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นิพนธ์ต้นฉบับ

Effects of *Lentinus edodes* extracts on platelet aggregation

ผลของสารสกัดจากเห็ดหอม *(Lentinus edodes)* ต่อการจับตัวของเกร็ดเลือด

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บทคัดย่อ : **เทอดพงษ์ ตรีรัตน์, รัชนี สุวรรณรักษ์ และ สุทธพรรณ ตรีรัตน์. 2535. ผลของสารสกัดจากเห็ดหอม *(Lentinus edodes)* ต่อการจับตัวของเกร็ดเลือด. วารสารวิจัยวิทยาศาสตร์การแพทย์ 6(1): 1-6**

การศึกษาผลของสารสกัดจากเห็ดหอมที่เพาะด้วยถุงขี้เลื่อยต่อการจับตัวของเกร็ดเลือด พบว่าสารสกัดจากเห็ดหอมในตัวทำละลาย เฮกเซน เมทธิลลีนคลอไรด์ เอทธานอล และน้ำ สามารถยับยั้งการจับตัวของเกร็ดเลือด ซึ่งถูกกระตุ้นโดย เอดีพี และคอลลาเจนได้ ผลจากการศึกษาในครั้งนี้ จะเป็นประโยชน์ในการศึกษาวิจัยเกี่ยวกับการป้องกันการอุดตันในท่อเลือด เนื่องจากการจับตัวของเกร็ดเลือดต่อไป

คำสำคัญ : *เห็ดหอม; เกร็ดเลือด; การจับตัว*

Abstract : **Terdphong Triratana, Rachanee Suwanuraks, and Suthaphun Triratana. 1992. Effects of *Lentinus edodes* extracts on platelet aggregation. Thai J Hlth Resch 6(1): 1-6**

*According to the studies on the effect of extracts of **Lentinus edodes** obtained from sawdust cultivation on platelet function, it was revealed that crude extracts in hexane, methylene chloride, ethanol and water could inhibit platelet aggregation induced by ADP and Collagen. The finding of substances in **L. edodes** might be useful for further studies on the prevention of thromboembolic disease in human.*

Key words: ***Lentinus edodes; platelet; aggregation***

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INTRODUCTION

In Thailand, a number of plants and mushrooms have been implicated in traditional system of medicine. The usage of these herbs is based upon the folklore knowledge. Therefore, scientific approaches are essentially carried on in order to select the suitable plants and to ensure in the application of these useful herbs.

Lentinus edodes or shiitake, is very popular among Asians because of its special taste, odor and medicinal properties. Many Japanese investigators have reported the presence of various substances in shiitake that have some medicinal properties including decreasing blood cholesterol (Suzuki and Oshima, 1976), antiviral (Yamamura and Cochran, 1976) and antitumor agent (Hamuro *et al.*, 1976; Taguchi *et al.*, 1985). However, the information on platelet aggregation is very limited, there has been only one report on this activity of shiitake (Hayakawa and Kuzuya, 1985). This study was performed to examine the effects of extracts from cultivated shiitake on platelet function as thrombogenic or antiplatelet agents.

MATERIAL AND METHOD

Mushroom extracts

Shiitake *(L. edodes)* strain No. MU4 was cultivated at the Mushroom Research Unit, Chulalongkorn University by sawdust bag method using pararubber sawdust mixture (Triratana and Tantikanjana, 1989). The mushrooms were harvested and dried in the oven at 60°C for 48 hours and milled to obtain fine powder. Each 120 grams of mushroom powder were extracted with solvents: methylene chloride, distilled water, hexane and ethanol. The procedures for all solvents were similar in most respects inthat the mushroom powder was macerated in the solvents for 7 days, percolated slowly at the rate of 2-3 ml/min., evaporated under reduced pressure in a Rotary Evaporator until near exhaustion of the solvent. Each extract was weighed and kept in an amble bottle for further use.

Platelet aggregation test

Human blood was taken from donor of Blood Bank Division, Department of Pathology, Ramathibodi Hospital. Nine volume of blood were mixed with one volume of 3.8% trisodium citrate. Platelet-rich plasma was seperated by centrifuging the blood at 1100 rpm for 10 minutes. The plasma then was stored at room temperature for used within 3 hours. The crude extracts of *L. edodes* were diluted with 20% ethanol in various concentrations.

