EFFECT OF CURCUMINOID NANOEMULSION EXTRACTED FROM Curcuma Xanthorrhiza ON HYPERCHOLESTEROLEMIC RATS

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ABSTRACT

The purpose of this study was to determine the effect of using curcuminoid nanoemulsions from Curcuma xanthorrhiza rhizome extract in rats fed a high-fat diet. Thirty male Rattus norvegicus rats were prepared under the same conditions for 7 days and then induced by feeding egg yolks of 10 mL/kg body weight (BW) each for 14 days. They were then divided into 3 groups, namely the negative control group, the positive control group, and the experimental group. The treatment of each group was as follows: the negative control group was not given drugs, the positive control group was treated with simvastatin at a dose of 0.9 mg/kg, while the experimental group (groups 1, 2, 3, and 4) were treated with curcuminoid nanoemulsion with doses of 3, 5, 7.5, and 10 mg/kg BW, consecutively every day for 26 days. Blood samples from each rat were taken before and after treatment, then a lipid profile analysis was carried out, which included cholesterol, LDL, HDL, and triglyceride levels by spectroscopic method. After 26 days of treatment, it was shown that there was an effect of giving curcuminoid nanoemulsion at a dose of 10 mg/kg BW to effectively reduce total cholesterol levels by 11.2%, triglyceride levels by 14.9%, LDL levels by 21.5% in rat blood serum. The results showed that curcuminoid nanoemulsion from C. xanthorrhiza rhizome extract has the potential to be developed as an antihypercholesterol drug.

Keywords: Nanoemulsion Curcuminoid, Curcuma xanthorrhiza, Hypercholesterolemia, Profil Lipid.

INTRODUCTION

Coronary heart disease is one of the main causes of death in the world, including in Indonesia. This disease is caused by the accumulation of cholesterol and lipids in the walls of blood vessels caused by the occurrence of hypercholesterolemia in the blood that exceeds normal limits.⁴ One of the herbal plants that are widely used to prevent hypercholesterolemia is C. xanthorrhiza. The rhizome of C. xanthorrhiza contains lots of curcuminoids and essential oils. Several curcuminoids found in C. xanthorrhiza include curcumin, demethoxycurcumin, bisdemethoxycurcumin, and 1,7-bis(4-hydroxy-3-methoxyphenyl)-hepten-3,5-dione.⁵ The essential oil of C. xanthorrhiza rhizome consists of various sesquiterpene compounds such as curzerenone and xanthorrhizol.⁶ Curcuminoids and C. xanthorrhiza rhizome essential oil can reduce blood cholesterol and triglyceride levels in rats.⁷ Likewise, research using C. xanthorrhiza rhizome powder can reduce cholesterol, LDL, HDL, and triglyceride levels in rats fed a high-fat diet.⁸ Curcumin combined with streptozotocin can prevent hyperglycemia and hyperlipidemia in the blood and liver caused by a high-fat diet in rats.⁹ Curcuminoids are compounds that are widely found in the rhizomes of plants of the Curcuma genus, such as C. xanthorrhiza, C. zedoria, and C. mango. Research shows that curcuminoids have various pharmacological activities such as anti-cancer, anti-inflammatory, anti-oxidant, anti-hypercholesterolemic, anti-alzheimer's, and anti-microbial. However, curcuminoids are poorly soluble in water and have low bioavailability, so they are currently being developed in the form of nanoparticles.¹⁰ Curcuminoids from ethanolic extract of C. xanthorrhiza can be formulated using chitosan and alginic acid to form nanocurcuminoids which show higher antioxidant activity than the free curcuminoids.¹¹ Curcumin formulations using a solid dispersion strategy in the form of nanoemulsions have the potential to improve solubility and stability.¹² The results showed that nano curcumin encapsulation can improve skin health.

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permeation and release kinetics, so it can be used for topical application.\textsuperscript{12} Previous studies have shown that curcuminoid nanoemulsions from \textit{C. xanthorrhiza} are effective as antioxidants and antimicrobials.\textsuperscript{13} Continuing the previous research, an experiment was conducted in this study to determine the effect of nanoemulsion curcuminoid extract of \textit{C. xanthorrhiza} rhizome in rats fed a high-fat diet.

