were (n=6) 1. Pylorus ligation model: After 4 days of pretreatment, fasted rats were anaesthetized and abdomen was incised and pylorus part was ligated. Immediately after ligation drug and vehicle was administered. 4 hours later, animals were sacrificed, abdomen was opened and stomach was removed and assessed; Indomethacin treated model: In this model after 4 days of pretreatment, indomethacin (50 mg/kg) was given to the fasted rats to induce ulceration. After 5 hour rats were sacrificed and stomach was removed and assessed; Stress induced ulcer model: After 4 days of GSPE pretreatment, on day 4th fasted animals were immobilized in restrainer. The restrainer was exposed to cold environment (4-7°C) to produce gastric ulcer. At the end of 3 hour stress exposure animals were sacrificed stomach was removed for ulcer assessment.

Results: Gastric acidity and ulcer index in pylorus ligation models were significantly decreased on GSPE (100 mg/kg) drug treatment. Administration of GSPE extract led to significant decrease in MDA levels, in both 50 and 100 mg/kg treated groups. Similar results were obtained in case nitrite/nitrate and MPO levels in each model. Furthermore, the obtained results revealed that pylorus ligation; indomethacin and stress models also significantly decreased the anti-oxidant levels like catalase, SOD and GSH. Antioxidant levels were lowered in vehicle treated control rats in each model and these levels were significantly increased in GSPE treated groups in each model.

Discussion: In the present study GSPE (100 mg/kg) prevented both the increase in markers of oxidative stress and the decrease in levels of endogenous oxidants. In contrast of Famotidine and omeprazole only prevented the increase in the markers of oxidative stress.

Conclusion: Hence, the GSPE higher doses are a better therapeutic option for the treatment of gastric ulcer in terms of better therapeutic health benefits and lesser side effects.

Keywords: Gastric ulcer; Indomethacin; Gastric acidity; Oxidative stress

Abbreviations: GSPE: Grape Seed Proanthocyanidin Extract; GSH: Glutathione; SOD: Superoxide Dismutase; CAT: Catalase; PL: Pylorus Ligation; UI: Ulcer Index; TBARS: Thio Barbituric Reactive Substances

Introduction

Herbs are safer to use and has fewer side effects, than synthetic western medicines, but only with the correct usages [1]. Grape seed extract, a waste product of wine and grape juice industry, contain various chemical compounds but the active chief chemical constituents are polyphenolic flavanoid called proanthocyanidin (PAs) [2,3]. PAs are of great interest in nutrition and medicine because of their potent antioxidant capacity. In addition to their free radical scavenging and

antioxidant activity, PAs have been reported to have antibacterial, antiviral, anticarcinogenic, antiallergic and vasodilatory actions [4].

GSPE, belongs to flavanoid group is obtained from the seeds of *Vitis vinifera* grapes (red), which contains 95% proanthocyanidins [2]. *In-vitro* studies using chemiluminescence assay demonstrated that GSPE exhibited superior antioxidant activity as compared to vitamin C, E and betacarotene [5,6]. GSPE shows protective effect against diabetic neuropathy in relation to its antioxidant properties [7]. GSPE suppresses ROS generation *in-vitro*, in human umbilical vein endothelial cells (HUVEC) [8]. Proanthocyanidins have been shown to exert a novel spectrum of biological, pharmacological, therapeutic and also chemoprotective properties against oxidative stress [9].

Proanthocyanidin from grape seeds dose-dependently inhibited the paw edema in rats induced by carageenan and ear edema of mice induced by croton oil, which may be due to inhibition of

prostaglandins, stabilization of lysosomal membrane and decreased formation of lipoperoxidation products. Also, GSPE has been shown to produce anti-arthritis effect in collagen-induced arthritis in mice through a reduction in the production of type 2 collagen specific IgG2a and inflammatory cytokines such as TNF- α and IL-17. In addition, GSPE also suppressed osteoclastogenesis in mice both *in-vivo* and *in-vitro* [10] and shown to produce protection against ethanol-induced ulcer in rats [11]. GSPE also shows protective effect in 2, 4, 6-trinitro benzene sulfonic acid (TNBS) induced ulcerative colitis in rats [12]. Hence the present study was designed to investigate the gastroprotective potential of GSPE in detail.

Gastric ulcer is a common global disease with increasing incidence and prevalence, despite significant medicinal advances. Despite the widespread use of conventional antiulcer therapy that effectively reduces gastric acid secretion, ulcers frequently recur, with 1 year recurrence rates estimated to range from 60% to 100% [13].

