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Lowering Cholesterol Effect of β -glucans Isolated of *Termitomyces eurrhizus* Extracts by Oral Administration to Rats

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ABSTRACT

Lowering cholesterol effect by oral administration in rats of *Termitomyces eurrhizus* extract has been conducted. The purpose of this study is to investigate the lowering cholesterol activity of water extract from *Termitomyces eurrhizus* in rats. By oral administration to rats, the lowering cholesterol activity has been showed that the boiling water extract of this mushroom respectively decreased the cholesterol level in rats after treated with this extract on certain concentration. Antihiperkolestrol test for isolated compounds were determined on 36 male white rats which were divided into six treatment groups. One group used as control and five other groups were induced to increase blood cholesterol levels and then given a β -glucan with a varied amount. Water extract of 330 g dried mushroom have yield 14.6038 g soluble β -glucan and measuring 72 mg kg⁻¹ of rats body weight had proven lowering cholesterol level of rats equivalent with giving cholestyramine 72 mg kg⁻¹ b.wt.

Key words: β -glucan, cholesterol analysis, *Termitomyces eurrhizus*, lowering cholesterol activity, water extract, triglyceride

INTRODUCTION

Several mushroom species have been exploited for life, some of which have been used as food materials while others used as medicine. In various region, mushroom have been utilized as traditional medicines for the treatment of cancer and metabolism disturbance of carbohydrate and fat (Ikekawa, 2000; Mizuno *et al.*, 1995). From various attempt of experiments, it has been known that some mushrooms contain polysaccharide compound in the form of (1,3/1,6)- β -glucan compound. In certain concentration of the compound can lower both cholesterol and blood sugar rate (Braaten *et al.*, 1994; Kalra and Jood, 2000).

Many studies have shown the beneficial health effects of β -glucan compound. Consuming β -glucan gives some good outcomes such as lowering the level of blood cholesterol and attenuate postprandial glucose response (Braaten *et al.*, 1994; Wood *et al.*, 1994, 2000). This is believed to be caused by the main component of soluble dietary fibre that called (1 \rightarrow 3), (1 \rightarrow 6)- β -D-glucan also referred to as β -glucan (Ripsin *et al.*, 1992; Drzikova *et al.*, 2005).

The positive health effects are believed to be caused by the viscosity of β -glucan (Guillon and Champ, 2000; Wood, 2004). Viscosity in turn is affected by concentration and molar mass. Consequently, these factors must be taken into account when health effects are to be considered. Therefore extractability and the effects that processing may have on it are important. An increase of faecal butyric acid and an improvement of symptoms in patients with ulcerative colitis has been reported by Nyman (2003). β -glucans from fungi and cereals have been shown to be immunostimulators β -D-glucan stimulators. They bind to receptors on macrophages and other white blood cells and activated them, thus they enhance the resistance to infections (Yun *et al.*, 2003).

Soluble β -glucan has been isolated and identified from Jamur Tanduk (local name), *Termitomyces eurhizus* (Mursito *et al.*, 2010). Aims of this current study is discovering nature chemistry of substance in Indonesian edible mushroom. This substance will be test for pharmacological effect, especially for lowering cholesterol effect in blood.

MATERIALS AND METHODS

This study was conducted at the Pharmacology Laboratory, Faculty of Pharmacy, Pancasila University, Jakarta, Indonesia from January 2008 to December 2009.

Plant material: All parts of mushrooms were collected from Sukabumi, West Java, Indonesia. It was sliced, sun-dried and gross powdered. Voucher plant specimen was deposited at Herbarium Bogoriense, Bogor, Indonesia.

Isolation of β -glucan compound: The isolation of β -glucan compound from *Termitomyces eurhizus* is carried out based on the method of Westerlund.

Animals: Male rats strain Wistar having normal activity about 3-4 months old, weight 250-300 g each were selected for the experimental study. Thirty rats have already inclimatation for 1 week to conditioning, checking of their healthy, weight and to homogenize their food intake. All rats have on hypercholesterol conditions by inducted orally with additional foods consists of high cholesterol level (cholesterol 1%, natural fat 1% and prophylthiouracil 0.01%) (Wood, 2004).