The effect of *L. edodes* on platelet function was detected by doing platelet aggregation. Equal volume of plasma and each diluted *L. edodes* extracts were mixed and tested against platelet aggregation, using 5 μM ADP or 0.28 mg/ml Collagen as inducer. The percentage of maximum platelet aggregation were detected by using Aggrecorder PA 3210. Mixture of an equal volume of plasma and 20% ethanol was used as control.

RESULTS

The effect of *L. edodes* extracts on platelet function shown in Table 1 and Table 2. The percentage of maximum platelet aggregation of plasma mixed with shiitake extracts in methylene chloride (0.2243 g), water (0.2436 g), hexane (0.1826 g) and ethanol (0.2030 g) were less than the control. It was concluded that shiitake have active ingredients which show strong potential in inhibiting platelet aggregation induced by ADP and Collagen. Each extract showed same pattern in inhibition of platelet aggregation since the maximum aggregation curve of each extract, methylene chloride, water, hexane and ethanol were similar as shown in Figs. 1, 2, 3 and 4 respectively.

Table 1. Comparison of platelet aggregation (% maximum aggregation) induced by ADP between *L. edodes* extracts and control in various solvents.

Solvent	Control N = 10	Extract N = 10	Statistics[a]	P value
Methylene Chloride	$\bar{X}$ = 131.60 SD = 4.20	$\bar{X}$ = 15.91 SD = 1.32	t = 83.02	P < 0.001
Water	$\bar{X}$ = 96.40 SD = 1.78	$\bar{X}$ = 3.70 SD = 1.12	t = 138.82	P < 0.001
Hexane	$\bar{X}$ = 106.70 SD = 6.92	$\bar{X}$ = 13.10 SD = 1.72	t = 41.46	P < 0.001
Ethanol	$\bar{X}$ = 97.40 SD = 2.68	$\bar{X}$ = 1.90 SD = 0.43	t = 111.26	P < 0.001

[a] = Unpaired t-test

Table 2. Comparison of platelet aggregation (% maximum aggregation) induced by Collagen between *L. edodes* extracts and control in various solvents

Solvent	Control N = 10	Extract N = 10	Statistics[a]	P value
Methylene Chloride	$\bar{X}$ = 48.80 SD = 4.42	$\bar{X}$ = 7.90 SD = 0.87	t = 28.66	P < 0.001
Water	$\bar{X}$ = 106.9 SD = 1.72	$\bar{X}$ = 13.10 SD = 0.92	t = 151.65	P < 0.001
Hexane	$\bar{X}$ = 97.30 SD = 5.90	$\bar{X}$ = 9.80 SD = 1.61	t = 44.78	P < 0.001
Ethanol	$\bar{X}$ = 57.30 SD = 4.02	$\bar{X}$ = 12.0 SD = 1.31	t = 111.26	P < 0.001