Gastric ulcer is a serious gastrointestinal (GI) disorder, and occurs when the gastric mucosa gets damaged, leading to perforations of the stomach lining and even bleeding. Typically, gastric ulcer is referred to that restricted to the stomach, while ulcers of the stomach and duodenum together are known as peptic ulcers [14]. Gastric and duodenal ulcers affect thousands of people and are currently considered a global health problem [15]. It is a complex pluricausal disease and is known to develop due to imbalance between aggressive and protective factors [16,17]. Several endogenous and exogenous factors are responsible for gastric ulceration. These include *H. pylori* infection, increased production of gastric acids, pepsin and stomach juices, certain types of medicines, notably the non-steroidal anti-inflammatory drugs (NSAIDs), and even personal factors such as consumption of tobacco, alcohol and caffeine, as well as emotional and physical stresses [18].

In traditional medicine, several plants and herbs have been used to treat gastrointestinal disorders, including gastric ulcers [19]. Herbal drugs obtained from the plant source are relatively less expensive, safe, and possess good tolerability even in higher doses [20].

Materials and Methods

Grape seed proanthocyanidin extract was purchased from Bio-gen extracts (P) Ltd Bangalore, India. Indomethacin was obtained from Jagsonpal Pharmaceuticals Ltd, Rudrapur, India; Omeprazole from Ranbaxy Labs., Mumbai, India and Famotidine from Cipla Ltd, India were used. All other reagents or chemicals used were of analytical grade. Animal feed was purchased from Ashirwad Industries, Ropar, India. Animals used were wistar rats of either sex weighing 200-240 g were procured from the Animal House, ISF College of Pharmacy, Moga. The animals were kept in polypropylene cages (3 rats in each cage) at an ambient temperature $25 \pm 2^\circ\text{C}$ and relative humidity 55-65%. A 12-12 hour light and dark schedule was maintained in the animal house. The rats were fed over commercially available feed (Aashirwad Industries, Ropar, and Punjab) and water ad libitum. Experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) and was conducted as per the CPCSEA guidelines for animal experimentation and care.

Indomethacin-induced ulcer in rats

All the rats except control rats were pre-treated with test drugs for 4 days. On day fourth, indomethacin (50 mg/kg, p.o.) suspended in 0.5% CMC was given to all the groups to induce acute gastric ulcers, after 30

min of test drug treatments. After 5 hour, the animals were sacrificed; stomach dissected out and was examined for ulcer index [21]. The fraction showing maximum efficacy in this model had been subjected to estimate its efficacy on pylorus ligation induced ulcer model in rat. Animals were divided into 5 different groups (n=6/grp). 5 groups were made in each model; normal control, Ind. Vehicle control, GSPE (50 mg/kg, p.o., 4 days), GSPE (100 mg/kg, p.o., 4 days) and Famotidine (40 mg/kg, p.o.).

Pylorus ligation induced ulcer model

Firstly, Animals were fasted for 24 hour and anesthetized with thiopental sodium (40 mg/kg, i.p.). Rats were pretreated with test drugs for 4 days. On day fourth, the abdomen was incised and pylorus ligated. Immediately after pylorus ligation, all the drugs and vehicle were administered. Four hours later, the animals were sacrificed; the abdomen was opened, and another ligation placed at the oesophageal end. The stomachs were removed, and the gastric contents collected and centrifuged at 3000 rpm [22]. In pylorus ligation induced ulcer model 5 groups were made, normal control, Ind. Vehicle control, GSPE (50 mg/kg, p.o., 4 days), GSPE (100 mg/kg, p.o., 4 days) and Omeprazole (50 mg/kg, p.o.).

Stress induced ulcer model

Rats were pretreated with test drugs for 4 days. On day fourth, 24-48 hour fasted animals were immobilized in rigid plastic devices that facilitated precise standardization of restraint volume and conditions [23]. Restraint was exposed to cold environment ($4-7^\circ\text{C}$) to produce gastric ulcers. At the end of the 3 hour stress exposure, the animals were killed by decapitation and stomachs were removed for measuring the areas of erosion [24]. The ulcer index was calculated as the sum of the length (mm) of each injury per rat. In this model, 5 groups were made; normal control, Stress Vehicle control, GSPE (50 mg/kg, p.o., 4 days), GSPE (100 mg/kg, p.o., 4 days) and Omeprazole (50 mg/kg, p.o.).

Drug administration

In pyloric ligation induced ulcer model, the grape seed proanthocyanidin extract and its fractions were suspended in 0.5% w/v carboxymethylcellulose (CMC) and administered orally with the help of gastric canula immediately after pylorus ligation.