\textbf{EXPERIMENTAL}

\textbf{Apparatus and Reagents}

The following main apparatus used in this work were a spectrophotometer, centrifuge, plain blood tube, microcapillary, Eppendorf-tube, microtube, micropipette, desk-glasser, object-glass, yellow tip, blue tip, syringe injection, and analytical balance. The main ingredients needed include curcuminoid extract from \textit{Curcuma xanthorrhiza}, tween-80, VCO (virgin coconut oil), phosphate buffer pH 7.0, Stanbio Kit cholesterol, triglycerides, and HDL, simvastatin, heparin, aqua dest, rat feed pellets, and yellow quail eggs.

\textbf{Animal Test}

There were 30 males \textit{Rattus norvegicus} Wistar strains aged 50 days (weight 150-200 grams). Rats were fed standard pellets and given access to drinking water ad libitum. All test animals have been treated in accordance with the Animal Care Institutional Protocol and have received approval from the Ethical Clearance Commission for integrated research and testing at Universitas Gadjah Mada with certificate number: 00056/04/LPPT/XI/2019. The grouping of rats and the treatment of each group are shown in Table-1.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
No. & Groups & Treatment & Number of mice \\
\hline
1. & Negative control (NC) & Standard feed + quail egg yolk (each day till day-14) \textsuperscript{[I]} & 5 \\
2 & Positive control (PC) & \textsuperscript{[I]} + simvastatin 0.9 mg/kg BW & 5 \\
3 & Experiment-1 & \textsuperscript{[I]} + curcuminoid nanoemulsion 3 mg/kg BW & 5 \\
4 & Experiment-2 & \textsuperscript{[I]} + curcuminoid nanoemulsion 5 mg/kg BW & 5 \\
5 & Experiment-3 & \textsuperscript{[I]} + curcuminoid nanoemulsion 7.5 mg/kg BW & 5 \\
6 & Experiment-4 & \textsuperscript{[I]} + curcuminoid nanoemulsion 10 mg/kg BW & 5 \\
\hline
\end{tabular}
\caption{The Grouping of Rats and the Treatment of Each Group}
\end{table}

\textbf{Preparation of Curcuminoid Nanoemulsion}

The curcuminoid nanoemulsion formulation used in this study was made by mixing 0.5 g of curcuminoid into a mixture of VCO and tween-80 (ratio 1:4) while stirring and heated at 70 °C for 20 minutes using a magnetic stirrer. Next, phosphate buffer at pH 7 was added dropwise to a final volume of 100 mL. The results of previous studies showed that the characteristics of the curcuminoid nanoemulsion had a particle size of 32.1 nm, a polydispersity index of 0.267, and a zeta potential of -7.7 mV.\textsuperscript{13}

\textbf{General Procedure}

As many as 30 test rats were grouped and put into cages according to their groups in a room with a temperature ranging from 25-30 °C. For 7 days all rats were given standard feed and enough water to drink. During the experiment every day the rats were always weighed to determine the development of the rats and the number of quail egg yolks or the dose of the drug to be given. From day-8 to day-21 (14 days), all mice were induced with a hypercholesterolemic diet (quail egg yolk) orally accompanied by standard feeding and drinking ad libitum. On day-22, blood was taken from the orbital sinus of the eye in all groups of mice. Before the blood was taken, the rats fasted for ± 12 hours while still being given water. Furthermore, the rat blood serum was separated and tested for total cholesterol, triglycerides, LDL (Low-density Lipoprotein), and HDL (High-density lipoprotein). From day-23 to day-48 (26 days) the negative control group was not treated, the positive control group was treated with simvastatin at a dose of 0.9 mg/kg BW, the experimental group-1, -2, -3, and -4 were treated with curcuminoid nanoemulsion from \textit{C. xanthorrhiza} extract at doses of 3, 5, 7.5, and 10 mg/kg BW, respectively. On day-49, the second stage of blood collection was carried out through the orbital sinus of the eye in all groups of rats. Before the blood was taken, the rats had to have fasted for ± 12 hours while still being given water. Furthermore, the analysis of total cholesterol, triglyceride, LDL, and HDL levels of rat blood serum was carried out using the same method as in the first blood collection. Briefly, the treatment procedure is depicted in Table-2.
Table-2: Treatment of the Samples and Procedure of Collecting Data

