Short Communication

Journal of Apiculture 33(4) : 303~306 (2018)
DOI: 10.17519/apiculture.2018.11.33.4.303

Anti-thrombosis Activity of Drone *Apis mellifera* Pupae Extracts

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(Received 16 November 2018; Revised 28 November 2018; Accepted 29 November 2018)

Abstract

Drones of honeybee (*Apis mellifera*) have been regarded as a useful value only when mating with queen bee. However, the drone pupae have been reported to be nutritionally valuable, and a potential beekeeping product. In this study, drone pupae extracted with 5% acetic acid were used to measure anti-thrombosis related fibrinolytic activity using Strup and Mullertz fibrin plate method. As a result, the drone pupae extract showed higher effect of fibrinolytic activity(clear zone diameter 20.83mm) compared to the human plasmin (clear zone diameter 12.93mm) used as a positive control. It was suggested that the extract of drone pupae can be developed as a functional material helping prevention or treatment of various vascular diseases.

Key words: *Apis mellifera*, Drone pupae, Anti-thrombosis, Fibrinolysis

INTRODUCTION

Currently, cardiovascular diseases are rapidly increasing in Korea due to westernized food habits and population aging (Lee *et al*., 1988; Park *et al*., 2007) In the past, people have been eating mainly vegetables, but nowadays it changed into eating mostly meats (Lee *et al*., 1988). These changes in dietary pattern cause various vascular diseases. Thrombosis, a typical symptom of blood-related diseases, is an abnormal increase in blood clots due to endogenous or exogenous factors that interfere with the flow of blood in the vessel (Hsieh, 1997; Butenas and Mann 2002). The over-production of blood clot leads to serious problems such as blood circulation disorder, blood flow rate reduction, and blood viscosity abnormality. The over-formation of thrombus in heart or brain may cause critical diseases including myocardial infarction, stroke, and pulmonary infarction (Carpiello *et al*., 1996; Butenas and Mann, 2002; Song *et al*., 2002).

The production of thrombus is caused by the abnormality of blood coagulation. In general, blood coagulation is the process by which blood turns from liquid to gel and forms blood clotting (David *et al*., 2009). In blood coagulation, activation of thrombin is the most important and final response. The activated thrombin changes fibrin into fibrinogen, an insoluble lattice polymer, that becomes blood clot and acts as a risk factor of vascular diseases.

Traditionally, herbal medicines or foods have been used as medicinal resources. However, recently, interests in insects as cure have also increased due to diversity and availability. Insects are found everywhere in the world with 1.8 million known species, in which more than 12,000 species live in Korea (Paek *et al*., 2010). Insect consumption is not a new concept and their pharmacological effects were also reported through various ancient documents. In fact, more than 100 kinds of insects have been used for

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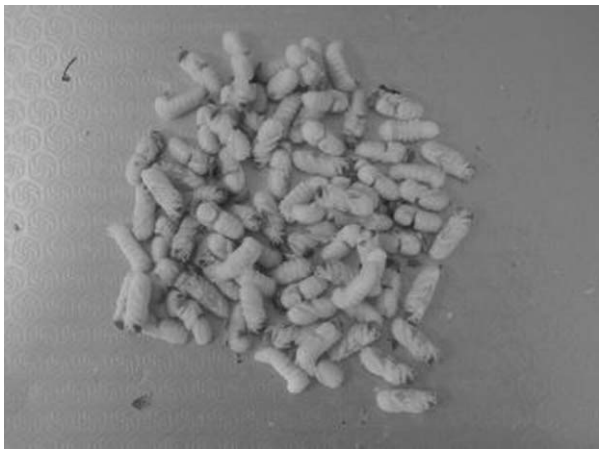

Fig. 1. The raw material of drone pupae of honeybee (*A. mellifera*).

medicinal purposes in Korea (Heo *et al*., 2006). Among them, drone pupae of honeybees have been traditionally used as food for immune enhancement in many countries for a long time (Winston 1991). Moreover, the pupae of drones have also been evaluated as a food source having high nutritional value and potential functional materials (Kim *et al*., 2018). The aqueous extract of honeybee (*Apis mellifera* L.) larvae and pupae had been reported having the anti-thrombotic effects (Kim *et al*., 2013). However, it is not clear whether the effects were caused by honeybee drone or worker.

This study tested the fibrinolytic activity using the drone pupae extract to investigate its potential as anti-thrombosis agent.

MATERIALS AND METHODS

Drone pupae samples

The drone pupae of honeybee (*A. mellifera*) were obtained from 3 regions in Korea namely Cheongyang, Gimje, and Changnyeong. The drone pupae were colonized by using the special drone hives and harvested on the 21th days (Fig. 1). All samples were immediately stored at −20°C after collection.