Assessment of ulcer index and gastric acidity

Ulcer index (UI) was determined as sum of the length (mm) of all lesions for each stomach and the protection percentage was calculated from the following formula [25]:

$$\% \text{ Protection} = \frac{(\text{UI}_{\text{control}} - \text{UI}_{\text{treated}})}{\text{UI}_{\text{control}}} \times 100$$

The total acid secretion in the gastric juice in the supernatant volume was determined by titration using a 0.01 mol NaOH solution, and phenolphthalein as indicator. Acidity was calculated by using the formula:

$$\text{Total Acidity} = \frac{(\text{vol of NaOH} \times \text{normality of NaOH})}{0.1} \times 100 \text{ mEq/L}$$

Determination of pH and gastric volume

The pH of the gastric juice was measured using the pH meter. Gastric volume was measured after centrifuging the gastric fluid,

allowed to stand, decant, and poured into the measuring cylinder of graduation 0.01 ml.

Biochemical estimations

Measurement of Lipid-peroxidation: Lipid-peroxidation, as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) was measured by using the thiobarbituric acid test of [26]. In brief, 0.2 ml of homogenate was added to 0.8% thiobarbituric acid, 8.1% sodium dodecyl sulfate (SDS) and acetic acid (20%) in distilled water. After heating for 60 min in a water bath at 95°C, the mixture was then cooled and extracted with a mixture of n-butanol/pyridine (15:1,v:v). The absorbance of the reaction product present in the upper organic layer separated by centrifugation was measured spectrophotometrically at 532 nm. The results were expressed in μM of MDA/mg of protein.

Estimation of antioxidant enzyme levels

Superoxide dismutase (SOD): SOD was assessed by utilizing the technique [27]. A single unit of enzyme was expressed as % inhibition of nitroblutetrazolium (NBT) reduction/min. The results were expressed in SOD units/mg of protein.

Catalase (CAT): CAT was assayed colorimetrically at 620 nm and expressed as μmoles of H_2O_2 consumed/min/mg protein as described by Sinha et al. [28]. In brief, a mixture (1.5 ml) contained 1.0 ml of 0.01 M phosphate buffer pH 7.0, 0.2 ml of tissue homogenate and 0.4 ml of 2 M H_2O_2 . The reaction was stopped by addition of 0.2 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). The results were expressed in H_2O_2 moles/mg of protein.

Reduced Glutathione (GSH): GSH was estimated by the method of Ellman [29]. In brief, 10% TCA was added to the homogenate and the mixture was centrifuged 1.0 ml of supernatant was treated with 0.5 ml of Ellmans reagent (19.8 mg of 5,5'-dithiobisnitro benzoic acid in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was measured spectrophotometrically at 410 nm. The results were expressed in mg of GSH/g of protein.

Estimation of nitrite/nitrate: Nitrite level in tissue homogenate was estimated using Griess reagent method. In brief, 200 μl of protein-free supernatant, 30 μl of 10% NaOH was added followed by 300 μl of tris-HCl buffer and mixed well. To this, 530 μl of Griess reagent (0.3% N-1[Naphthyl-ethylene diamine-dihydrochloride] in distilled water+3% Sulphanilamide in 1 M HCl) was added and incubated in the dark for 10-15 minutes, and the absorbance was read at 540 nm (Spectrophotometer, Beckman DU 640B, and Switzerland). The results were expressed in μM of nitrite/mg of protein.

Estimation of Myeloperoxidase (MPO) activity: MPO activity, an indicator of polymorphonuclear leukocyte (PMN) accumulation was determined as described previously [30]. Firstly tissue was homogenized in a solution containing 0.5% (w/v) hexadecyltrimethyl-ammonium bromide (Sigma-aldrich) dissolved in 10 mM potassium phosphate buffer (pH 7), and centrifuged for 30 min at 20,000 g at 4°C. An aliquot of the supernatant was then allowed to react with a solution of tetramethylbenzidine (1.6 mM) and 0.1 mM hydrogen peroxide. The rate of change in absorbance was measured spectrophotometrically at 650 nm. MPO activity was defined as the quantity of enzyme degrading 1 μmol peroxide min⁻¹ at 37°C and was expressed in milliunits per g wet tissue [30].

Statistical Analysis

The results were expressed as mean \pm standard deviation (S.D) and analyzed using analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. P-value <0.05 was considered as statistically significant. Statistical analysis was performed using Graph Pad Instant Software, (Version 3.01).

Results

Combined results of three models (Indomethacin induced, pylorus ligation induced and stress induced model for gastric ulcer).

Effect of various doses of grape seed proanthocyanidin extract on ulcer index in 3 models of gastric ulceration

The ulcer index was increased markedly in the vehicle treated indomethacin induced (Figure 1), pylorus ligation induced (Figure 2) and stress induced gastric ulcer (Figure 3) group as compared to the respective normal control group. However, repeated administration of GSPE (50 and 100 mg/kg, p.o) for 4 days shown significant and dose dependent decrease in ulcer index. Similarly, standard drug treatment (Omeprazole 50 mg/kg or Famotidine 40 mg/kg, p.o) significantly decreased the ulcer index as compared to the vehicle control group Figures 1b, 2b and 3b.

