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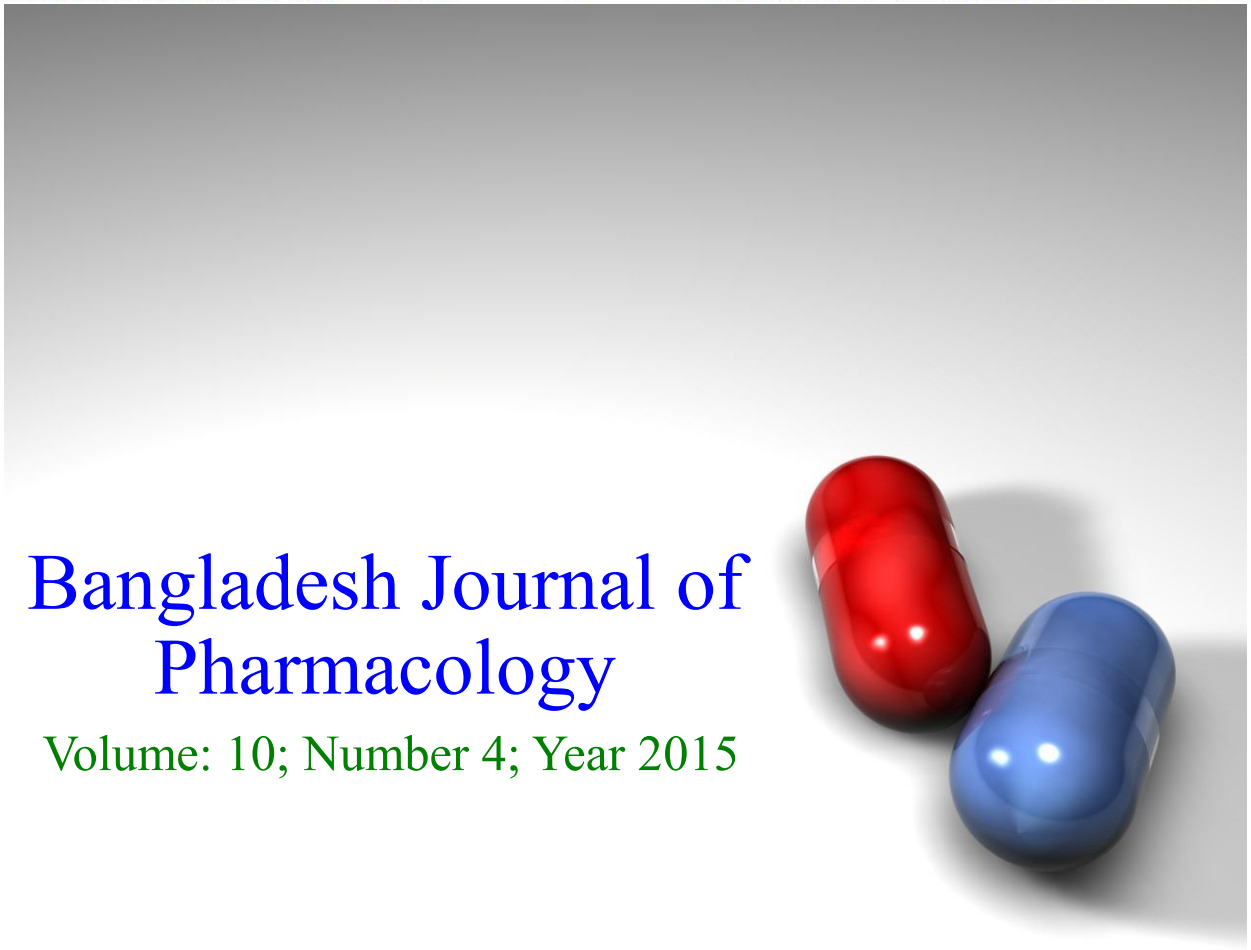