Determination of β -glucan dose and positive control cholestyramine: It was used as positive control. For human being, its dose was 4000 mg day⁻¹. According to the conversion factor (F : 0,018), cholestyramine dose for rats was 72 mg b.wt.⁻¹. According to FDA β -glucan can be used to decreasing cholesterol level for human who has hypercholesterol conditions. For this purpose, 3 g day⁻¹ doses of β -glucan should be consumed.

For this study, dose for β -glucan was applied in 3 category, low dose (8 mg b.wt.⁻¹), medium dose (24 mg b.wt.⁻¹) and high dose (72 mg b.wt.⁻¹).

Evaluation of hypercholesterolemia activity. In this experiment 30 healthy rats were divide into 6 groups, group A-F.

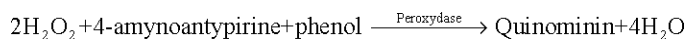
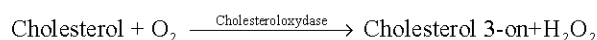
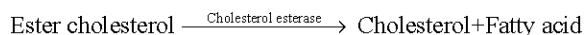
- **Group A:** Consist of normal rats, each rat was consumed with standard foods and suspension of 0.5% sodium carboxyl methyl cellulose
- **Group B:** Consist of hypercholesterolaemie rats which induced with high cholesterol foods consists of cholesterol 1%, animal fat 1% and prophylthiouracil 0.01%

- **Group C:** Consist of hypercholesterolaemie rats as positive control, each rats were consumed orally with cholestyramine 72 mg b.wt.⁻¹ for 4 weeks
- **Group D:** Consist of hypercholesterolaemie rats, each rats were consumed orally with β -glucan of mushroom 8 mg b.wt.⁻¹ for 4 weeks
- **Group E:** Consist of hypercholesterolaemie rats, each rats were consumed orally with β -glucan of mushroom 24 mg b.wt.⁻¹ for 4 weeks
- **Group F:** Consist of hypercholesterolaemie rats, each rats were consumed orally with β -glucan of mushroom 72 mg b.wt.⁻¹ for 4 weeks

Cholesterol analysis: Stipulating of cholesterol rate (total cholesterol, HDL, triglyceride, VLDL and LDL) conducted the time of giving and 28 days after giving of additional high cholesterol foods. Before the blood taken, all rats were fasted for 16 h. Rat's blood taken through tail was dropped at about 1 mL, centrifuged at 7500 rpm. Obtained blood serum used for stipulating the total cholesterol, HDL, triglyceride, VLDL and LDL level based on color forming resulted from enzymatic reaction. Formed ruddle young color will be specified its intensity by using spectrophotometer at wavelength ± 520 nm.

Procedure: Serum (10.0 μ L) react with diagnostic kit reagent, incubate for 10 min on 37°C and the absorbance is measured at 500 nm. Its intensity has correlation with concentration of total cholesterol on the sample.

Reaction:



Evaluation of cholesterol concentration in blood rats using cholesterol diagnostic reagent: From each rat, the blood was taken from 70% v/v alcohol cleaned tail and the blood was dropped at about 1 mL. In order to get this serum, this blood was centrifuged at 7500 rpm for 5 min. It was used as sample for calculation cholesterol includes HDL, triglyceride, VLDL and LDL concentration on blood.

Analysis data: Data of the experiment results were analyzed statistically using complete randomized design. When there was a significant difference, the analysis was continued with Duncan test, at $\alpha = 0.05$. (Mendenhall and Sincich, 1992).

RESULTS AND DISCUSSION

Water extract from *Termytomices aurrhizus* mushroom yield soluble fiber equal to 4.425% w/w. Total cholesterol rate analysis in early attempt conducted to recognize rat's cholesterol rate before inducted by high cholesterol food. The rate range between 69.36-74.33 mg dL⁻¹ which spread over to entire animal group.

