

Pharmacological Test of Herbal Products from Temulawak (*Curcuma Xanthorrhiza*) As Antihypercholesterol by *In Vivo*

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ABSTRACT

The purpose of this research was to observe the effects of *temulawak* (*C. xanthorrhiza*) powdered and instant herbal products on hypercholesterol by *in vivo*. This research began with the manufacture of *temulawak* powdered and instant medicinal products from standard raw materials. The activity assay by *in vivo* using female Wistar strain rats aged 50 days with a weight around 150 grams which was fed with pellets and quail egg yolk. There were five rats in each group and were placed in a cage with a temperature of 25-32° C, and 98% relational humidity. The treatment to the tested animals was done to observe the rat blood lipid profile due to the treatment with *temulawak* powdered and instant herbal products, compared to the negative control (without quail egg yolk) and the positive control which was given the quail egg yolk and simvastatin as an antihypercholesterol. The treatment of each group of rats lasted for 50 days. All the rats were taken the blood from sinus orbitalis for the lipid profile analysis which includes determining total cholesterol levels, triglycerides, HDL (high density lipid), and LDL (low density lipid), before and after giving the Quail egg yolk, after a two week and a four week administration of *temulawak* powdered and instant herbal products. The results show that there are effects of the dose and four week duration of administration of *temulawak* powdered and instant herbal products on the levels of cholesterol, triglycerides, HDL, and LDL in the rats' blood.

Key words: *temulawak herb, C. xanthorrhiza, antihypercholesterol, in vivo*

INTRODUCTION

Herb is one of the Indonesian local wisdom. Empirically, herb can be used to prevent or treat diseases. The family of Zingiberaceae plant spread in South Asia and Southeast Asia, consists of 47 genera and about 1000 species¹. Several species of this family are widely used in all traditional medicinal herbs, such as herbal medicine. Economically important species among the plant families of the Zingiberaceae, which are perennial rhizomatous herbs, contain volatile oil and other important compounds of enormous medicinal values². The plants are usually aromatic and carminative, and are used to treat indigestion, hepatitis, jaundice, diabetes, atherosclerosis, diarrhea, irregular menstruation, tuberculosis, gingivitis, skin diseases, tumors, malaria, and bacterial infections²⁻⁴. The plants grow naturally in damp, shaded parts of the low land or on hill slopes, as scattered plants or thickets.

Some plants of the family Zingiberaceae which are widely used as a traditional medicine among others are *Curcuma*, *Kaempferia*, and *Alpinia*. The use of the rhizome of *Curcuma* medicinal plants has been studied clinically, as well as the genus of *Kaempferia*. The current state of research of some species on *Kaempferia* genus clearly shows that the isolated bioactive compounds have a high potential in treating many diseases. Chemical constituents that have been reported for the some species of *Kaempferia* include cyclohexane oxide derivatives, chalcone

derivatives, cinnamates, diterpenes, monoterpenes, and flavonoids. Some bioactive compounds show high cytotoxic effects against some cancer cell lines, and so these plants have the bioprospecting for cancer treatment⁵⁻⁸. Some studies on pharmacological effects of curcuminoid compounds found from a plant of family Zingiberaceae have been reported that they are antioxidant, anti-inflammatory, antimutagenic, anticarcinogenic, antiviral, and antihepatotoxic activities⁶⁻¹¹. The chemical content of the plant's essential oil also indicates traits as insect repellent, antibacterial, and antifungal⁸.

The major constituent of *Curcuma* species is curcuminoid. Many studies show that curcuminoid can lose triacylglycerols in the liver, cholesterol concentrations and VLDL (very low density lipoprotein) plasma in rats¹². According to Shin *et al.*¹³, curcumin can lower the cholesterol levels, triglycerides, LDL cholesterol and Apo B in rats' blood plasma which is proportional to the effect of simvastatin.

