Research Article

Potency of turmeric (Curcuma longa L.) extract and curcumin as anti-obesity by inhibiting the cholesterol and triglycerides synthesis in HepG2 cells

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ABSTRACT

Background: Adipocytes accumulate triacylglycerol when excessive food consumption. Adipocyte dysfunction plays an important role in the obesity development. People with a body weight 40 % heavier than the average body weight population at risk of death two times greater than the average body weight. The use of anti-obesity drugs have many side effects, so it is necessary to find the anti-obesity drug with low toxicity. This ex vivo study was conducted to determine the activity of C. longa L. extract in inhibiting triglycerides and cholesterol synthesis and lipid droplet formation on HepG2 cells compared to curcumin.

Methods: Anti-obesity activity includes reduced formation of lipid droplet in HepG2 cells can be observed using oil red O staining method. The measurement of triglyceride level was performed according to Randox protocol using Randox TR 210 assay kit. Lipolytic activity by measuring cholesterol levels was performed based on Randox CH 200 kits.

Results: This study suggested that the extract of C. longa L. and curcumin have potential anti-obesity compounds. C. longa L. extract have higher activity in inhibiting triglycerides and cholesterol synthesis compared to curcumin with inhibition activities 70.43% and 66.38% respectively in the highest concentration.

Conclusion: The C. longa extract posses the anti-adipogenesis potential on inhibiting the synthesis of triglycerides and cholesterol and lipid droplet formation in HepG2 cell as anti-obesity parameters better than curcumin.

Keywords: Anti-obesity, Triglycerides, Cholesterol, C. longa L., Curcumin, HepG2

INTRODUCTION

Preventing the epidemic of obesity become one of the greatest public health challenges in the first half 21st century. In Indonesia, diabetes, which is associated with the increase in over weight and obesity is a major disease in terms of health costs, and this replacing infectious diseases.¹ Obesity is characterized by cellular fat content increased both the number and size of adipocytes cells that undergo differentiation of cells in tissue pre-adipocyte.² Adipocyte tissues as a part of endocrine and paracrine organ that releases active cytokines and a large number of active peptides including leptin and adiponectin, Plasminogen Activator Inhibitor-1 (PAI-1), angiotensinogen, IL-6, and TNF-α.³

The accumulation of fat can be determined by measuring the balance of synthesis and degradation of fat. Lipogenesis includes the process of fatty acid synthesis and subsequent triglyceride synthesis in the liver and
adipose tissue. Synthesis occurs in the cytoplasm, while degradation occurs in the mitochondria. Several enzymes involved in fatty acid synthesis enzymes are grouped into multi complex called fatty acid synthesis enzymes include: sterolregulatory element binding protein-1c (SREBP-1c), Malonyl CoA, Decarboxylase (MCD), Acetyl CoA Carboxylase-l (ACC-1), Fatty Acid Synthase (FAS). Lipolysis is the decomposition of fat in fat cells, during the process of decomposition of the free fatty acids are released in the bloodstream and circulated in the body. Triglycerides undergo lipolysis (hydrolysis by lipases) into fatty acids. The enzyme Carnitine Palmitoyl Transferase lipolysis include-I (CPT-1), Acyl CoA Oxidase-1 (ACO-1), uncoupling proteins (UCPS). Excessive adipose tissue can cause insulin resistance, thereby increasing the risk of cardiovascular diseases and diabetes melitus-2, so it is important to inhibit proliferation and apoptosis preadipocytic network. Various types of drugs have been studied, these days, many research studies that focus on herbal search as antiobesity because herbal remedies are natural, cheap and low side effects. Various types of herbs studied as antiobesity is safe materials and low toxicity.