Indomethacin induced gastric ulcer

Values are expressed as mean \pm S.D; n=6; a denotes for P<0.05 vs. normal control, b denotes for P<0.05 vs. vehicle control; c denotes for P<0.05 vs. GSPE (50 mg/kg) Repeated drug treatment of GSPE and standard control drug (Famotidine 40 mg/kg) was given. GSPE: Grape Seed Proanthocyanidin Extract treated rats.

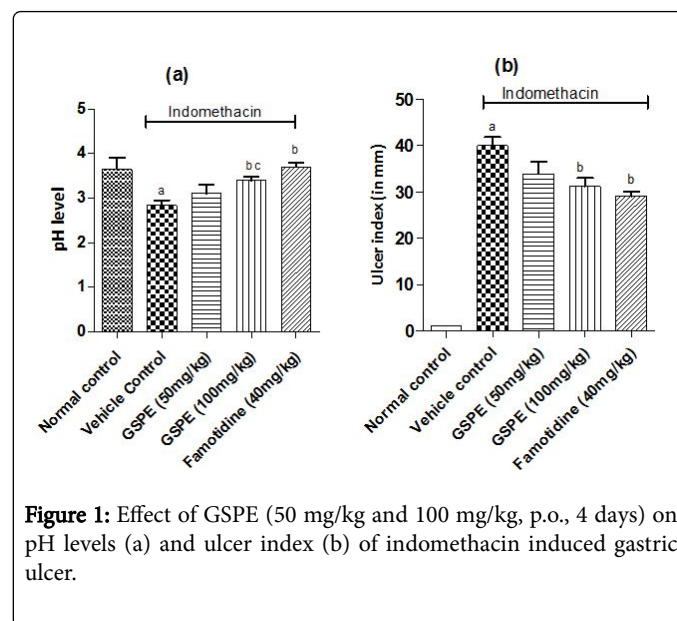


Figure 1: Effect of GSPE (50 mg/kg and 100 mg/kg, p.o., 4 days) on pH levels (a) and ulcer index (b) of indomethacin induced gastric ulcer.

Pylorus ligation induced gastric ulcer

Values are expressed as mean \pm S.D; n=6; a denotes for P<0.05 vs. normal control, b denotes for P<0.05 vs. vehicle control; c denotes for P<0.05 vs. GSPE (50 mg/kg) Repeated drug treatment of GSPE (50 mg/kg or 100 mg/kg) and standard control drug (Omeprazole 50 mg/kg) was given. GSPE: Grape Seed Proanthocyanidin Extract treated rats.

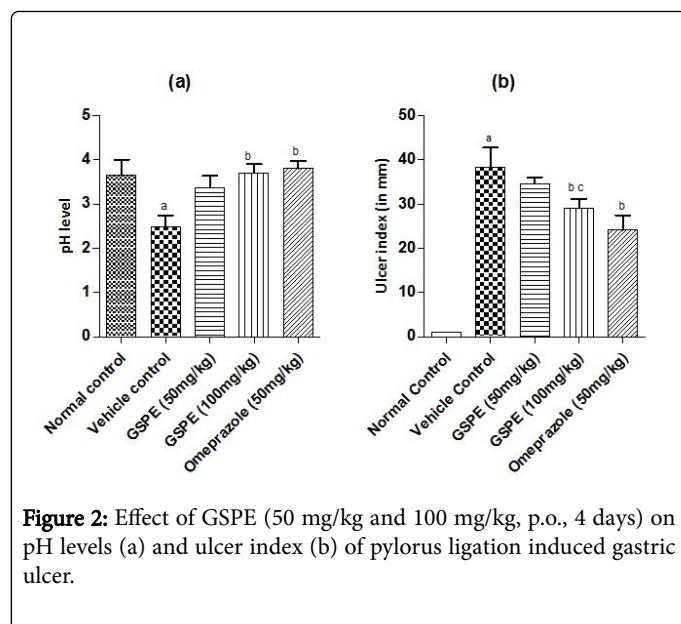


Figure 2: Effect of GSPE (50 mg/kg and 100 mg/kg, p.o., 4 days) on pH levels (a) and ulcer index (b) of pylorus ligation induced gastric ulcer.