One of the herb materials is *temulawak* (*Curcuma xanthorrhiza* Roxb). The rhizome of this plant has been used for centuries in traditional system of medicine to treat several diseases such as hepatitis, liver complaints, diabetes, anti haemorrhoids, and also to lower the cholesterol. It has been consumed as food supplement and "jamu" as a remedy for hepatitis. Recently, herb products

Table 1: The groups of the experiment

| Group | Experiment |
|-------|--|
| 1 | Normal Group: the rats were only given pellet feed. |
| 2 | Negative Control Group: the rats were given pellet feed and Quail egg yolks. |
| 3 | Positive Control Group: similar to group II and were given simvastatin 4 mg/Kg body weight. |
| 4 | Treatment Group: similar to group II and were given powdered <i>temulawak</i> 50 mg/kg body weight. |
| 5 | Treatment Group: similar to group II and were given powdered <i>temulawak</i> 100 mg/kg body weight. |
| 6 | Treatment Group: similar to group II and were given powdered <i>temulawak</i> 200 mg/kg body weight. |
| 7 | Treatment Group: similar to group II and were given instant <i>temulawak</i> 50 mg/kg body weight. |
| 8 | Treatment Group: similar to group II and were given instant <i>temulawak</i> 100 mg/kg body weight. |
| 9 | Treatment Group: similar to group II and were given instant <i>temulawak</i> 200 mg/kg body weight. |

or traditional medicine from *temulawak* in both single and mixed on the market are in the form of capsules, instant drinks, and bottled drinks. Many studies on the efficacy of *temulawak* have also been done; one of them is the result of *in vivo* experiment using white rats which indicates that curcumin compounds and essential oil of rhizome *C. xanthorrhiza* can lower the levels of cholesterol and triglycerides in the blood of rats¹⁴⁻¹⁵. *C. xanthorrhiza* contains bioactive compounds, such as curcuminoids, camphor, geranyl acetate, zerumbone, β -curcumene, zingiberene, α -curcumene, and xanthorrhizol. Xanthorrhizol and crude extract of *C. xanthorrhiza* showed as antihyperglycemic and anti-inflammatory activities might be used as potent antidiabetic agents for the treatment of type 2 diabetes¹⁶.

The existing researches usually use rats as the animal testing with the tested substances in the form of ethanol extract or pure compound from the isolation of its extracts. In this research, anti-hypercholesterol test was done using rats as the animal testing and *temulawak* as the tested substance, which are produced in the form of ready-to-drink supply, i.e. powdered *temulawak* and *temulawak* juice cooked with sugar to produce crystallite (instant *temulawak*) of herbal products.

MATERIAL AND METHOD

Apparatus and reagent

The following apparatus used in this work were glassware, desk glasser, ependorf, object glass, yellow tip, blue tip, syringe injection, centrifuge, spectronic 20 (Genesys), and analytical balance. The materials used in this study were powdered *temulawak* and *temulawak* juice to produce crystallite (instant *temulawak*) of herbal products of Yogyakarta, cholesterol Stanbio Kit, triglyceride Stanbio Kit, HDL Stanbio Kit, standard cholesterol, standard triglyceride, positive control (simvastatin), heparin, aquadest, rats feed pellets, and quail egg yolk.

Animal test

The experiments were treated on males Wistar strain rats of 50-day-old obtained from LPPT, Gadjah Mada University, Indonesia, weighing about 150 g and were kept under a stable environmental condition with 12:12 light-dark cycle at 23-25 °C. The rats were fed by standard granulated chow (pellets 789) and had an access to drinking water *ad libitum*. Thus, the animal test was done

in accordance with Institutional Protocols of animal care. For about 4 weeks, the animals were then fed on an experimental diet containing 5% of either cellulose as a control or Indonesian plants which were added to the basal diet. The body weight and feed and water intake were recorded daily.

In vivo test

In the *in vivo* test, the corresponding males Wistar strain rats were fed with pellets, given drink of tap water, and also given Quail egg yolk every day. The rats were divided into 9 groups, and each group was composed of 5 rats, as shown in Table 1. The content of cholesterol levels, Triglyceride and HDL were recorded four times: (1) before giving the Quail egg yolks, (2) after giving the Quail egg yolks, (3) after a two week administration of *temulawak* (powdered or instant), and (4) after a four week administration of *temulawak* (powdered or instant).

Analysis of total cholesterol, triglycerides, HDL (High-density lipoprotein), and LDL (Low density Lipoprotein) blood Rat

The tested rats were stunned using the addition of dimethyl ether prior to blood sampling process. The blood was drawn from the heart of the rats about 5 ml using a syringe and then inserted into a vacuum tube containing the anticoagulant EDTA (Ethylene Diamine Tetra Acetate). The serum lipids were extracted in a chloroform-methanol mixture (2: 1, V/V) and analyzed for cholesterol, triglyceride, HDL and LDL as described on Stanbio Laboratory procedure Kit. Blood serum that has been obtained was centrifuged at 1000 rpm for 10 minutes. The separated plasma was taken using a pipette and put into a tube ependorf, which was then closed. LDL cholesterol fraction was precipitated from the serum by the addition of a magnesium chloride or dextran sulfate reagent. HDL cholesterol was then determined in the supernatant fluid, using a cholesterol reagent and the derived dilution factor in calculation. Cholesterol esterase hydrolyzes esters to free cholesterol and fatty acids. The free cholesterol produced plus the preformed cholesterol were then oxidized in the presence of cholesterol oxidase (CO_x) to cholest-4-en-3-one and hydrogen peroxide. A quinoneimine chromogen with maxima absorption at 500 nm, should be produced when phenol was oxidatively coupled with 4-aminophenazone in the presence of peroxidase with hydrogen peroxide. The intensity of the final red color

