

Bangladesh Journal of Pharmacology

Volume: 10; Number 4; Year 2015



Cite this article as: Varadharajan V, Shanmugam S, Ramaswamy A. Thrombolytic activity of the ethanolic extract of *Vitis vinifera* seeds. Bangladesh J Pharmacol. 2015; 10: 964-65.



Letter to the Editor

Thrombolytic activity of the ethanolic extract of *Vitis vinifera* seeds

Sir,

Grape and its seed are rich in some important vitamins, minerals, proteins, carbohydrates and a variety of polyphenolic compounds (Nowshehri et al., 2015). Grape seed extract (GSE) is derived from whole grape seed and it is used as a nutraceutical agent (Zhu et al., 2015). Apart from excellent antioxidant potential (Jayaprakasha et al., 2001), they have antimicrobial (effective against Gram positive bacteria) (Cvetnić and Knežević, 2004) and anti-cancerous activities (Shrotriya et al., 2012). The major aim of this research was to determine the thrombolytic property of ethanolic extract of grape seeds from Indian gulabi grapes (*Vitis vinifera*) using *in vitro* clot lysis method.

For grape seed extract preparation, the extraction was carried out using a solvent mixture of ethanol and water in the ratio of 80:20 (v/v). 0.4 g of the grape seed powder was extracted with 20 mL of solvent at 45°C for 2 hours in a shaking incubator under shaking condition

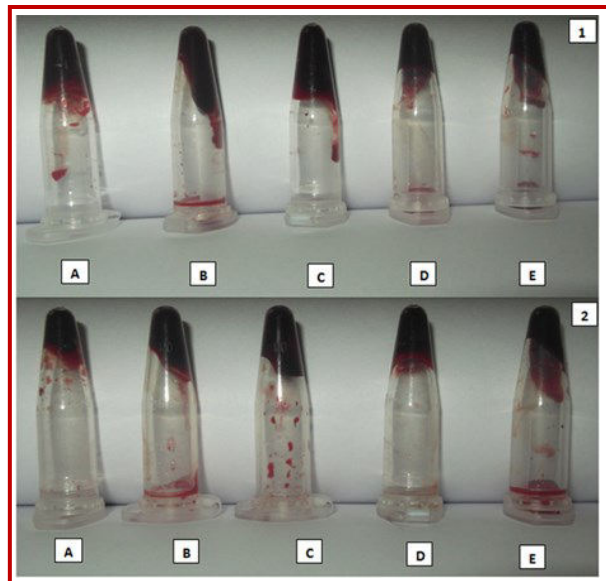


Figure 1: Clot lysis of blood sample of positive and negative control. Tubes with control clot (1) and tubes with remnants of clot after lysis (2). Tube A - D was lysed by four different concentrations of grape seed extract with decreasing order. Tube E was a negative control clot to which water was added

(150 rpm). After incubation, the mixture was centrifuged at 5,000 xg for 10 min and decanted subsequently. The pellet was re-extracted for 2 hours and the supernatants were combined. The extract was concentrated using rotary evaporator (R/150/01 SUPERVAC, Superfit, India) and the concentrated extract was dried under shade condition. The solid residue left behind was collected and washed thrice with chloroform (5,000 x g for 5 min) to remove lipid materials present in the extract. The pellet was again dried under room temperature and the powder obtained was stored at +4°C. For further thrombolytic experiments, the extract solution was prepared by dissolving 0.5 g of grape seed extract in 50 mL of 80% ethanol (10 mg/mL).

For the clot lysis experiments, the samples were prepared on the basis of the following protocol. To the lyophilized streptokinase containing vial, 5 mL of sterile distilled water was added and mixed gently to prepare the stock solution. One hundred microliter (30,000 I.U.) from stock solution was used for *in vitro* clot lysis. The blood sample (4 mL each) was collected from healthy volunteers (n=20) without the history of oral contraceptive or anticoagulant therapy based on the guidelines provided by the Institutional ethical committee of K. S. Rangasamy College of Technology, Tiruchengode. A written consent was taken from all the volunteers.