Stress induced gastric ulcer

Values are expressed as mean \pm S.D; n=6; a denotes for P<0.05 vs. normal control, b denotes for P<0.05 vs. vehicle control; c denotes for P<0.05 vs. GSPE (50 mg/kg) Repeated drug treatment of GSPE (50 mg/kg or 100 mg/kg) and standard control drug (Omeprazole 50 mg/kg, p.o) treatment group was given. GSPE: Grape Seed Proanthocyanidin Extract treated rats.

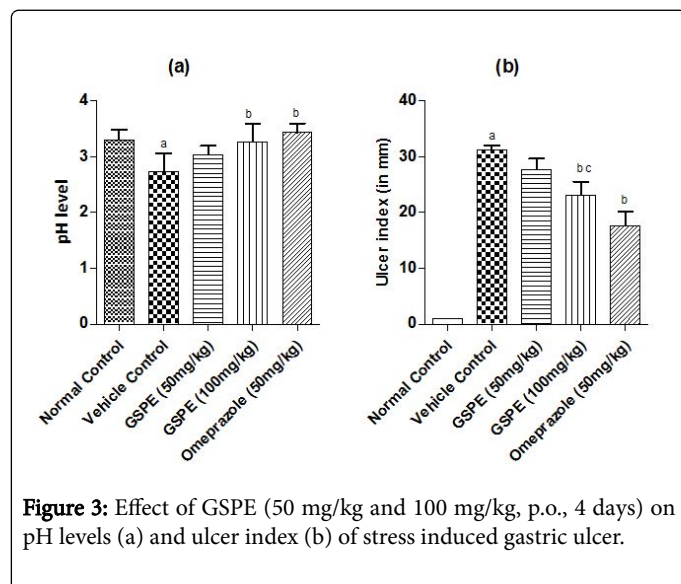


Figure 3: Effect of GSPE (50 mg/kg and 100 mg/kg, p.o., 4 days) on pH levels (a) and ulcer index (b) of stress induced gastric ulcer.

Effect of various doses of grape seed proanthocyanidin extract on pH levels

The pH levels were significantly acidic in vehicle treated indomethacin induced, pylorus ligation induced and stress induced gastric ulcer group as compared to the respective normal control group shown in Figures 1a, 2a and 3a. However, repeated administration of GSPE (100 mg/kg, p.o) for 4 days shown remarkable increase the pH levels. GSPE (50 mg/kg and 100 mg/kg, p.o) significantly increased the

gastric pH level towards the normal. Standard drug treatment (Omeprazole 50 mg/kg or Famotidine 40 mg/kg, p.o) also increased the gastric pH greatly to significant levels as compared to vehicle control group.

Effect of various doses of grape seed proanthocyanidin extract on TBARS level

There was a significant increase in mucosal TBARS level (measured as MDA) was observed in vehicle control group in each model to the levels of 14.98 in indomethacin induced model, 16.09 in pylorus ligation induced and 15.57 in stress induced model for gastric ulcer. Treatment with GSPE (50 mg/kg and 100 mg/kg, p.o) produced a dose dependent decrease in the level of TBARS. GSPE treatment has produced greater decrease in TBARS level even as compared to that of standard drug treatment group (Omeprazole 50 mg/kg or Famotidine 40 mg/kg, p.o).

Effect of various doses of grape seed proanthocyanidin extract on nitrite/nitrate levels

A significant increase in nitrite/nitrate level was observed in the vehicle control rats as compared to the normal control rats. Nitrite/nitrate levels were reduced on treatment with GSPE (50 mg/kg, p.o) in a significant and dose dependent manner. However this decrease was significant on GSPE (100 mg/kg, p.o) treatment in various ulcer models. The decrease in nitrite/nitrate levels was greater in GSPE (100 mg/kg, p.o) treatment group as compared to standard drug (Omeprazole 50 mg/kg or Famotidine 100 mg/kg, p.o) treatment group, but the difference was not statistically significant.

Effect of various doses of grape seed proanthocyanidin extract on myeloperoxidase levels

The MPO activity in mucosal tissue was significantly increased in vehicle control group as compared to the normal control group. Treatment with GSPE 50 mg/kg and 100 mg/kg significantly decreased the MPO levels as compared to the vehicle control group. MPO levels were noted 0.69 in indomethacin induced model, 0.78 in pylorus ligation model and 0.70 in stress induced model for gastric ulcer on GSPE (100 mg/kg, p.o) drug treatment. Compared to that of vehicle control group, GSPE (100 mg/kg) treatment was effective as the standard drug treatment group.

Effect of various doses of grape seed proanthocyanidin extract on SOD levels

SOD levels in various groups are shown in indomethacin induced model, pylorus ligation model and stress induced model in gastric ulcer. SOD levels were significantly decreased in vehicle control group in each model as compared to the normal control, and levels were slightly recovered on GSPE (50 mg/kg, p.o) drug treatment.