Extraction

The stored drone pupae were homogenized by grinding after freeze drying. The drone pupae powder (1g) were extracted with 10mL of 5% acetic acid using a stirrer in 4°C for 18h and then filtered using a 0.45μM filler (Whatman, Simga-Aldrich, MO, USA). Each extract was dispensed in a 700~800μL into 1.5mL tubes and then concentrated using a rotary vacuum evaporator (CVC-3100, Eyela, Japan) for 24 hours. The concentrated extract was diluted in 0.01% acetic acid as suitable concentration for experiment.

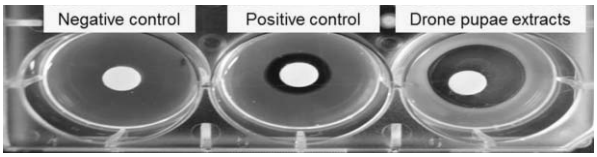

Fig. 2. The fibrinolytic activity of the drone pupae extract compared with control groups. Negative control is 5% acetic acid. Positive control is human plasmin (20U/mL). Concentration of drone pupae extracts is 0.12mg/mL in 5% acetic acid.

Anti-thrombosis activity

Anti-thrombosis activity was measured using Astrup and Mullertz fibrin plate method (Astrup and Mullertz 1952). 20U/mL Thrombin (Sigma-Aldrich, USA) and 0.5% (w/v) concentration of fibrinogen (Sigma-Aldrich, USA) were used in phosphate-buffered saline (PBS). 100μL of thrombin and 1mL of fibrinogen were mixed in a 6-well plate to prevent the formation of air bubbles. After, plates were completely sealed and put in a drying oven at 37°C for 1 hour to form a fibrin membrane. Fibrin plate method (Astrup and Mullertz, 1952) was used to measure the area where the fibrin hydrolyzed and become transparent. The relative area of fibrin dissolved by sample and plasmin were measured. The experiments were repeated with three times. Human plasmin (20U/mL, Simga-Aldrich) was used as a positive control while 5% acetic acid was used as a negative control.

RESULTS AND DISCUSSION

Fibrinolytic activity

In Fig. 2, the drone pupae extract (0.12mg/mL) was

Table 1. The numerical values of the fibrinolytic activity of drone pupae extract. Negative control is 5% acetic acid. Positive control is human plasmin (20U/mL). Concentration of drone pupae extracts is 0.12mg/mL in 5% acetic acid.

Treatment	The diameter of the dissolved ring (mm)		
	Negative control	Positive Control	Drone pupae extract
Mean±SD	ND	12.93±0.6	20.83±0.3

*ND : Not detected.

more effective in fibrinolytic activity than the human plasmin used as a positive control, thus its extract was excellent in thrombolytic activity. The results are shown in Fig. 2, and the numerical values are shown in Table 1.

Thrombolytic agents are very important because thrombus accumulation in blood vessels causes various vascular diseases. Currently, the kinds of available thrombolytic agents are tissue plasminogen activator (t-PA), urokinase, streptokinase, and staphylokinase. Of these, only urokinase is possible to be administered orally (Weitz and Crowther 2002; Choi *et al*., 2013). However, these thrombolytic agents are expensive, low thrombosis selectivity, and have side effects such as systemic hemorrhage and allergic reactions. For this reason, there is a growing need for economical and safe thrombolytic agents. Drone pupae extract had a sufficient value as a thrombolytic agent since it can be orally administered and it had excellent fibrinolytic activity. Moreover, it is possible to develop drone pupae extract not only as a medicine but also as a health food supplement.

The whole fibrinolytic activity test was carried out in two replicated experiments. In Table 1, the upper treatment was the first treatment and the lower treatment was the second treatment. The diameter of the ring dissolved in the positive control was 12.93mm and the diameter of the drone pupae extract was 20.83mm. Based on these results, it was confirmed that the extract of the pupae had better anti-thrombotic efficacy than human plasmin.

Commonly, fibrinolysis is directly related to anti-thrombosis. Thrombosis is formed when fibrinogen is converted to fibrin by thrombin. Blood clots accumulate in blood vessels and interfere with blood circulation by blocking nutrients and oxygen supply to each tissue resulting in an increase in blood pressure (Butenas and Mann, 2002). Therefore, not only inhibiting the production of fibrin but also dissolving the generated thrombus contributes to improvement of vascular diseases (Ryu *et al*., 2008).

CONCLUSION

Drone honeybees have been regarded as worthless for a long time because their only function is mating with queen bee. However, drone pupae have been reported to be nutritionally valuable, and potential beekeeping product. This study suggested that drone pupae extract can be developed as a functional material capable of preventing or treating various vascular diseases such as arteriosclerosis and stroke.

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea (Project No. : PJ01314201).

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