Extract of turmeric (Curcuma longa L.) are safe herbal, low toxicity so that it can be developed as an anti-obesity. Turmeric extract containing 70-76% curcumin, 16% demethoxycurcumin, and 8% bisdemethoxycurcumin. Curcumin showed a broad-spectrum therapeutic agent are antiinflammatory, antitumor, antiviral, antifungal and hepatoprotective. Curcumin dose of 100-200 mg/kg in mice were able to inhibit the antiinflammatory arachidonic acid metabolism, cyclooxygenase, lipoxygenase, cytokines product (IL and TNF). Plant extracts as obesity drug candidates have high activity when the antilipase activity is about 75%-100% (high activity), moderate activity (50%-70%), low activity (25%-50%) and no antilipase activity if it is less than 25%. Lipase catalyses the hydrolysis of triglycerides that produces fatty acids. Ingredients or plant extracts as antiobesity have high activity if it is less than 25%. Incubated for 24 hours at 37°C humidified atmosphere and 5% CO₂. Cells then digested by trypsin-EDTA after confluent and harvested in 2500 rpm centrifuge for 4 minutes. Cell cultures were grown in 6 well plate (5x10⁵ cells/well) then grown in DMEM containing 2% calf serum. Medium then discharged and supplemented with starving medium (DMEM + 1% antibiotic solution) then incubated for 24 hours. Starving medium then discharged and supplemented with induction medium (DMEM, 1:2 of 1mM palmitic acid: 1 mM linoleic acid, BSA, and treatment). Curcumin extract was used for the treatment in various concentrations (250 µg/mL; 125 µg/mL; 62.5 µg/mL; 31.25 µg/mL; and 15.63 µg/mL). Cells then incubated in 37°C humidified atmosphere and 5% CO₂.

Oil red-O staining

The HepG2 cells after adipocyte differentiation induction and C. longa extract and curcumin treatment were

**METHODS**

**Materials**

The materials used in the study consisted of turmeric extract. Dimethylsulfoxide (DMSO) was purchased from Merck Co. (Darmstadt, Germany). Dulbecco’s modified Eagle’s medium (DMEM - High glucose), bovine calf serum, and antibiotic mixture (penicillin-streptomycin). The HepG2 cells obtained from Stem Cells and Cancer Institute, Jakarta, Indonesia, crude porcine pancreatic lipase type II (Sigma EC 3.1.1.3), tris - HCl buffer, p-nitrophenol butyrate (PNPB), acetonitrile (Sigma Aldrich), triglycerides kit (Randox - TR - 210), cholesterol kit (Randox CH 200), high glucose DMEM w/L-glutamine w/sodium pyruvate (Biowest), Calf serum, fetal bovine serum (Chile), oleic acid 10 mL, 10 mL palmitic acid, phosphate buffer saline, DMSO 100%, formaldehyde, lysis buffer, 70% ethanol, oil red O, trypsin EDTA, penicillin-streptomycin, Bovine Serum Albumin (BSA) (Biowest). The tools used in this study are the autoclave (All American), CO₂ incubator (ESCO), laminar air flow (ESCO) cabinet, sixwellplate, blue tip, yellow tip, serological pipette 5 ml, 2 ml serological pipette, 15 ml falcon, falcon 50 ml, 5 ml syringe, syringe filter, microtube 1.5 ml, cuvette 1 box, T flask 25 ml, 75 ml T flasks.

**Extraction procedure and sample preparation**

Turmeric rhizomes (C. longa L.) were collected from farmer plantation location in Bogor, West Java, Indonesia (May 2009). The plants were identified by staff herbarium, department of biology, school of life Science and technology, Bandung institute of technology, Bandung, West Java, Indonesia. Rhizomes were chopped and dried using drying device (40°C-45°C) until achieving the stable water level (±13%). The dried rhizomes were milled to produce 60 mesh sizes of flour. Dried, flour materials were extracted with distilled ethanol by maceration extraction, filtered and evaporated using rotatory evaporator at 40°C-45°C.

**HepG2 cell culture and adipocyte differentiation induction**

HepG2 cells (human liver hepatocellular carcinoma cell line) cultured in DMEM (Dulbecco’s Modified Eagle Medium, Biowest) supplemented with 10% FBS (Fetal Bovine Serum, Biowest) and 100 U/mL penicillin-streptomycin (Biowest) then incubated for 24 hours at 37°C humidified atmosphere and 5% CO₂. Cells then digested by trypsin-EDTA after confluent and harvested in 2500 rpm centrifuge for 4 minutes. Cell cultures were grown in 6 well plate (5x10⁵ cells/well) then grown in DMEM containing 2% calf serum. Medium then discharged and supplemented with starving medium (DMEM + 1% antibiotic solution) then incubated for 24 hours. Starving medium then discharged and supplemented with induction medium (DMEM, 1:2 of 1mM palmitic acid: 1 mM linoleic acid, BSA, and treatment). Curcumin extract and curcumin was used for the treatment in various concentrations (250 µg/mL; 125 µg/mL; 62.5 µg/mL; 31.25 µg/mL; and 15.63 µg/mL). Cells then incubated in 37°C humidified atmosphere and 5% CO₂.
washed with PBS 2X. The cells then fixed for 30 s with 70% ethanol in PBS 2X. Cells were washed with double distilled sterile (dds) water and stained for 2 hours by complete immersion in a working solution of Oil Red O (Milipore ECM950). Cells then washed in 50% ethanol for 3 seconds and dds. Cell then observed under the inverted light Olympus microscope after the Oil-red O staining to compare the lipid droplet formation of normal cells and treated cells.\(^{15,16}\)