SOD levels were significantly and dose dependently increased on GSPE drug treatment as compared to vehicle control group. The increased level was significantly greater than that with standard drug treatment (Omeprazole 50 mg/kg or Famotidine 40 mg/kg, p.o).

Effect of various doses of grape seed proanthocyanidin extract on catalase levels

A significant decrease in the catalase levels was noted in the vehicle control rats. The levels of catalase in vehicle control were 12.33 in indomethacin induced model, 12.4 in pylorus ligation model and 10.7 in stress induced model for gastric ulcer and these levels were increased to the levels of 17.08, 17.18 and 19.9 in respective models on GSPE (100 mg/kg) treatment.

GSPE (100 mg/kg) treatment markedly increased the catalase levels as compared to the vehicle control group. Therefore, GSPE treatment has shown dose dependent increase in catalase levels. The maximum increase in the catalase levels was shown by GSPE (100 mg/kg) treatment group.

Effect of various doses of grape seed proanthocyanidin extract on glutathione levels

The levels of glutathione were significantly decreased in the vehicle control group as compared to the normal control group. These levels were significantly and dose dependently increased on GSPE (50 mg/kg) and GSPE (100 mg/kg) treatment group. GSPE (100 mg/kg) treatment group increased the GSH levels to a similar extent as that of standard drug treatment group. GSH levels got recovered on GSPE treatment.

Pylorus ligation model for gastric ulcer

Effect of various doses of grape seed proanthocyanidin extract (GSPE) on gastric volume and gastric acid secretion in pylorus ligation model of gastric ulcer: In pylorus ligated vehicle control group gastric vol. was significantly decreased as compared to the normal control group shown in Figure 4a. Administration of GSPE (50 mg/kg and 100 mg/kg, p.o; 4 days) significantly increased the gastric volume as compared to vehicle control rats.

The effect of GSPE (100 mg/kg) was not statistically significant from that of standard drug Omeprazole (50 mg/kg, p.o) treatment. A marked increase in the levels of gastric acidity was seen in pylorus ligated vehicle control rats Figure 4b. Repeated administration of GSPE (50 mg/kg) decreased the gastric acidity, and was more significant on treatment with GSPE (100 mg/kg) dose. Further on treatment with omeprazole (50 mg/kg) significantly decreased the gastric acidity levels. Gastric acidity levels in pylorus ligation model are depicted in Figure 3b.

Percentage protection shown by various doses of gspe on experimental models of gastric ulcer (Figure 5): Percentage protection shown by indomethacin induced model of ulcer was 15.31 on GSPE (50 mg/kg) treatment, 21.85 on GSPE (100 mg/kg) treatment and 27.35 on Famotidine (40 mg/kg) drug treatment.

In case of pylorus ligation induced gastric ulcer % protection was 9.89 in GSPE (50 mg/kg), 24.14 in GSPE (100 mg/kg) and 37.30 in Omeprazole (50 mg/kg) treatment group. In stress induced model of gastric ulcer % protection was 11.42, 25.98 and 43.52 in the respective groups.

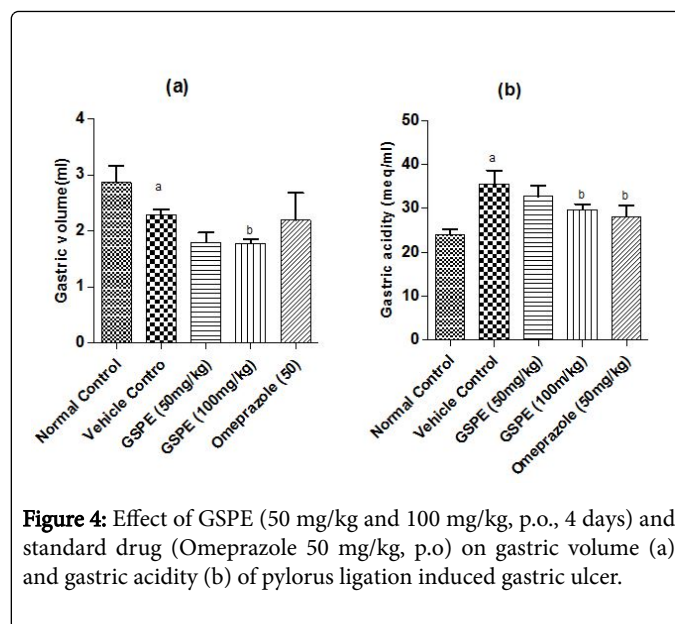


Figure 4: Effect of GSPE (50 mg/kg and 100 mg/kg, p.o., 4 days) and standard drug (Omeprazole 50 mg/kg, p.o) on gastric volume (a) and gastric acidity (b) of pylorus ligation induced gastric ulcer.