Table 1: Concentration of total cholesterol, HDL, triglycerid, VLDL and LDL on 0th days

Group of treatment	Total cholesterol		HDL		Triglyserid		VLDL		LDL	
	Conc. (mg dL ⁻¹)	SD	Conc. (mg dL ⁻¹)	SD	Conc. (mg dL ⁻¹)	SD	Conc. (mg dL ⁻¹)	SD	Conc. (mg dL ⁻¹)	SD
A (control)	72.04	6.84	40.47	5.33	135.05	17.01	27.01	3.40	4.57	12.13
B	71.45	6.37	40.38	1.17	136.81	21.08	27.36	4.22	3.70	9.18
C	72.33	6.44	43.94	3.80	139.55	18.58	27.91	3.72	0.49	10.00
D	69.36	4.76	46.91	2.01	142.71	17.46	28.54	3.49	0.00	6.14
E	73.63	4.95	44.10	3.07	141.44	17.63	28.29	3.53	1.24	5.30
F	74.33	3.46	40.92	6.23	140.12	18.36	28.02	3.67	5.39	9.47

Table 2: Concentration of total cholesterol, HDL, triglycerid, VLDL and LDL on 14th days

Group of treatment	Total cholesterol		HDL		Triglyserid		VLDL		LDL	
	Conc. (mg dL ⁻¹)	SD	Conc. (mg dL ⁻¹)	SD	Conc. (mg dL ⁻¹)	SD	Conc. (mg dL ⁻¹)	SD	Conc. (mg dL ⁻¹)	SD
A (control)	77.45	6.00	29.92	3.64	158.22	19.94	31.64	3.99	15.89	6.25
B	254.08	4.84	28.07	2.79	353.94	26.02	70.79	5.20	155.22	9.68
C	253.90	18.95	28.20	4.39	355.69	11.72	71.14	2.34	154.56	20.45
D	246.57	13.10	33.01	4.78	356.96	12.27	71.39	2.45	142.17	12.32
E	246.63	16.84	30.72	3.76	346.13	29.03	69.23	5.81	146.69	10.88
F	252.57	13.51	26.76	3.12	343.46	20.62	68.69	4.12	157.12	8.17

Table 3: Concentration of total cholesterol, HDL, triglycerid, VLDL and LDL on 42th days

Group of treatment	Total cholesterol		HDL		Triglyserid		VLDL		LDL	
	Conc. (mg dL ⁻¹)	SD	Conc. (mg dL ⁻¹)	SD	Conc. (mg dL ⁻¹)	SD	Conc. (mg dL ⁻¹)	SD	Conc. (mg dL ⁻¹)	SD
A (control)	85.08	6.77	30.46	4.28	148.82	35.37	29.76	7.07	24.86	10.15
B	293.81	24.04	24.77	3.25	432.66	33.59	86.53	6.72	182.51	20.76
C	215.20	14.21	30.23	2.23	416.52	15.30	83.30	3.06	101.67	12.28
D	235.78	12.68	30.77	3.82	389.07	26.59	77.81	5.32	127.19	15.37
E	232.28	22.80	32.23	3.60	397.35	17.54	79.47	3.51	120.58	23.75
F	224.28	9.18	30.34	5.04	408.43	15.21	81.69	3.04	112.26	10.92

Cholestyramine is used as standard because its mechanism as antihypercholesterol is equal with β -glucan. This mechanism will be done by increasing bile acids excretions and attenuates of cholesterol's absorption's in small intestine.

Antihypercholesterol activity of glucan was done on 28 days. It calculated by statistical methods. Using SPSS methods programs on 16th version, t-test data to evaluate increasing cholesterol level. Studying increasing of cholesterol is done by t-test methods and than ANOVA-test is used to evaluate effect of hypocholesterolaemic. Concentration (mg mL⁻¹) and Standard Deviation (SD) on day of 0, 14 and 42 for total cholesterol, HDL, triglycerides, VLDL and LDL can be readed on Table 1-3.

Effect on the total cholesterol: In Table 1-3 shows that the addition of beta-glucan can decrease total cholesterol. After given additional food (day 14), total cholesterol has increased and on the day 42 has decreased by approximately 11.20%.

Effect on HDL level: In the Table 1-3, it can be seen the difference of normal HDL level prior to treatment in which on day compared to day 14, there is a decline in HDL level because of the hypercholesterolemia condition. In day 42, group V and VI (test group) have increase percentage of 4.94 and 13.38%, respectively. Whereas in group III (comparison group) gives 7.18% increase in HDL.