**Triglycerides assay**

The triglyceride levels was measured from cell lysate after *C. longa* extract and curcumin treatment as sample.

\[
\text{Triglyceride level} = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \times \text{standard concentration (mM)…. (equation 1).}
\]

**Cholesterol assay**

Randox CH 200 kits was used for the quantitative *in vitro* determination of cholesterol level in cell lysate treated by *C. longa* extract and curcumin as sample based on a standard. The cholesterol was determined after enzymatic hydrolysis and oxidation.\(^{11}\) Briefly 500 µL mix reagent was added into 24 well plate. 5 µL sample was added into the sample well. 5 µL of ddH\(_2\)O was used as blank sample. 5 µL standard solution was added into the standard well. Standard reaction was prepared in seven different concentrations using serial dilution (5.170; 2.585; 1.293; 0.646; 0.323; 0.162; and 0.081 mmol/L). The absorbance was measured in 500 nm of wave length. Cholesterol concentration was calculated using the equation 1. The triglyceride inhibition activity was measured based on the triglyceride level in the positive control.

\[
\text{Cholesterol concentration (mg/dL)} = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \times \text{standard concentration (mM)…. (equation 2).}
\]

**Statistical analysis**

Data was presented as means ± standard deviation. Statistical comparisons were performed using One Way ANOVA statistics methods and Tukey’s HSD Post Hoc test by SPSS software (version 20.0). P values <0.05 were considered as statistically significant.

**RESULTS**

**Comparison of lipid droplet formation on HepG2 cells treated by *C. longa* L. extract and curcumin using oil red O staining**

Assessment of the anti-obesity potential by anti-adipogenesis parameters of *C. longa* L. extract compared to curcumin in this study was also performed using oil red O staining method to detect lipid droplet formation in HepG2 cells line (Figure 1, 2, 3) The results show that the number of lipid droplet formation in HepG2 cells treated with the extracts of *C. longa* L. and curcumin were less than the control. It shows that both treatments can inhibit the formation of lipid droplet in HepG2 cells.

The measurement was performed according to Randox protocol using Randox TR 210 assay kit. The triglyceride level was determined after enzymatic hydrolysis with lipases.\(^{17,18}\) Briefly 450 µL reagent with 5 µL sample was added into the 24-well plate then incubated at 37°C for 5 minutes. ddH\(_2\)O was used in blank well and standard reagent was used for standard well. Standard reaction was prepared in seven different concentrations using serial dilution (2.180; 1.090; 0.545; 0.273; 0.136; 0.068 and 0.034 mmol/L). The absorbance was measured in 500nm of wavelength. Triglyceride concentration was calculated using the equation 1. The triglyceride inhibition activity was measured based on the triglyceride level in the positive control.

Staining results also indicate a difference in the number of lipid droplets generated on *C. longa* L. extract and curcumin treatment. Lipid droplets in HepG2 cells treated with *C. longa* L. extract lower than curcumin. This result shows the potential of *C. longa* L. extract as anti-adipogenesis relatively better compared with curcumin.

*Figure 1: Oil red O-staining to detect the lipid droplet formation (showed by the black arrow) for control in HepG2 cells line.*
Figure 2: Oil red O-staining to detect the lipid droplet formation (showed by the black arrow) for *C. longa* in HepG2 cells line.

Figure 3: Oil red O-staining to detect the lipid droplet formation (showed by the black arrow) for curcumin in HepG2 cells line.