Values are expressed as mean \pm S.D; n=6; a denotes for $P < 0.05$ vs. normal control, b denotes for $P < 0.05$ vs. vehicle control. Repeated drug treatment of GSPE (50 mg/kg or 100 mg/kg) and standard control drug (Omeprazole 50 mg/kg) was given. GSPE: Grape Seed Proanthocyanidin Extract treated rats.

Percentage protection shown by various doses of gspe on experimental models of gastric ulcer

Values are expressed in mean \pm S.D; n=6; a denotes for $P < 0.05$ vs. vehicle control, b denotes for $P < 0.05$ vs. vehicle control, c denotes for $P < 0.05$ vs. vehicle control. Repeated drug treatment of GSPE (50 mg/kg and 100 mg/kg, p.o; 4 days) and Standard control drug control drug (Omeprazole 50 mg/kg or Famotidine 40 mg/kg, p.o) was given. GSPE: Grape Seed Proanthocyanidin Extract treated rats.

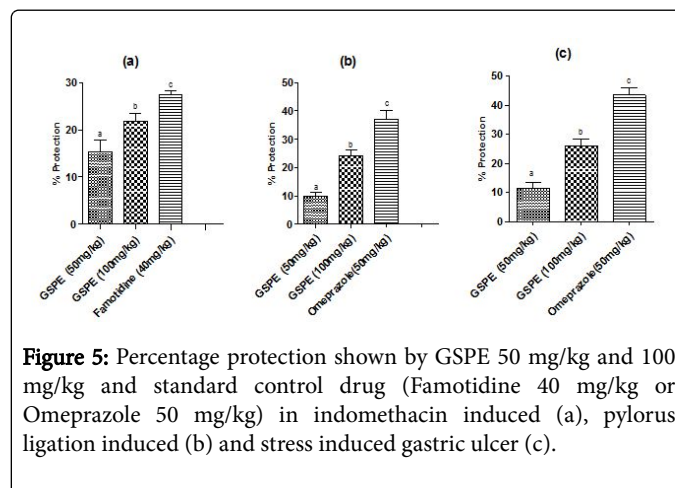


Figure 5: Percentage protection shown by GSPE 50 mg/kg and 100 mg/kg and standard control drug (Famotidine 40 mg/kg or Omeprazole 50 mg/kg) in indomethacin induced (a), pylorus ligation induced (b) and stress induced gastric ulcer (c).

Discussion

Medicinal plants are among the most attractive sources of new drugs, and have been shown to produce promising results for the treatment of gastric ulcer [31]. Grape seed proanthocyanidin extract (GSPE) is safe for consumption as its LD50 was found to be higher

than 5000 mg/kg, when administered orally to fasted male and female albino rats. In addition, 2000 mg/kg body weight was found to be a no-observed-effect-level (NOEL) for systemic toxicity [6,32]. A large number of herbal extracts are used in folk medicine to treat various types of digestive disorders [19,33]. Grape seed proanthocyanidin extract is an herbal extract containing 95% proanthocyanidin having potent anti-oxidant properties [34].

Indomethacin, a NSAID induced ulcer in rat is a widely used experimental model to assess the pathophysiology of ulcer induced by NSAIDs and screening of gastroprotective agents. It induces gastric lesions through a number of mechanisms which includes inhibition of prostaglandin synthesis, increased expression of interleukin-1 (IL-1), generation of reactive oxygen species (ROS), and induction of apoptosis [35,36]. Although various mechanisms are involved in the pathogenesis of pyloric ligation induced peptic ulcer, gastric acid secretion and accumulation are thought to be the most important factors. In addition it is well established that various antisecretory agents, such as H₂ receptor antagonists and proton pump inhibitors prevent gastric lesions [37].

Stress can arise from prolonged anxiety, tension, and emotion, severe physical discomfort, haemorrhage and surgical shock, burns and trauma, thereby resulting in severe gastric ulceration. The mechanism of gastric ulceration is poorly understood. Recently research has shown that cold stress causes severe haemorrhage ulcer through derangement of the mucosal antioxidant enzyme such as super oxide, dismutase and peroxides. NSAIDs are known to induce gastric ulcer not only by denaturing mucous glycoproteins but also by free radical formation [38]. Stress causes an ischemic condition in the gastric mucosa by the activation of the parasympathetic and sympathetic nervous systems resulting in vasoconstriction, which in turn causes free radical generation.