[a] = Unpaired t-test

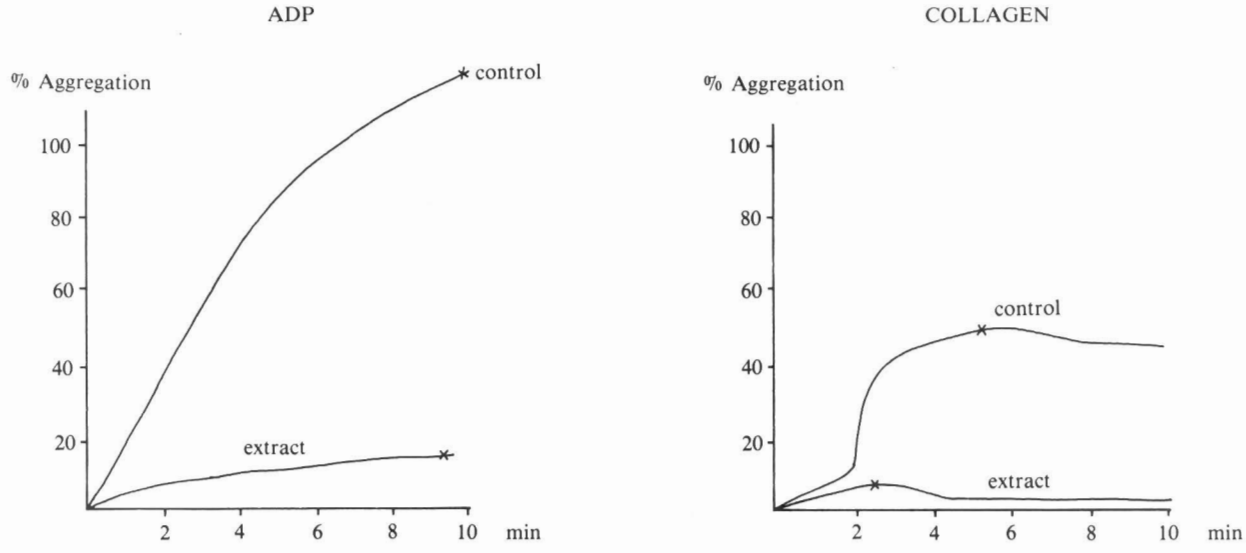


Fig. 1. Effect of *L. edodes* extract from methylene chloride on platelet aggregation (% maximum aggregation at time interval.)

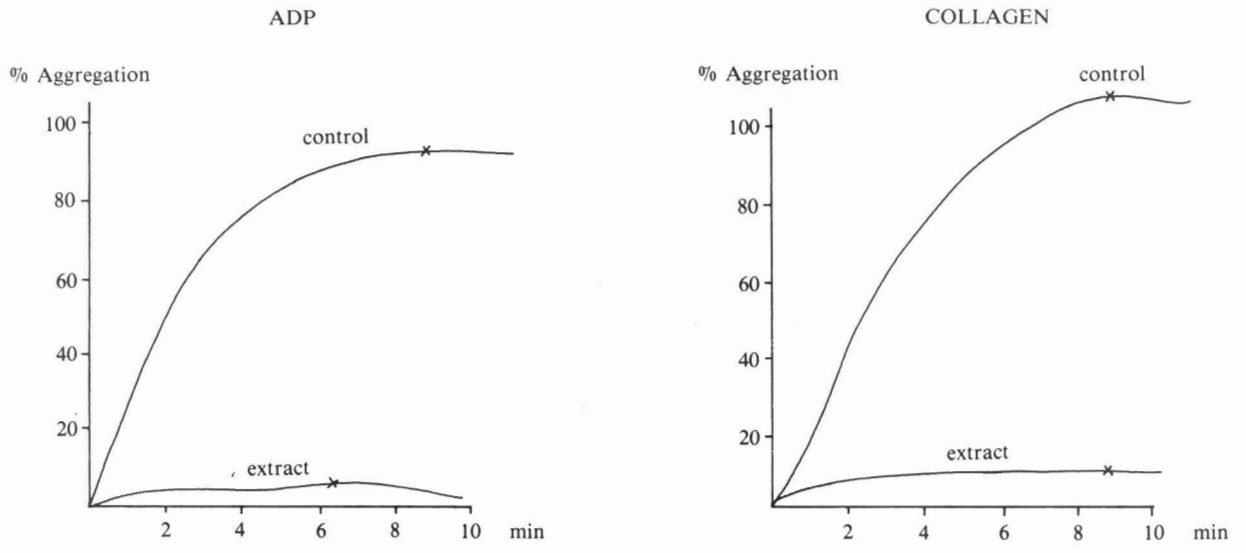


Fig. 2. Effect of *L. edodes* extract from water on platelet aggregation (% maximum aggregation at time interval)