<table>
<thead>
<tr>
<th>Pra condition of mice (7 days)</th>
<th>Treatment-I (Day-8 to Day-21)</th>
<th>Day-22</th>
<th>Treatment-II (Day-23 to Day-48)</th>
<th>Finish (Day-49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>from day 1 till day 7 (7 days), all rats were induced with a hypercholesterolemic diet (quail egg yolk) orally</td>
<td>- all mice were induced with a hypercholesterolemic diet (quail egg yolk) orally</td>
<td>After fasting (12 h) the blood was taken (I)</td>
<td>- No medication was given to the NC group</td>
<td>After fasting (12 h) the blood was taken (II)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- simvastatin (0.9 mg/kg BW) was given to the PC group</td>
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<td></td>
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<td></td>
<td>- nanoemulsion curcuminoid (3 mg/kg BW) was given to the Exp. group-1</td>
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<td>- nanoemulsion curcuminoid (5 mg/kg BW) was given to the Exp. group-2</td>
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<td>- nanoemulsion curcuminoid (7.5 mg/kg BW) was given to the Exp. groups-3</td>
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<td>- nanoemulsion curcuminoid (10 mg/kg BW) was given to the Exp. groups-4</td>
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</tbody>
</table>

Detection Method

Analysis of Total Cholesterol, Triglycerides, LDL, and HDL

Lipid profile analysis using the laboratory Stanbio Kit procedure. Measurement of total cholesterol level using the enzyme cholesterol-oxidase phenol aminophenazone (CHOD-PAP), and triglyceride levels using the enzyme glycerol-3-phosphate oxidase phenol aminophenazone (GPO-PAP) were recorded spectrophotometrically. The blood serum obtained was centrifuged at 1000 rpm for 10 minutes. The corresponding plasma that has been separated was taken using a pipette and put into an Eppendorf- tube and tightly closed. LDL cholesterol fraction was precipitated from serum by the addition of magnesium chloride reagent. HDL cholesterol was then determined in the supernatant liquid using a cholesterol reagent, and the dilution factor was considered in the calculation. The principle of the CHOD-PAP method is that cholesterol esters are hydrolyzed into free cholesterol by the enzyme of cholesterol esterase. Free cholesterol is oxidized by the enzyme of cholesterol oxidase so that it turns into cholesterol-4-en-3-on along with the production of H₂O₂ which then reacts by oxidative coupling with 4-amino antipyrine and phenol with the help of a peroxidase catalyst to form a red colored quinoline chromogen compound. The absorption of quinoline compounds was measured with a spectrophotometer at a maximum wavelength of 546 nm and the amount of quinoline compound was proportional to the level of cholesterol in the serum. Cholesterol, triglyceride, and HDL levels were measured using an automated clinical analyzer, while LDL was obtained using the formula:

\[
LDL (\text{mg/dL}) = \text{Total cholesterol} - \text{HDL} - (\text{Triglycerides}/5)
\]

Statistical Analysis

All data were presented as the mean ± standard deviation (SD), and they were then analyzed descriptively and statistically with SPSS 26.0 program. One Way Analysis of Variance (ANOVA) was followed by the Paired Sample t-test to determine the differences between each treatment group before and after treatment, with a significance level of 5% (p<0.05).

RESULTS AND DISCUSSION

The results of the analysis of rat blood serum lipid profiles consisting of total cholesterol, triglycerides, LDL, and HDL of rat blood serum before (I) and after (II) treatment-II are presented in graphical form in Fig.-1.

