Ear fungus (Auricularia auricula) Infusion Reduces Blood Cholesterol Level in Dyslipidemic Rats

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Abstract

Background: Hypercholesterolemia is a major risk factor for coronary heart disease. Ear fungus (Auricularia auricula) contains soluble fiber (beta glucan) which is believed to have an effect of reducing cholesterol level. The aim of this study was to determine the effect of fungus infusion in reducing blood cholesterol.

Methods: This study used the laboratory experimental method conducted in the pharmacology laboratory of Dr. Hasan Sadikin General Hospital Bandung. The subjects were 25 male Wistar rats which were randomly divided into 5 groups. The first group was given only distilled water as a negative control, the remaining groups were induced to be dyslipidemic and received fungus infusion with different doses of 18%, 36%, 72% and one group received no treatment.

Results: The comparison of different doses showed all had the effect of reducing cholesterol levels compared to the positive control group ($p<0.05$). Each dose showed slight differences in their effectiveness, 18% ($p=0.007$; $p<0.05$), 36% ($p=0.002$; $p<0.05$), and 72% ($p=0.014$; $p<0.05$).

Conclusions: ear fungus infusion had the effect of reducing cholesterol with the most effective concentration was 36%. [AMJ.2015;2(1):153–61]

Keywords: Beta glucan, cholesterol, ear fungus

Introduction

In this era of globalization, people in the world especially in the urban areas experienced significant lifestyle changes, especially in terms of eating behavior. Changes in lifestyle can lead to health problems that cause a shift in the characteristic pattern of disease. In this case, there was a shift in the pattern of disease of which infectious disease used to dominate the mortality rate while nowadays it is replaced by non-communicable diseases such as coronary heart disease.[1]

The prevalence rate of coronary heart disease, especially in developing countries such as Indonesia, increases from year to year. Data from the World Health Organization (WHO) Non-Communicable Disease Country Profile 2011 showed that 30% of the proportion of mortality are due to cardiovascular disease.[2] Profil Kesehatan Indonesia 2009 also states that 11.06% major disease causing death in hospital are diseases of the circulatory system.[3]

Coronary heart disease has multiple risk factors such as dyslipidemia that could be controlled by lipid lowering drugs. These drugs have a variety of working mechanism consisting of several groups such as 3-hydroxy-3-methyl-glutaril-CoA reductase/HMG-CoA reductase inhibitors (statins), niacin, cholesterol absorption inhibitors, fibrates and bile acid resins.[4] However, although these have been proven effective in lowering blood lipid levels, lipid-lowering drugs have side effects, long time intake, and financially uneconomical.

Ear fungus (Auricularia auricula) is a type of fungus that comes from China's traditional medicine and people believed that it has many health benefits. A lot of people consume mushroom as food supplement such as for soup. Some literatures has proved the health effects of this fungus, among which, as anti-thrombotic, anti-carcinogenic, hypoglycemic, and hypocholesterolemic.[5,6] Based on pharmacological evidence and its use in

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society, the researchers were interested in conducting a research to determine the effects of ear fungus on cholesterol reduction.

Methods

This study used the laboratory experimental methods which took three weeks in the pharmacological laboratory of Dr. Hasan Sadikin General Hospital Bandung. Fresh mushrooms were obtained from cultivation in the biology building of the Indonesian Institute of Sciences (Lembaga Ilmu Pengetahuan Indonesia /LIPI), Cibinong-Bogor, West Java. Cultivation time began from July to August 2012. After been harvested the ear mushrooms were then dried. Infusion was made by cutting up the ear fungus finely and put it into an Erlenmeyer flask and 100 ml of distilled water was added. The mixture was put into a pot previously filled with heated (90oC) water. Next, the mixture was boiled in an infusion pot for 15 minutes and stirred occasionally. Afterward, the ear fungus was removed from the pot and waited to cool. Then it was filtered by using a filter paper to obtain ear fungus infusion.