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# Letter to the Editor

## 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 (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).

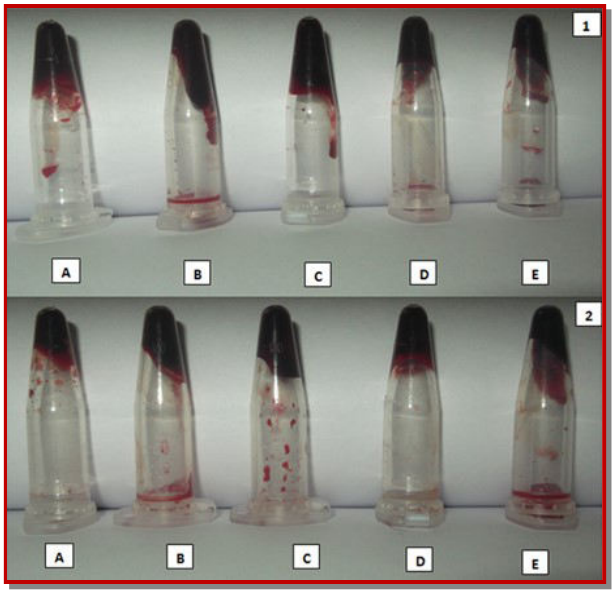


Figure 1: Clot lysis of blood sample of positive and negative control. Tubes with control clot (1) and tubes with remnants of clot after lysis (2). Tube A – D was lysed by four different concentrations of grape seed extract with decreasing order. Tube E was a negative control clot to which water was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

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 µL) 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 µL 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 µL) 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 $\eta_{Lysis}$ %, 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,

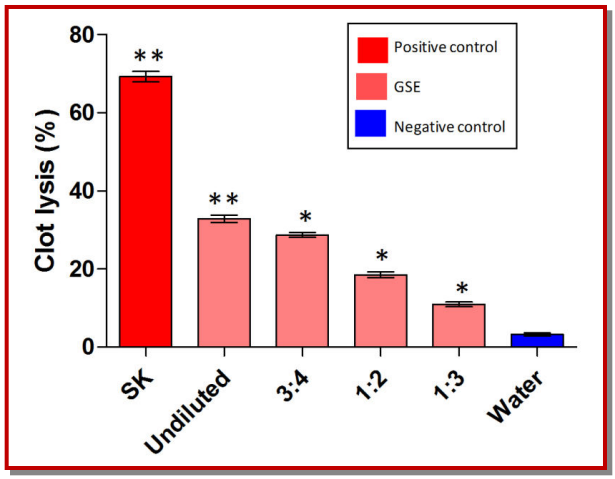


Figure 2: %Clot lysis of normal subjects by various dilutions of grape seed extract. *indicates p<0.05 and **indicates p<0.01

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 ± standard deviation.

From the clot lysis experiments, negligible clot lysis of 3.0 ± 0.2% was observed in the control clot added with 100 µL 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 ± 1.3% ($p<0.001$). Maximum clot lysis was observed in clot treated with undiluted (123.9 µg) grape seed extract ($p<0.001$) and other three dilutions (92.9 µg, 61.9 µg and 41.3 µg) 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 ± 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.

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## References

Cvetnić Z, Knežević SV. Antimicrobial activity of grape fruit seed and pulp ethanolic extract. Acta Pharm. 2004; 54: 243–50.

Jayaprakasha GK, Singh RP, Sakariah KK. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. Food Chem. 2001; 73: 285–90.

Nowshehri JA, Bhat ZA, Shah MY. Blessings in disguise: Bio-functional benefits of grape seed extracts. Food Res Int. 2015.

Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Development of an in vitro model to study clot lysis activity of thrombolytic drugs. Thrombosis J. 2006; 4: 14.

Shrotriya S, Deep G, Gu M, Kaur M, Jain AK, Inturi S, Agarwal R, Agarwal C. Generation of reactive oxygen species by grape seed extract causes irreparable DNA damage leading to G2/M arrest and apoptosis selectively in head and neck squamous cell carcinoma cells. Carcinogenesis 2012; 33: 848–58.

Zhu F, Du B, Li J. Recent advance on the antitumor and antioxidant activity of grape seed extracts. Int J Wine Res. 2015; 7: 63–67.