*C. longa* L. extract and curcumin effect on levels of triglycerides in HepG2 cells

High plasma triglycerides (TG) is associated with obesity.\(^\text{19}\) Antiobesity potential of *C. longa* L. extract inhibits the production capability or the synthesis of TG in HepG2 cells induced by linoleic and palmitic acids. TG level and activity of TG inhibition can be seen in Table 1.

The research results showed that the effect of turmeric extract on the synthesis of triglycerides compared to curcumin in HepG2 cells. Turmeric extract may inhibit the production of triglycerides in HepG2 cells stronger than the curcumin in a concentration dependent manner. The greatest inhibition of TG production shown by concentration of 250 µg/mL, while the lowest inhibition shown by the smallest concentration of 15.63 µg/mL.

Table 1: TG level (mg/dl) and TG inhibition (%) of *C. longa* L. extract and curcumin.

<table>
<thead>
<tr>
<th>Samples</th>
<th>TG level (mg/dL)</th>
<th>TG inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. longa</em> L. extract 250 µg/mL</td>
<td>27.43±3.12 a</td>
<td>70.43±3.37 h</td>
</tr>
<tr>
<td><em>C. longa</em> L. extract 125 µg/mL</td>
<td>36.72±2.82 ab</td>
<td>60.41±3.04 fg</td>
</tr>
<tr>
<td><em>C. longa</em> L. extract 62.5 µg/mL</td>
<td>50.48±5.01 cd</td>
<td>45.57±5.40 de</td>
</tr>
<tr>
<td><em>C. longa</em> L. extract 31.25 µg/mL</td>
<td>58.93±3.62 de</td>
<td>36.46±3.90 cd</td>
</tr>
<tr>
<td>Curcumin 250 µg/mL</td>
<td>33.47±4.77 ab</td>
<td>63.92±5.14</td>
</tr>
<tr>
<td>Curcumin 125 µg/mL</td>
<td>42.64±5.59bc</td>
<td>54.00±6.03</td>
</tr>
<tr>
<td>Curcumin 62.5 µg/mL</td>
<td>56.91±2.30 de</td>
<td>38.64±2.48 cd</td>
</tr>
<tr>
<td>Curcumin 31.25µg/mL</td>
<td>65.14±5.91 e</td>
<td>29.77±6.37 c</td>
</tr>
<tr>
<td>Curcumin 15.63 µg/mL</td>
<td>78.15±4.66 f</td>
<td>15.74±5.03 b</td>
</tr>
<tr>
<td>Positive control</td>
<td>92.75±4.55 g</td>
<td>0.00±4.90 a</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. Different letters in the same column (among samples in TG level and TG inhibition) are significant at P <0.05 (Tukey’s HSD post hoc test)

*C. longa* L. extract and curcumin effect on levels of cholesterol in HepG2 cells

The total cholesterol level measurement, is one of the most commonly examined measurement in lipid.\(^\text{20}\) *C. longa* L. extract and curcumin posses the inhibitory activity on cholesterol level in concentration dependent manner. *C. longa* posses the stronger cholesterol inhibitory activity compared to curcumin.

Table 2: Cholesterol level (mg/dl) and cholesterol inhibition (%) of *C. longa* extract and curcumin.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Cholesterol level (mg/dL)</th>
<th>Cholesterol inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. longa</em> extract 250 µg/mL</td>
<td>46.70±1.11 a</td>
<td>66.38±0.80 g</td>
</tr>
<tr>
<td><em>C. longa</em> extract 125 µg/mL</td>
<td>62.63±1.40 b</td>
<td>54.91±1.01 f</td>
</tr>
<tr>
<td><em>C. longa</em> extract 62.5 µg/mL</td>
<td>70.70±3.13 bc</td>
<td>49.10±2.26 ef</td>
</tr>
<tr>
<td><em>C. longa</em> extract 31.25 µg/mL</td>
<td>78.06±2.87 cd</td>
<td>43.80±2.07 de</td>
</tr>
<tr>
<td><em>C. longa</em> extract 15.63 µg/mL</td>
<td>86.03±2.88 de</td>
<td>38.06±2.07 cd</td>
</tr>
<tr>
<td>Curcumin 250 µg/mL</td>
<td>48.15±0.57 a</td>
<td>65.33±0.41 g</td>
</tr>
<tr>
<td>Curcumin 125 µg/mL</td>
<td>78.36±3.13 cd</td>
<td>43.58±2.25 de</td>
</tr>
<tr>
<td>Curcumin 62.5 µg/mL</td>
<td>87.63±5.22 de</td>
<td>36.91±3.76 cd</td>
</tr>
<tr>
<td>Curcumin 31.25µg/mL</td>
<td>96.26±2.50 e</td>
<td>30.74±1.80 c</td>
</tr>
<tr>
<td>Curcumin 15.63 µg/mL</td>
<td>110.98±7.13 f</td>
<td>20.09±5.13 b</td>
</tr>
<tr>
<td>Positive control</td>
<td>138.89±4.56 g</td>
<td>0.00±3.28 a</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation. Different letters in the same column (among samples Cholesterol level and Cholesterol inhibition) are significant at P <0.05 (Tukey’s HSD post hoc test)
DISCUSSION