Twenty five male Wistar rats with 200–250 grams of weight were randomly divided into 5 groups. Sick rats were excluded if they have lost 10% of body weight during the first week of adaptation. On the second week, each group was given different treatment. Group 1 was given a normal diet and aquadest as the negative control group. Group 2 was given a high lipid diet and propiltiouracil (PTU) 0.01% as the positive control group. Group 3, group 4 and group 5 were given a high lipid diet and PTU 0.01% with additional ear fungus infusion with 18%, 36%, and 72% concentration respectively. A schematic diagram of this study is shown in Figure 1.

After two weeks of treatment, the rats were incubated in a warm basket in order to vasodilate their blood vessels, then blood was taken from each rat from their tails. Next, the plasma sample was taken by centrifugation for 10 minutes in 12000 rpm.

The cholesterol level was measured by using the enzymatic photometric test. The blood plasma was mixed with reagent which contained cholesterol oxidase, and peroxidase. This end product was quinoneimine. The cholesterol levels were proportional to the quinoneimine concentration. It was measured by using the spectrophotometer with 546 nm wavelength.

The Saphiro-Wilk test was used to determine the distribution of data and the Levene's test to determine homogenity of variance. Next, the One-way ANOVA test was performed to compare the group means and LSD test to determine which group had the best effect to reduce cholesterol levels.

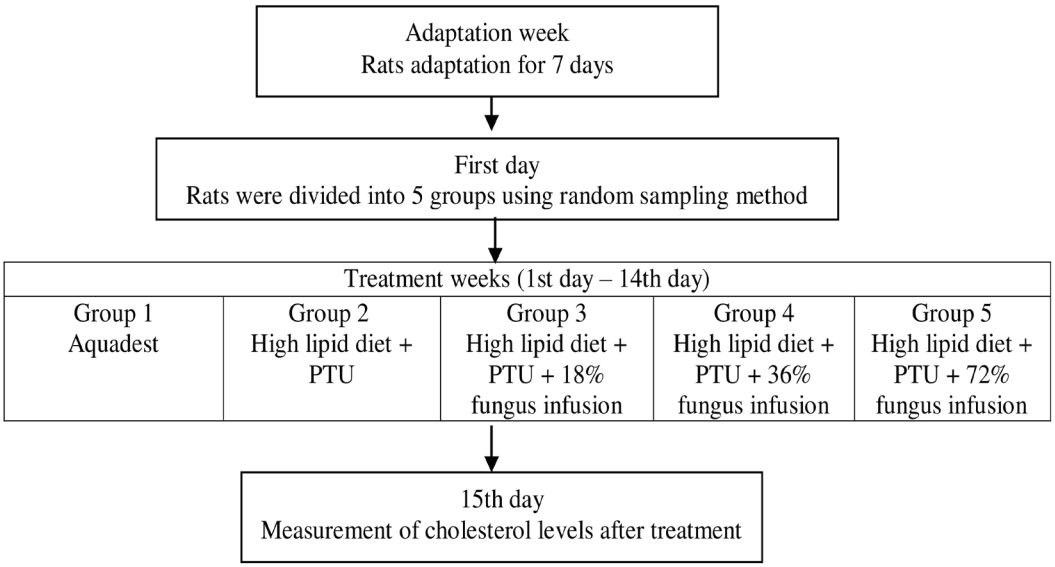

Figure 1 Schematic Diagram of the Research

Table 1 Blood Cholesterol Level after Treatment

No. Sample	Group 1 (mg/dL)	Group 2 (mg/dL)	Group 3 (mg/dL)	Group 4 (mg/dL)	Group 5 (mg/dL)
1	82.4	126.4	76	68.4	96.6
2	59.8	98.5	106.6	106.1	128
3	64.5	133.6	96.9	83.8	92.6
4	84.2	126.5	77.1	86	66.9
5	65.7	118.8	102.5	83.3	89.2
Means	71.32	120.76	91.82	85.52	94.66

Note: Group 1: Negative control, Group 2: Positive control, Group 3: Test group 1 (18% infusion), Group 4: Test group 2 (36% infusion), Group 5: Test group 3 (72% infusion)