Rat blood was taken twice, namely after being given egg yolks for 14 days (before treatment II) and after being given treatment II for 26 days. On day 25, one rat died in experimental group 3, so this rat was removed from the group and not included in the calculation. Data were analyzed by quantitative descriptive and one-way ANOVA statistical analysis. The mean levels of total cholesterol, triglycerides, LDL, and HDL of rat blood serum before and after treatment were compared and the decreasing/increasing percentage was calculated. This data shows a decrease in cholesterol by 10.8% in the positive control group.
In experimental group-4, the administration of curcuminoid nanoemulsion at a dose of 10 mg/kg BW shows a decrease of 11.2%, while the other groups did not. There was also a decrease in triglyceride levels by 14.9% and LDL by 21.5%, while HDL increased by 0.5%. The lipid profile of rat blood serum before and after treatment-II was then statistically analyzed using Way ANOVA. The results of the analysis showed that there was no significant difference (p>0.05) in total cholesterol and HDL levels before and after treatment-II in each group of rat blood serum, so the analysis was not continued with a different test in each group. For the triglyceride and LDL levels, there was a significant difference (p<0.05) between the groups before and after treatment. Triglyceride levels before and after treatment showed an increase in each group, except the experimental group-4 which was given a curcuminoid nanoemulsion at a dose of 10 mg/kg BW. This indicates that the administration of curcuminoid nanoemulsion at a dose of 10 mg/kg BW gave a better effect in reducing triglyceride levels in the blood serum of rats. Hypercholesterolemia can occur when cholesterol levels in the blood exceed normal limits it will cause an accumulation of cholesterol in the walls of blood vessels. Hypercholesterolemia can be caused by a combination of environmental and genetic factors. Environmental factors include dietary regulation, and in this study, each group of rats was fed quail egg yolk for 14 days. The condition of hypercholesterolemia in rats is comparable with high levels of total cholesterol, LDL cholesterol, and triglycerides in the blood and low levels of HDL cholesterol. Mice with total cholesterol levels of more than 54 mg/dL and LDL levels of more than 27.2 mg/dL indicated that they had hypercholesterolemia. Based on these data, it shows that after being induced with a high-cholesterol diet in the form of egg yolks for 14 days, all groups of rats showed total cholesterol levels of more than 54 mg/dL. Meanwhile, the LDL level was still lower than 27.2 mg/dL except for the rats in the negative control group. Similarly, the measurement of LDL levels after treatment showed low concentrations in all groups of rats except the negative control group which showed very high LDL levels, namely 60.58 ± 14.46 mg/dL.
and the experimental group-1 was 58.55 ±24.39 mg/dL. Simvastatin is a statin drug that is widely used in the treatment of hypercholesterolemia. The mechanism of action of simvastatin is to inhibit cholesterol biosynthesis through the inhibition of the enzyme 3-hydroxy-3-methylglutaryl reductase. Simvastatin administration to humans with hypercholesterolemia is 10 mg/kg BW, so the dose given to rats is 0.9 mg/kg BW. After the treatment, it is expected that the blood serum cholesterol levels of rats in the positive control and experimental groups will decrease. This cholesterol reduction includes a decrease in total cholesterol, LDL, and triglycerides as well as an increase in HDL levels. Previous research using C. xanthorrhiza powder in vivo in the test of animals showed a decrease in blood cholesterol, triglyceride, HDL, and LDL levels in rats during four weeks of treatment at a dose of 200 mg/kg BW. In this study, administration of curcuminoïd nanoemulsion at a dose of 10 mg/kg BW for 26 days of treatment resulted in a decrease in cholesterol levels by 11.2%, triglycerides by 14.9%, and LDL by 21.5%. The administration of simvastatin at a dose of 0.9 mg/kg BW in the positive control group showed a decrease in cholesterol levels of 10.2%, triglyceride levels increased by 38.7%, LDL decreased by 8.8%, and HDL increased by 1.7%. These data indicate that the administration of curcuminoïd nanoemulsion 10 mg/kg BW is more effective in improving the lipid profile of rat blood serum compared to the administration of simvastatin 0.9 mg/kg BW. Curcuminoïd compounds can reduce hypercholesterolemia in rats induced by a high-fat diet causing an increasing rate of cholesterol catabolism, increasing fecal excretion, and inducing changes in the expression of genes involved in cholesterol homeostasis, as well as inhibiting LDL oxidation. In addition, curcuminoïd can reduce serum lipid peroxide levels, increase HDL-C, and lower serum total cholesterol, as well as regulate LDL receptor expression in cells. The administration of 10 mg/kg BW of curcuminoïd nanoemulsion showed a more effective reduction in cholesterol, triglyceride, and LDL levels compared to the administration of C. xanthorrhiza powder in a previous study. Several other secondary metabolites that have the potential to act as antihyperlipids include flavonoids, saponins, and tannins. This is because curcuminoïd in the form of nanoemulsions can increase absorption, help the dissolution of lipophilic drugs, and are able to increase bioavailability.

CONCLUSION

Curcuminoïd nanoemulsion of C. xanthorrhiza rhizome extract has activity in lowering total cholesterol, triglyceride, and LDL at a dose of 10 mg/kg BW. Administration of curcuminoïd nanoemulsion at a dose of 10 mg/kg BW reduced total cholesterol levels by 11.2%, triglyceride levels by 14.9%, and LDL levels by 21.5% in rat blood serum with fed a high lipid diet. The results showed that curcuminoïd nanoemulsions from C. xanthorrhiza rhizome extract have the potential to be developed as an antihypercholesterol drug.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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