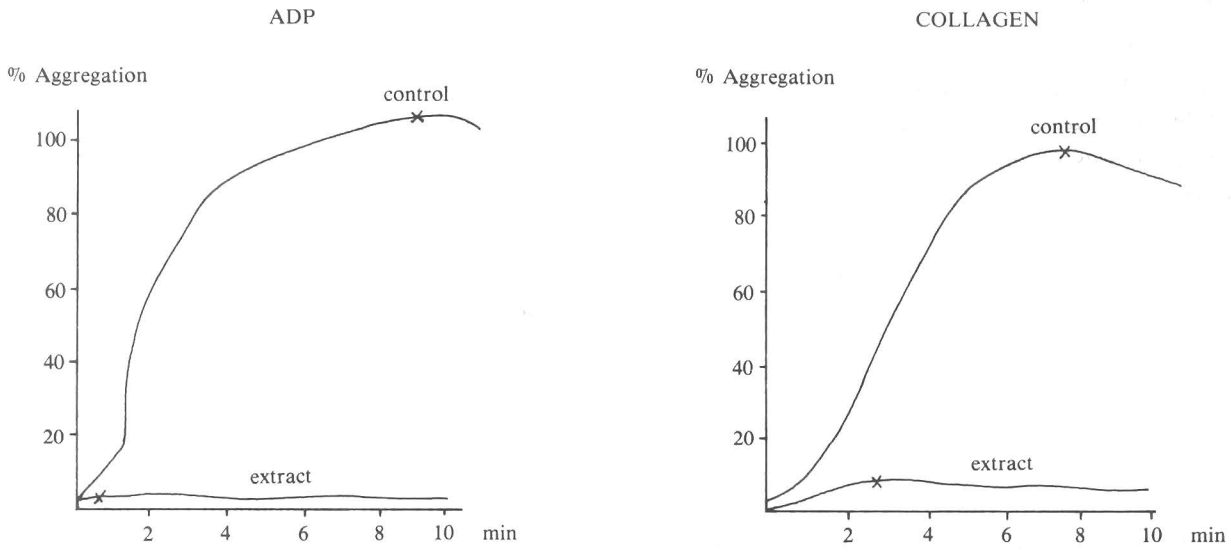


Fig. 3. Effect of *L. edodes* extract from hexane on platelet aggregation (% maximum aggregation at time interval)

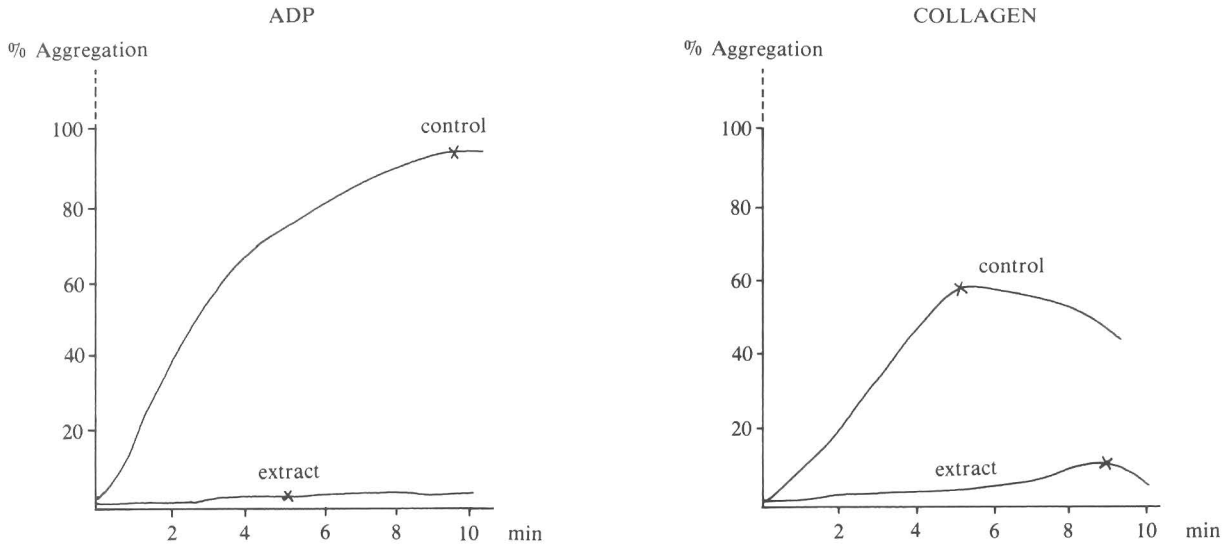


Fig. 4. Effect of *L. edodes* extract from ethanol on platelet aggregation (% maximum aggregation at time interval)

DISCUSSION

Our study showed that shiitake extracts inhibit platelet aggregation induced by ADP and Collagen *in vitro*. This findings are consistent with those of Hayakawa and Kuzuya (1985) who reported that water soluble substances from shiitake inhibited thrombin induced platelet aggregation and suggested that substance was Guanosine-5-monophosphate. It is possible that the substances would affect through deactivation of thrombin. Although the inhibitory mechanism is questioning, it is evidenced that *L. edodes* might be useful not only as a protective agent for atherosclerotic disease but also may be used as antithrombotic agent for patients with thromboembolic diseases. Further studies are needed to find out the mechanism of shiitake extracts in inhibiting platelet aggregation.

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