Results

The results of this study are presented in Table 1. The means of positive control showed effective experimental-induced dyslipidemia. The intake of ear infusion showed lower mean cholesterol level in all doses. Statistic analytical was done, and the result is shown in Table 2

Successful induction was shown by the negative control group which had a significant difference compared to the positive control group ($p = 0.00$, $p < 0.05$). All treatment groups showed significant hypocholesterolemic effect compared to group 2 ($p < 0.05$)

Discussions

This study aimed to determine the effects of ear fungus infusion on blood cholesterol levels of dyslipidemic rat models. Based on the results showed on Table 1, successful induction of dyslipidemia was indicated by significant difference of cholesterol level between the negative and positive control groups given high lipid diet and 0.01% PTU ($p=0.00$, $p < 0.05$). The treatment group with ear fungus infusion showed that all three concentrations of treatment: 18% ($p = 0.007$, $p < 0.05$), 36% ($p = 0.002$, $p < 0.05$), and 72% ($p = 0.014$, $p < 0.05$) had a significant difference compared to the positive control group. From these results we could affirm that the treatment of fungus had an effect of lowering cholesterol. The cholesterol-lowering effect was presumably due to its soluble fiber (beta glucan) contents on the mushroom. Soluble fiber (beta glucan) can lower blood cholesterol levels by several mechanisms such as increasing the excretion of bile salts, increased catabolism of LDL and inhibit the absorption of cholesterol in the intestine.[7, 8] Increased excretion of bile salts can lower blood cholesterol levels due to the fact that cholesterol is the precursor of bile salt synthesis. With an increase in the excretion of bile salts will cause a reduction in reabsorption of bile salts in the ileum and liver will increase the production of bile salts that will increase cholesterol use. This supports some previous research on mushroom beta glucan which has the effect of lowering cholesterol levels in blood.[9]

The results of a comparative analysis of

Table 2 Post-hoc LSD Test Result

Compared Group (I)	Comparing Group (J)	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Upper Bound	Lower Bound
2	1	49.44	0.000	29.2192	69.6608
	3	28.94	0.007	8.7192	49.1608
	4	35.24	0.002	15.0192	55.4608
	5	26.1	0.014	5.8792	46.3208

Note: Group 1: Negative control, Group 2: Positive control, Group 3: Test group 1 (18% infusion), Group 4: Test group 2 (36% infusion), Group 5: Test group 3 (72% infusion)

the effectiveness of three different treatment concentrations in this study suggested that ear fungus infusion with a concentration of 36% had the best effect in reducing cholesterol levels compared to the other treatment groups with different concentrations. Giving higher concentrations in this study was not accompanied by increased blood cholesterol-lowering effects. This was indicated by a statistical test that there was no significant difference among the three treatment groups. This condition was likely due to other active substances contained in the fungus, so that the effects were not only cholesterol-lowering, but could also inhibit cholesterol reduction effect. So that the effects obtained from this study was the effect of the accumulation of substances contained in ear fungus infusion. Another factor that might affect the outcome of the study was an error in the laboratory and technical errors in both the induction and the provision of infusion in the treatment groups. In the treatment groups, we determined that the volume of infuse given to each rat was as much as 3 ml per day via oral administration. This did not rule out giving infusion could be reduced, because after giving infusion subjects were likely to vomit or not all infusion entered the body so that the volume of ear fungus infusion was less than the specified volume

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retrospectively. Diagnosis of tetanus was based on Patel and Joag grading. Patients were divided into five grades based on the criteria such as incubation period, period of onset, fever, and spasm and lock jaw. Fever is defined as temperature > 37.5. Grade I consisted of one of these criteria. For every additional criterion there will be alteration of the grade by one criterion. If all five criteria were present, it indicated grade V. While mild is range from grade I-II, moderate range from III-IV and severe was grade V.