Table 2: Average of cholesterol levels, triglycerida, HDL, and LDL

| Profile of lipid | Peri-ode | Groups | | | | | | | | |
|---------------------------------------|----------|-----------------|-----------------|-----------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | | I | II | III | IV | V | VI | VII | VIII | IX |
| Average of cholesterol levels (mg/dL) | 1 | 53,91± 13.3 | 84,09± 12.9 | 58,36± 6.2 | 66,73± 18.3 | 70,90± 13.2 | 61,68± 7.9 | 57,95± 10.9 | 63,17± 10.6 | 51,27± 7.8 |
| | 2 | 88,17± 16.4 | 86,92± 10.43 | 88,24± 19.7 | 69,11± 12.6 | 94,24± 14.3 | 70,46± 11.3 | 69,46± 9.9 | 84,83± 14.6 | 84,52± 15.4 |
| | 3 | 88,47± 15.6 | 85,65± 8.94 | 88,80± 20.4 | 73,27± 9.5 | 80,05± 10.4 | 65,17± 13.7 | 60,64± 18.2 | 60,64± 12.7 | 82,64± 9.2 |
| | 4 | 70,75± 12.7 | 72,88± 10.8 | 75,84± 9.9 | 57,27± 10.6* | 64,50± 8.1* | 62,68± 12.4* | 54,34± 5.9* | 54,34± 8.7* | 71,86± 10.9 |
| Average of Triglyceride (mg/dL) | 1 | 93,12± 17.6 | 78,65± 17.1 | 89,46± 25.1 | 66,82± 34.8 | 52,30± 2.4 | 74,56± 19.1 | 86,30± 31.3 | 68,43± 21.3 | 118,93± 38.2 |
| | 2 | 68,91± 18.7 | 69,28± 15.3 | 90,56± 13.8 | 81,83± 37.7 | 155,45± 16.3 | 140,19± 52.8 | 191,72± 76.1 | 91,51± 22.6 | 95,01± 28.1 |
| | 3 | 72,07± 19.5 | 85,12± 16.1 | 61,76± 11.7 | 60,97± 16.2 | 63,49± 21.1 | 47,64± 14.6 | 79,39± 24.7 | 89,61± 25.3 | 116,89± 47.2 |
| | 4 | 61,68± 11.4 | 85,01± 14.6 | 72,47± 10.1* | 107,21± 14.5 | 87,11± 4.2 | 67,83± 15.6* | 135,16± 20.9 | 86,48± 22.1 | 91,45± 44.3 |
| Average of HDL (mg/dL) | 1 | 10,93± 3.4 | 10,32± 1.5 | 7,76± 1.3 | 9,67± 2.1 | 10,92± 1.5 | 8,87± 2.1 | 7,33± 1.8 | 5,98± 0.3 | 6,83± 1.9 |
| | 2 | 42,19± 4.3 | 52,52± 13.2 | 37,80± 7.9 | 35,75± 4.9 | 49,85± 9.2 | 42,89± 20.9 | 36,66± 13.4 | 3,06± 6.4 | 35,76± 8.9 |
| | 3 | 36,10± 9.0 | 42,04± 5.5 | 39,61± 4.6 | 32,96± 4.9 | 42,85± 4.4 | 39,90± 15.2 | 32,43± 8.1 | 28,50± 6.0 | 35,93± 3.4 |
| | 4 | 33,37± 6.9 | 33,39± 6.6 | 29,94± 4.5 | 24,56± 2.6 | 33,43± 4.8 | 27,99± 6.9 | 22,05± 3.2 | 25,62± 5.5 | 25,62± 1.5 |
| Average LDL (mg/dL) | 1 | 24,35± 9.7 | 58,04± 11.6 | 32,70± 8.5* | 43,69± 14.2* | 49,51± 12.1* | 37,89± 6.6* | -0,47± 13.6* | 15,73± 11.4* | 21,12± 11.1* |
| | 2 | 32,20± 12.6 | 20,55± 22.1 | 32,32± 11.2 | 16,99± 10.0 | 13,29± 7.4 | 33,36± 16.4 | -5,54± 20.2 | 12,32± 14.9 | 5,24± 13.9 |
| | 3 | 37,95± 12.9 | 26,58± 9.9 | 36,12± 18.3 | 28,11± 4.7 | 24,50± 7.1 | 43,50± 13.9 | 28,45± 13.6 | 25,45± 7.7 | 28,07± 9.6 |
| | 4 | 25,040± 14.5 | 22,48± 10.8 | 31,41± 7.7 | 11,269± 11.1* | 13,65± 6.8 | 20,65± 12.7 | 29,76± 4.6 | 23,33± 5.1 | 28,24± 6.5 |