Further, stress has also been found to inactivate mucosal prostaglandin synthase by accumulating H₂O₂, which in turn inhibits the synthesis of prostaglandins known to favour the generation of reactive oxygen species [39]. Cold restraint stress model is commonly used for evaluating drugs having gastroprotective activity by virtue of its anti-stress effect. Pylorus ligation method is used for evaluating drugs which are effective by decreasing acid and pepsin secretion [22]. Thus it can be assumed from above observation that the gastroprotective activity of GSPE is unique as compared to most conventional anti-ulcer drugs as it is not based mainly on acid and pepsin secretion, or acid does not play major role in stress related mucosal disorder.

The present study confirms observations in our earlier study on gastro-protective potential of grape seed proanthocyanidin extract in NSAIDs-induced gastric ulcer in wistar rats [40]. In the present study preliminary results showed that GSPE single dose treatment did not show any significant effect in pylorus ligation-induced ulcer model (data not shown). However, repeated pre-treatment for 4 days in all the models of gastric ulcer showed positive results. Administration of investigated extract led to significant decrease in MDA levels, in both GSPE 50 mg/kg and GSPE 100 mg/kg treated groups. Standard control drug treatment showed even lesser decrease in MDA units as compared to the GSPE 100 mg/kg treatment group. Similar results were obtained in case nitrite/nitrate and myeloperoxidase levels in each model.

Sulfhydryl compounds such as GSH play an important role in scavenging reactive oxygen species and have been implicated to protect

the gastric mucosa [41]. The treatment of experimental animals with doses of 50 mg/kg and 100 mg/kg of Grape seed proanthocyanidin extract produced a moderate, but significant and dose-dependent increase in activity of GSH in gastric mucosa. Increase in GSH shows that extract was able to diminish the excessive production of free radicals provoked by indomethacin and pylorus ligation. Furthermore, the obtained results revealed that pylorus ligation; indomethacin and stress models also significantly decreased the anti-oxidant levels like catalase, SOD and GSH. Antioxidant levels were lowered in vehicle treated control rats in each model and these levels were significantly increased in GSPE treated groups in each model.

Several studies have demonstrated that nitrite/nitrate production is involved in the genesis of intestinal inflammation and that its inhibition can reduce inflammatory damage [42,43]. The increase in nitrite/nitrate in ulceration models was associated with an elevated expression of iNOS and NO production [44,45]. NO, could rapidly interact with oxygen free radicals to yield a variety of cytotoxic nitrogen species that induce lipid peroxidation and other cellular oxidative stress [46]. The present study showed that GSPE remarkably decrease the elevated levels of nitrite/nitrate. This may be due to GSPE induced decrease in iNOS and NO activity in gastric mucosa [47]. In similar study anti-ulcer activities of grape seed extracts GSE-1 (with low flavanol content), GSE-2 (with high flavanol content) and procyanidins at a dose of 200 mg/kg shown to inhibit the stomach mucosal injury induced by ethanol. The observed protection by free radical scavenging activity and the defence action of procyanidins covering the stomach surface by strong ability to bind protein.

It is known that a variety of cytokines such as interleukin -1 β , interleukin-8 and tumour necrosis factor α are expressed in ulcerated mucosa and they exaggerated the inflammatory response. Increased H₂O₂ production has also been reported to increase the production of IL-17 and TNF- α [48,49]. GSPE reduces the production of intracellular H₂O₂ by T-cell-receptor-stimulated CD4+ splenocytes [6,50-55]. GSPE induce decrease in TNBS (2,4,6-trinitrobenzoic acid) was associated with decreased production of pro-inflammatory cytokine IL-1 β in the colon in rats [7]. Similarly GSPE might suppress the production of these pro-inflammatory cytokine and subsequent oxido-nitrostatic stresses.

Taken as a whole, these data provide evidence that reactive oxygen species play an important role in the pathogenesis of gastric ulceration [56-58]. Thus, scavenging free radicals, reducing the depletion of endogenous anti-oxidant enzymes, and counteracting GSH deficiency could be the target for therapeutic intervention in gastric ulcer [59]. In addition, GSPE (50 mg/kg and 100 mg/kg) treatment facilitated the recovery of damaged mucosal tissue [60]. These studies have implicated the generation of oxygen-derived free radicals and lipid peroxidation as one of the most important mechanisms involved in the pathogenesis of gastric ulcer [61-63]. Therefore, the grape seed proanthocyanidin might exert its activity in one or more aforementioned assays. This provides favourable results showing gastroprotective potential of GSPE in treatment of disorders caused by oxidative stress [64,65].

Conclusion

On the basis of above data, it may be concluded that various doses of GSPE have shown anti-ulcer effect in wistar rats. Also, GSPE treatment produced significant gastro-protective effects against indomethacin-induced, pylorus ligation induced and stress induced gastric damage. Hence, the GSPE higher doses are a better therapeutic

option for the treatment of gastric ulcer in terms of better therapeutic health benefits and lesser side effects.

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