The collected data included patient's details, age and sex, incubation period, period of onset, site of wound, immunization background, passive immunization, classification, grading, the comorbidity such as autonomic storm, cardiac arrest, aspiration pneumonia, and stress ulcer; and the use of mechanical ventilation. The incubation period was defined as a time period from time of injury to onset of symptoms, and the period of onset was defined as the time period from onset of symptoms to time of full blown disease / onset of general spasms. The patients also received passive immunization with anti tetanus serum 10,000 IU and active immunization with tetanus toxoid. Patients were graded based on the severity of the disease: mild, moderate and severe according to Patel and Joag.5 The data will be analyzed using Chi-squared tests or, when needed, the Fisher's exact test was used, to analyze the association between mortality and categorical variables.[6]

Results

Out of a total of 132 cases of tetanus only 87 patients with complete records were included in this study. Out of the 87 patients, 86 of them had generalized tetanus and only one of them had cephalic tetanus. All patients had spasms and lockjaw. There were 54 (67.07%) male and 33 (37.93%) female patients. The age range was between 20–80 years. The highest number of patients presented with tetanus was between 35–49 years which was 34 (39.08%).

Table 1 The Distribution of the Demographic data of Tetanus Patients

Demographic data	No. of patients	%
Sex		
Male	54	67.07
Female	33	37.93
Age (years old)		
20–34	10	11.49
35–49	34	39.08
50–64	29	32.18
>65	14	16.09
Wound site		
Extremities	74	85.07
Face/nose/mouth	11	12.65
Trunk	2	2.30
Vaccine after injury		
Yes	21	24.14
No	66	75.86
Grading*		
2	12	13.79
3	45	51.72
4	23	26.44
5	7	8.05

Notes: * Grading is according to Patel and Joag: 0-2=mild, 3-4=moderate, 5=severe

Table 2 The relationship between mortality and prognostic factors for tetanus

Factors	Total (n=87)	Died (n=28)	Mortality rate (%)	P-value
Sex				
Male	54	23	42.59	0.189
Female	33	5	15.15	
Age				
<50	45	14	16.09	0.503
≥50	42	14	16.09	
Wound site				
Extremities	74	24	85.72	0.850
Face/nose/mouth	1	4	14.28	
Trunk	2	0	0.00	
Vaccine after injury				
Yes	21	9	42.86	0.286
No	66	19	28.79	
Incubation period*				
< 7days	32	15	46.88	0.033
>7days	55	13	23.64	
Period of onset*				
< 48 hours	59	21	35.59	0.28
>48 hours	28	7	25.00	
Fever				
yes	27	14	51.85	0.013
no	60	14	23.33	
Co morbidity				
stress ulcer	13	6	46.15	0.002
respiratory distress	2	0	0.00	
respiratory failure	9	9	100.00	
autonomic storm	15	12	80.00	
cardiac arrest	2	2	100.00	
aspiration pneumonia	2	2	100.00	
none	44	0	0.00	
Grading				
2	12	2	16.67	0.031
3	45	11	24.44	
4	23	10	43.48	
5	7	5	71.43	
Use of mechanical ventilation or tracheostomy				
yes	61	22	36.07	0.318
no	26	6	23.08	

Note:*Incubation period is the period from injury to first symptom. Period of onset is the duration of time between first symptom and occurrence of spasm

The second highest was between 50–64 years which was 29 (32.18%). The lowest was presented between 20–34 years which was 10 (11.49%), followed by the age > 65 years which was 14 (16.09%). The most common wound site was extremities which were presented in 74 (85.07%) patients, followed by face/nose/mouth 11 (12.65%). The least common wound site was trunk which was two (2.30%). The absence of post-injury vaccination was shown as majority 66 (75.86%). Only 21 (24.24%) were vaccinated after injury. Based on the grading, most patient were presented with tetanus grade 3 which is 45 (51.73%) of them. It was followed by tetanus grade 4 which was 23 (26.44%), grade 2 which was 12 (13.79%) and grade 5 which was 7 (8.05%) of them.