Data are expressed as mean ± SD (n = 5)

*Significant at p < 0.05 with negative control group (group II)

would be proportional to the total cholesterol concentration. The kit used in the analysis of triglycerides and total cholesterol was Standbio. Levels of cholesterol, triglycerides, and HDL were measured by using an automated clinic analyzer. LDL obtained was calculated using the formula:

$$\text{LDL (mg/dl)} = \frac{\text{total cholesterol} - \text{HDL} - (\text{triglycerides})}{5}$$

Statistical analysis

All data were presented as the mean ± standard deviation (SD). Data analysis was performed by one way analysis of variance (ANOVA). The differences between the treated and control groups were also post-hoc analysed by the Duncan test (SPSS 13.0). The value of p < 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

The content of cholesterol, Triglyceride, HDL, and LDL levels (Table 2 and Graphs 1) were recorded four times: 1. before giving the Quail egg yolks, 2. after giving the Quail egg yolks, 3. after a two week administration of

temulawak, and 4. after a four week administration of *temulawak*. To find out the influence of both *temulawak* and simvastatin after administration for two weeks and four weeks, and also to see the influence of the dosage form and dosage of *temulawak* on cholesterol, triglyceride, HDL and LDL levels in the rat's blood, the ANOVA statistic test were done. The profile of lipid effect test, which is used to determine the effect of powdered *temulawak* and instant *temulawak* in lowering cholesterol, triglyceride, HDL and LDL levels in the rat's blood, was conducted in dosages with three different ratings. The positive control was treated with simvastatin form of 4 mg/kg BW daily.

According to the significance of the ANOVA statistic test, the results are as follow: there is a group that is affected toward cholesterol, triglyceride, HDL and LDL levels (p < 0.05); there is a treatment that is affected toward cholesterol triglyceride, HDL and LDL levels (p < 0.05); and there is an interaction between the groups with preferential treatment in the determination of the cholesterol triglyceride, HDL and LDL levels (p < 0.05).