Effect on triglyceride level: In the Table 1-3, it can be seen the difference of normal triglyceride levels prior to treatment in which on days 0 compared to day 14, there are upsurges in triglyceride levels along with elevated levels of cholesterol. On Day 42, there was an increase in group IV-VI (test group) at 9.00, 14.80 and 18.92%, respectively. Whereas in group III (comparison) gives 17.10% increase in triglyceride.

Effect of LDL level: In the Table 1-3, it can be seen the difference of normal LDL levels prior to treatment in which on days 0 compared to day 14, there are upsurges both in LDL and cholesterol levels. On Day 42, there is a decrease in group IV-VI (test group), respectively, for 10.53, 17.80, 28.55%. The decrease of LDL with questran (comparison) group III at 34.22%.

Analysis of early total cholesterol, HDL, triglyceride, VLDL, dan LDL level of hypercholesterolemia conducted to know the increases of cholesterol level after induction with high cholesterol foods. The rate vary among 246.57-254.08 mg dL⁻¹ for total cholesterol, 26.76-33.01 mg dL⁻¹ for HDL and 142.17-157.12 mg dL⁻¹ for LDL.

After added by cholestyramine at group III or β -glucan at group IV-VI for 28 days successively, on day 42 the total cholesterol level at group III 72.33 \pm 6.44 mg dL⁻¹, group IV 69.36 \pm 4.76 mg dL⁻¹, group of V 73.63 \pm 4.95 mg dL⁻¹ and group VI 74.33 \pm 3.46 mg dL⁻¹.

There were significantly different values between group of normal control, negative control, group of treatment with β -glucan and group of positive control.

Data of lowering cholesterol level at treatment with β -glucan was significant at treatment with measuring 215 mg kg⁻¹ b.wt., equal to 26.14%.

By comparing with other studies, such as Braaten *et al.* (1994) b-glucan compound from oat can reduce blood cholesterol concentration in hypercholesterolemic subjects. Davidson *et al.* (1991) reported that there is hypercholesterolemic influence by the b-glucan in outmeal and bran. Behall *et al.* (1997), Nicolosi *et al.* (1999) and Bell *et al.* (1999) pointed out that the b-glucan compound isolated from yeast extracts can affect the fiber and blood lipids in both women and men. Furthermore, recently, Kerckhoffs *et al.* (2003) reported similar results regarding the cholesterol lowering effect of b-glucan compound from oat bran. Overall results of above researches showed after at least four weeks for using approximately 10% for cholesterol and 8% for LDL (bad) cholesterol, with elevation in HDL (good) cholesterol ranging from zero to 16%.

Comparison of total cholesterol, triglycerides, HDL and LDL among the same group on day 14 and 42 can be either a decrease (-) or increase (+) concentration. Analysis of increase or decrease percentage can be seen in Table 4.

Giving questran was effective enough to reduce 15.24% of total cholesterol and to increase triglycerides by 7.18% as well as to reduce LDL cholesterol by 34.22%. At the high dose test group (group VI), cholesterol was decreased by 11.20%, an increase of 18.92%, triglyceride levels and lowered LDL levels by 28.55%. However, changes in cholesterol and LDL levels are still far compared with normal values. Whereas for HDL cholesterol levels did not have much effect.

Table 4: Percentage change in total cholesterol, triglycerides, HDL and LDL from day 14 until day 42

Groups	Percentage change (%)			
	Cholesterol	HDL	Triglyceride	LDL
I	9.85	1.79	-5.94	56.49
II	15.64	-11.77	22.24	17.58
III	-15.24	7.18	17.10	-34.22
IV	-4.38	-6.78	9.00	-10.53
V	-5.82	4.94	14.80	-17.80
VI	-11.20	13.38	18.92	-28.55

The mechanism of lowering cholesterol level by polysaccharide compound not clearly known yet, but at least there are 4 mechanism able to be used to explain the process of lowering cholesterol level: increasing of bile acid, degrading fat absorption, resistance of cholesterol synthetic by fat acid short chain which yielded from soluble fiber ferment in colon and fast degradation of carbohydrate absorption which causing degradation of serum insulin level so that degrade synthetic stimulant of cholesterol and lipoprotein. In this experiment the degradation is seemingly strong caused by fast degradation of carbohydrate absorption (Morgan, 2000).

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