Journal of Life Science 2006 Vol. 16. No. 2. 357~359

- 단 보 -

Studies on Fibrinolytic Activity of Jujube (*Zizyphus mauritiana*) Extract

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Received January 10, 2006 / Accepted February 14, 2006

Crude extract prepared from jujube (*Zizyphus mauritiana*) possseses fibrinolytic activity hydrolyzing fibrin. A clear transparent region is observed where fibrin is hydrolyzed, and its diameter increased as the added jujube extract increased. The fibrinolytic activity of jujube extract seems to be heat and acid stable since its activity was retained after heat treatment at 100℃ for 10 min or an acid treatment by incubating at pH 2.0, 3.0, and 4.0 for 3 hours. But the jujube extract dialyzed with a cellulose membrane with molecular weight cutoff of 12,000 and 2,000 lost its activity, which suggests the fibrinolytic activity might be compounds of low molecular weight.

Key words – Jujube extract, fibrinolytic activity, stability

Fibrin is the primary protein component of a blood clot. Blood clots are formed by the conversion of fibrinogen into fibrin via the proteolytic action of thrombin and subsequently, the formation of insoluble fibrin clots[1]. The hydrolysis of fibrin is also known as fibrinolysis. Fibrin clot formation and fibrinolysis are normally well balanced in biological systems[2]. Disorders of blood clotting and fibrinolysis are serious medical problems[3]. When accumulated in the blood vessels, fibrin causes thrombosis leading to myocardial infarction and other cardiovascular diseases[4].

Jujube (*Zizyphus mauritiana*) has been commonly used as a traditional Korean medicine and also commonly used as food for thousands of years. It is mainly distributed in the subtropical regions of Asia[5]. Recently, antithrombotic, anticoagulant and thrombolytic reagents from various sources have been investigated. However, few studies have been conducted on fibrinolytic activity in jujube. The aim of the present study is to investigate the fibrinolytic activity and its stability in jujube.

Materials and Methods

Materials

Jujube (*Zizyphus mauritiana*) was purchased from a local market in Busan, Korea. Fibrinogen and thrombin were obtained from Sigma Chemical Co.

Preparation of jujube extract

Jujube (100 g) was homogenized with 200 ml of a 0.1 M Tris-HCl buffer at pH 7.6 for 3 min. The homogenate was centrifuged at 15,000×*g* for 20 min, and the supernatant was collected. All steps were carried out at 4℃.

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Measurement of fibrinolytic activity

Fibrinolytic activity was determined by a slightly modified method of Astrup and Mullertz[6] as follows. A total of 9 ml of 0.06 g fibrinogen in 0.1 M Tris-HCl buffer (pH 7.6) was poured into a 10 cm Petri dish and then clotted by the addition of 1 ml of thrombin (20 unit) in the same buffer. The clot was allowed to stand for 1 hour at 37℃. Then 50ul of sample solution was carefully placed on the paper disk (8 mm diameter) of plate. The plate was incubated for 30 minutes at 37℃ and the diameter of the lytic circle was measured. One unit of fibrinolytic activity was defined as 1 mm^2 of a clear transparent region per 1 min.

Results and Discussion

Fig. 1 shows the effect of added jujube extract amount on fibrinolytic activity. In the fibrin plate method, a clear transparent region was observed where fibrin was hydrolyzed, and its diameter increased as the added jujube extract amount increased. The effect of heat and acid on fibrinolytic activity of jujube is shown in Table 1. The jujube extract subjected to a heat treatment at 100℃ for 10 min or to an acid treatment at pH 2.0, 3.0, and 4.0 for 3 hours still retained fibrinolytic activity. But the jujube extract dialyzed with a cellulose membrane (Sigma Chemical Co.) with molecular weight cutoff of 12,000 or 2,000 lost its activity(Table 2).

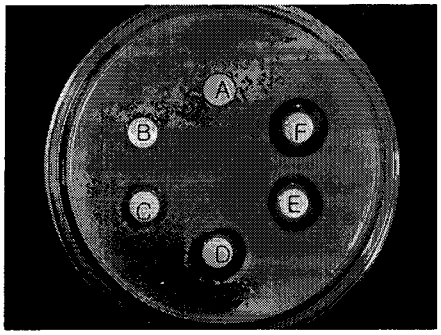

Fig. 1 The effect of added jujube extract amount on fibrinolytic activity.
A : Control
B : Addition of 10ul of jujube extract
C : Addition of 20ul of jujube extract
D : Addition of 30ul of jujube extract
E : Addition of 40ul of jujube extract
F : Addition of 50ul of jujube extract

Table 1. The heat and acid stability of jujube extract on fibrinolytic activity

Condition		Area of lytic circle (cm^2)
Control		1.75±0.04
After heat treatment		1.52±0.05
After acid treatment at	pH 2.0	1.42±0.03
	pH 3.0	1.43±0.02
	pH 4.0	1.45±0.02

The jujube extract was treated at 100℃ for 10 min or at pH 2.0, 3.0 and 4.0 for 3 hours. The plate was incubated for 30 minutes at 37℃ and the area of the lytic circle was measured. Data are mean±SD values(n=3).

Table. 2 The heat and acid stability of jujube extract on fibrinolytic activity

Condition	Area of lytic circle (cm^2)
Control	1.75±0.04
After dialysis	ND*
Ultrafiltration	1.73±0.01

ND* : Not detected
The jujube extract was dialyzed with a cellulose membrane with molecular weight cutoff of 12,000. Also jujube extract was ultrafiltrated with molecular weight cutoff 10,000. Centricon YM-10(Millipore Korea Co. Ltd) was used. The plate was incubated for 30 minutes at 37℃ and the area of the lytic circle was measured. Data are mean±SD values(n=3).

Otherwise, the jujube extract ultrafiltrated with molecular weight cutoff of 10,000 retained fibrinolytic activity. This finding suggests that the ingredients responsible for fibrinolytic activity in jujube have low molecular weight.

The fibrinolytic activity of crude extracts from mantis egg case has been reported previously[7]. Recently many food derived fibrinolytic enzymes have been found in various traditional Asian foods[8]. Korean traditional anecdotes suggest that mushrooms can be and have been used in the treatment and prevention of thrombosis[9]. Nonaka also have described fibrinolytic activity occurring in some edible mushrooms[10]. Bordia reported that garlic reduced significantly total serum cholesterol and triglyceride, and increased significantly HDL-cholesterol and fibrinolytic activity[11]. Further study is needed to identify the structure of the active components for the fibrinolytic activity in jujube.

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초록 : 대추 추출물의 fibrinolytic activity에 관한 연구

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대추 추출물에서 fibrin을 가수분해 하는 생리활성 물질이 존재함을 알고 이 추출물의 열과 산에 대한 안정성 및 그 특징을 조사 하였다. Fibrin plate method 를 사용하여 측정된 fibrinolytic activity 는 대추 추출물의 첨가량이 증가할수록 높았으며, 이 추출물은 100℃ 에서 10분간 열처리시에 안정한것으로 나타났다. 또한 pH 2.0, 3.0, 4.0 에서 3시간 보관후 fibrinolytic activity 를 측정한 결과 control의 80% 이상의 활성을 나타내었다. 한편 투석후 (분자량 12,000) 에는 활성이 나타나지 않았으며, 이는 대추에서 추출한 fibrin 분해 생리활성 물질이 분자량이 낮은 저분자 물질임을 알 수 있었다.