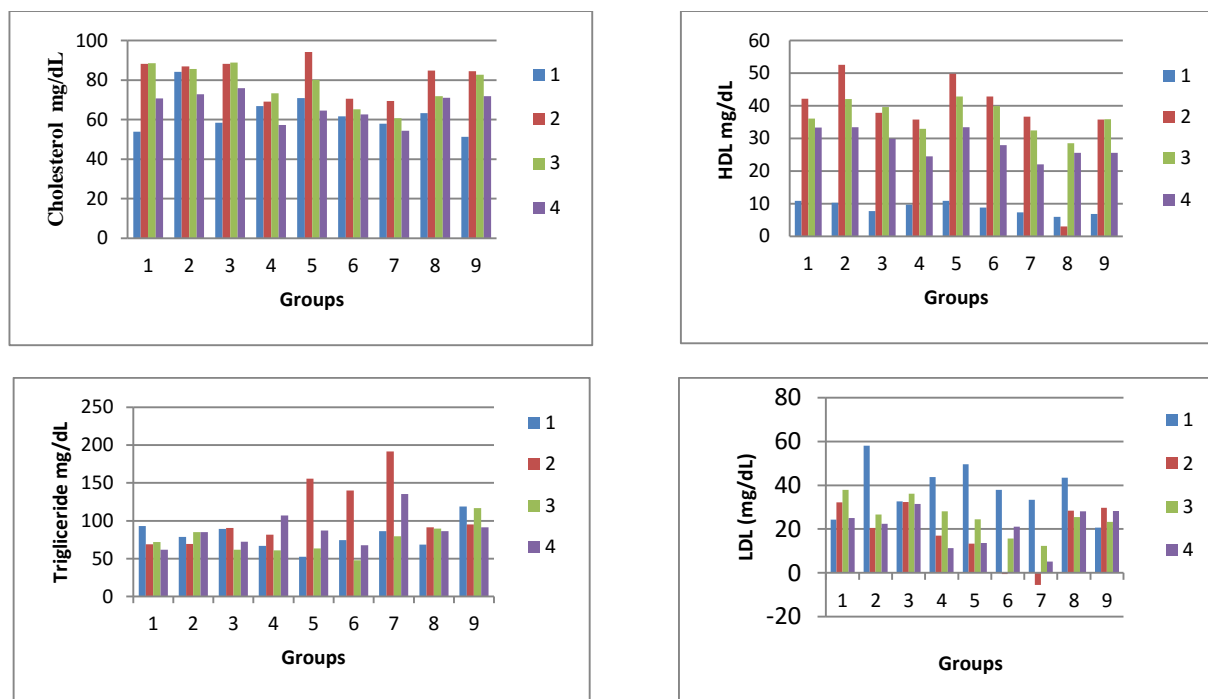


Figure 1: Graphs of average of cholesterol levels, triglycerida, HDL, and LDL

The results of post-hoc analysis are as follow: the cholesterol, triglyceride, HDL and LDL levels in each treatment shows that group I < group II > group IV. The cholesterol level in treatment IV tends to decreasing. The triglyceride level in treatment III is decreased but increased again in treatment IV. In the analysis of LDL of each group according to the post-hoc results, the LDL level in treatment IV tends to decrease. The data of cholesterol level after a four week administration of *temulawak* show a lowering in the treatment of group IV, VII, and VIII. The HDL level showed a lowering in the treatment of all groups after a four week administration of *temulawak* powdered and instant. The data show that there is influence of dosage and the duration of administration of powder and instant *temulawak* for four weeks in rat blood cholesterol; triglycerides ; HDL and LDL levels.

Hypercholesterolemia is a risk factor for coronary heart disease, because it can lead to atherosclerosis. Therefore, it is necessary to reduce the excess cholesterol in the body fat. The concentration of plasma cholesterol in the body can be set via the biosynthesis of cholesterol, the removal of cholesterol from the circulation, the absorption of dietary cholesterol or cholesterol excretion via the bile and feces. One herbal remedy commonly used in cholesterol-lowering diet is ginger. Some researchers have found levels of *Curcuma* in ginger that causes plasma LDL peroxidation¹⁵. Curcumin can increase faecal excretion of bile acids and cholesterol in normal mice and hypercholesterolemia¹⁷. Piyachaturawat *et. al.*¹⁸ reported that the administration of the extract weight of 100-400 mg/kg rhizome of *C. comosa* Roxb results in decreasing LDL-c concentrations in mice.

Curcuminoid is an active compound that is widely available in the rhizome of the *Curcuma* plant. The compounds show various biological activities such as

antioxidant, anti-inflammatory, anticancer, and antihypercholesterol. Many plant extracts have been reported to have multiple biological effects, including antioxidant, antimutagenic, antiinflammatory, anticholesterol, and antihepatotoxic.

The active compounds corresponding to the antihypercholestermia activities of *temulawak* were xanthorizol. Research conducted by Kim¹⁶ reported that the extract of *C.xanthoriza* and also xanthorizol isolated compounds showing activity as antihyperglycemic and anti-inflammatory. *C.xanthoriza* containing 16.64 % of xanthorizol, did not show substantial differences as compared to xanthorizol. This is probably because *C. xanthoriza* contains other bioactive compounds, such as curcuminoids, camphor, geranyl acetate, zerumbone, β -curcumene, zingiberene, and ar-curcumene as well as xanthorhizol. The influence of the extract of *Curcuma comosa* Roxb (Zingiberaceae) on lipid metabolism has been investigated in hypercholesterolaemic hamsters. Intra gastric administration of the ethyl acetate extract of *C. comosa* rhizome (0–500 mg/kg per day) to hypercholesterolaemic animals for 7 days decreased both plasma triglyceride and cholesterol levels in a dose-dependent manner¹⁸.

CONCLUSION

There is an effect of dosage and the four week duration of administration of powder and instant *temulawak* in rat blood cholesterol, triglyceride, HDL and LDL levels. The results obtained from the study suggest a potential application of *temulawak* rhizome for treatment of anti-hypercholesterolaemic.

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Conflict of interest statement

We declare that we have no conflict of interest.

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