High fat diet associated with obesity correlated with elevated level of the cholesterol and triglycerides that caused adipogenesis. Differentiation process of fat cell known as adipogenesis is controlled by hormonal-induced coordinate expression and activation of peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer-binding protein (C/EBP). Accumulation of intracellular triglycerides ultimately gives rise to the morphologically distinct fat cell. Inhibiting the synthesis of cholesterol and triglycerides as anti-adipogenesis become an important role in anti-obesity agent. Several modes of action from natural products that have potential to treat obesity, such as metabolic stimulants, appetite suppressants, starch blockers, glucose/insulin metabolism, lipid metabolism, and adipocyte-specific effects.

In this ex vivo study using HepG2 as a model showed that the extract of turmeric and curcumin have potential anti-obesity. It is shown from the results of studies showed that turmeric extract and curcumin can inhibit the synthesis of TG and cholesterol and also the lipid droplet formation in HepG2 cells. Human hepatoma HepG2 cells is the most suitable and accessible human-derived cells that retain many of the biochemical functions of human liver parenchymal cells for the ex vivo study including anti-obesity screening. Curcumin is a polyphenol which can be isolated from Curcuma longa (turmeric). Tumeric contains curcumin as the main active constituent (77%), in addition to demethoxycurcumin, bisdemethoxycurcumin and cyclocurcumin. All four components together are termed curcuminoids. In this study, turmeric extract showed the higher inhibitory activity on TG and cholesterol synthesis that caused adipogenesis compared to curcumin.

The decreasing of TG level indicated the inhibition of adipogenesis caused obesity. Adipocyte normally contain free cholesterol and will redistributed from the plasma membrane to the lipid droplet as the increasing of TG storage. The increasing of adipocyte cholesterol level was proportional with TG level. The potential of curcumin in turmeric extract in preventing obesity is well documented. The previous study showed that curcumin, at a dose of 0.05 g/100 g, produces a hypolipidemic effect on hamsters fed on a high-fat diet. There was also a significantly decrease in hepatic cholesterol and TG level, and an increase in fatty acid beta-oxidation. Curcumin was reported to down-regulate the genes expression involved in energy metabolism and lipid accumulation, and decreasing the level of intracellular lipids. Moreover, curcumin suppresses angiogenesis, which is essential for tissue growth. Several key genes responsible for adipogenesis and lipogenesis, such as PPARγ and C/EBPα, were also observed to express at a much lower level. In an ex vivo study on 3T3-L1 adipocytes, curcumin, at 5-20 μM, prevented differentiation and caused apoptosis of the adipocytes. In the same report, supplementing high-fat diet mice with 500 mg/kg of curcumin for 12 weeks resulted to lower body weight gain, adipocyte and microvessel growth in adipose tissue. There was also a notable to increase the fatty acid oxidation. The effect of anti-obesity turmeric were also studied in mouse model. The result significantly lower the body weight gain and retroperitoneal and epididymal adipose tissue weights compared to the control group. Additionally, total cholesterol and TG levels in serum and liver were significantly decreased when compared to those of the control group, whereas the high-density lipoprotein-cholesterol level was significantly increased. Our ex vivo study suggests that C. longa L. extract has potent anti-obesity effects by inhibit the cholesterol and TG that led adipogenesis in HepG2 cells better than curcumin. However, in vivo test in an animal model still needed to confirm the anti-obesity activity of the C. longa extract. The further mode of action test, preclinical and clinical studies should be pursued before pharmaceutical applications.

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