The mortality rate among cases with an incubation period of < 7 days was higher (46.88%). Fourteen (51.85%) patients died with the presence of fever. In several co-morbidities of autonomic storm have shown the highest mortality rate with 12 (80%) patients. Mortality was high in patients for the period of onset < 48 hours which was 21 (35.59%). Patients who had been through mechanical ventilation and tracheostomy and died were 22 (36.07%).

Table 2 showed the relationship between mortality and prognostic factors for tetanus. Higher mortality rate was significantly associated with incubation period <7 days, presence of fever, co-morbidity of autonomic storm and more sever disease according to Patel and Joag grading. There were no significant association between age and sex, wound site, vaccine after injury, period of onset, and use of mechanical ventilation.

Discussion

Even though with the invention of tetanus toxoid, tetanus still cannot be eradicated in developing countries.[7,8] The mortality rate is considered high at 32.18%. Mortality was also proven high in a previous study by Polhaupessy.[9] A high rate of tetanus is shown in male than in female which is in line with the study by Patel and Mehtra[10], also in the study by Polhaupessy.[9] This is due to higher exposure rate in dirty environment at work in male than in female.[9,11] Besides, female are immunized before pregnancy.[1,12] In this study, gender was not shown significant to mortality rate. It is only common that tetanus occurres more in male than in female.

The highest mortality rate of tetanus was shown in the age range between 35–49 years, 39.08%. This may be due to the patients' activities at this age. Therefore, they are more prone to risk of injury which can be related to their work or other causes.13 In previous studies it was shown that there were associations of older age with mortality due to the poor adherence to immunization schedule.[6] The study by Udwadia[11] has stated that the response towards tetanus immunization declines at age more than 50 years old.

Vaccination after injury is important for preventing tetanus but in this study, the number of patients who were absence of post-injury vaccination is high (75.86%).6 It is also proven in the study by Widjaya.[13] Post-injury vaccinations are important in tetanus patients to prevent complications.

The factor wound site showed the highest number of patients was extremities with mortality rate 85.72%. The results differences could be because the patients in this study who were from allow socioeconomic background and also had low education were not aware about the safety at work. Therefore, they were more likely to injure their extremities. Usually tetanus is more severe if the site of wound is at the face or cranial than at extremities.

The factor incubation period <7 days (46.88 %; p 0.033) had a higher mortality compared to >7 days. This result was in line with the previous studies by Saltoglu et al.[5,6,10] The shorter the incubation period, the higher the risk of getting an infection showed that the toxin production by C. tetani had not neutralized. This factor was proven to be significant.

The patients who were presented with fever were higher than those without fever. It is significantly related to mortality in tetanus as the previous studies also showed presence of fever is related to mortality.6 In this study, fever was present because of the presence of microbiological organisms. Therefore, until the C. tetani was not eradicated the fever would not subside.

The co morbidity of autonomic storm had a p value of 0.002. This showed it was significantly related to mortality. The study by Polhaupessy has proven that autonomic storm is significant to mortality with p<0.001.[9] For severity of the disease by grade which is tetanus grade V shows as the highest mortality which is in line with the previous studies, the severer the disease the higher the mortality rate.[6,10,14] Grade V is the severest stage where there is presence of the five criteria as stated earlier. Therefore, this shows the patient is

in critical condition that will possibly lead them to further complications. This is proven significant to the mortality in tetanus.

The deaths from tetanus can be related to the prognostic factors. The factors which were significantly related to mortality were incubation period, presence of fever ($p<0.05$), co morbidity ($p<0.01$) and severity by grade ($p < 0.05$). Hopefully, this study could provide guidance for the physician to put much more effort on the prevention aspect of tetanus to reduce the mortality rate.

The limitation to this study was management as a factor was not identified. A multivariate logistic analysis could be performed to identify the most significant factors affecting the mortality simultaneously.

In conclusion, the mortality rate of adult tetanus was high (32.18%). The factors that were significantly affecting the mortality were incubation period less than 7 days, presence of fever, co morbid of autonomic storm and severity based by grade.

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