The thrombolytic property of grape seed extract was determined using *in vitro* clot lysis method (Prasad et al., 2006). The whole venous blood (500 μ L) from each individual was allowed to form clots in a pre-weighed sterile microfuge tubes at 37°C for 45 min. The serum was removed and the clots were weighed. After lysis by 100 μ L of grape seed extract at 37°C for 90 min, the fluid obtained was removed and the remaining clots were again weighed along with the microfuge tubes. Streptokinase (100 μ L) and distilled water were used as positive and negative thrombolytic controls. The difference obtained in weight taken before and after blood clot lysis gives %clot lysis, denoted by η_{Lysis} %, was expressed as:

$$\eta_{Lysis}\% = \left(\frac{\text{Weight of clot before lysis} - \text{Weight of clot after lysis}}{\text{Weight of clot before lysis}} \right) * 100$$

The statistical analysis for the clot lysis test was made using StatPlus Professional ver. 5.8.4.3 (AnalystSoft,

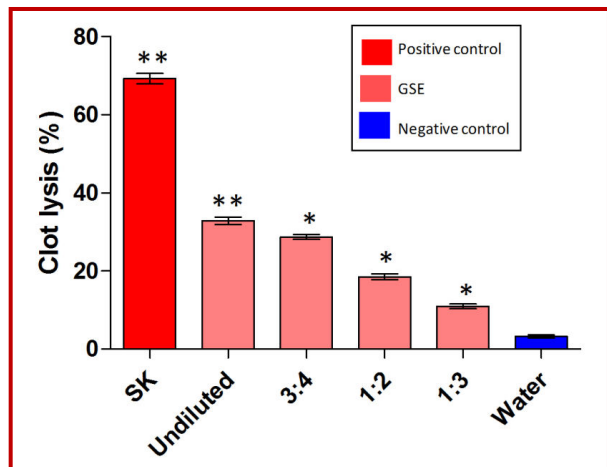


Figure 2: %Clot lysis of normal subjects by various dilutions of grape seed extract. *indicates $p < 0.05$ and **indicates $p < 0.01$

Inc., Vancouver, Canada). The mean %clot lysis of streptokinase and different concentrations of grape seed extract was compared with distilled water using one-way ANOVA with Tukey HSD test. All results were expressed as mean \pm standard deviation.

From the clot lysis experiments, negligible clot lysis of $3.0 \pm 0.2\%$ was observed in the control clot added with $100 \mu\text{L}$ of water. Significant clot lysis was seen on the tubes containing different dilutions of grape seed extract (Figure 1 and 2). The standard streptokinase showed the highest clot lysis of $69.1 \pm 1.3\%$ ($p < 0.001$). Maximum clot lysis was observed in clot treated with undiluted ($123.9 \mu\text{g}$) grape seed extract ($p < 0.001$) and other three dilutions ($92.9 \mu\text{g}$, $61.9 \mu\text{g}$ and $41.3 \mu\text{g}$) of grape seed extract also showed significant clot lysis ($p < 0.05$) when compared with the control. The clots treated with four different dilutions of grape seed extract, i.e., undiluted, 3:4, 1:2 and 1:3 showed clot lysis of 33.3 ± 1.3 , 28.6 ± 0.9 , 18.1 ± 1.2 and $10.9 \pm 0.9\%$. The

percentages of clot lysis by different dilutions of grape seed extract ranges from 11–33%.

In conclusion, grape seed extract has significant thrombolytic activity.

Venkatramanan Varadharajan, Sethupathi Shanmugam and Arulvel Ramaswamy

Department of Biotechnology, K. S. Rangasamy College of Technology, Tiruchengode 637-215, Tamilnadu, India.

Corresponding author:

email: mail4venkat1992@gmail.com ; Tel: +917667726062, Fax: 04288 2471471

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