Global Medical and Health Communication

Online submission: https://ejournal.unisba.ac.id/index.php/gmhc
DOI: https://doi.org/10.29313/gmhc.v11i3.10993

GMHC. 2023;11(3):159–163
pISSN 2301-9123 | eISSN 2460-5441

RESEARCH ARTICLE

The Effect of Turmeric and Mangosteen Peel on Rat PPARα Gene Expression Induced by High-Fat Diet

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Abstract

High levels of fat in the blood are a risk factor for nonalcoholic steatohepatitis liver disease. Indonesian medicinal plants that can decrease blood fat levels are turmeric and mangosteen peel. One of the mechanisms of blood fat-lowering drugs is to increase the expression of the PPARα gene. The purpose of this study was to assess the effect of turmeric and mangosteen peel on the expression of the PPARα gene in the rat liver induced by a high-fat diet. This research was conducted at Maranatha Biomedical Research Laboratory in February–October 2021, using male Wistar rats that were divided into 5 groups (n=5): negative control groups (no treatment), positive control groups (high-fat diet), turmeric, mangosteen, and fenofibrate groups, that was given high-fat diet continued by ethanol extract of turmeric, ethanol extract of mangosteen peel, and fenofibrate. At the end of the study, the animals were terminated, and the liver was extracted for RNA extraction and semi-quantitative PCR. The results showed that there was an increase in PPARα gene expression in the turmeric group and fenofibrate group, which were significantly different from the positive control group that received a high-fat diet ($p<0.05$) and between the fenofibrate group compared to negative controls that received standard chow diet ($p<0.05$). In conclusion, turmeric and fenofibrate are suggested to increase the expression of the PPARα gene in the liver induced by a high-fat diet.

Keywords: Mangosteen peel, PPARα, turmeric

Introduction

High levels of fat in the blood are a risk factor for cardiovascular diseases such as coronary heart disease, stroke, and nonalcoholic steatohepatitis liver disease.[1] Therefore, high-fat content must be overcome. Drugs that can reduce fat levels include fibrate-group drugs, such as fenofibrate.[2] Indonesians often use medicinal plants to lower blood fat levels. Examples of medicinal plants that can decrease blood fat levels are turmeric and mangosteen peel.[3–5] One of the mechanisms of blood fat-lowering drugs is by increasing the expression of the PPARα gene.[6]

Peroxisome proliferation activating receptors-alpha (PPARα) is a part of the superfamily of nuclear hormone receptors. PPARα is expressed in many body cells, such as cardiomyocytes, brown adipocytes, renal tubular cells, and hepatocytes in the liver. The liver is essential in energy homeostasis, especially in regulating lipid metabolism.[7] Its role in lipid metabolism is influenced by PPARα, which can induce oxidation of liver fatty acids, which reduces excess lipid accumulation in the liver and eventually prevents fatty liver.

Previous studies have shown that PPARα upregulated medium-chain and long-chain acyl coenzyme-A (CoA) dehydrogenase, carnitine palmitoyltransferase 1A (CPT1A), and fatty-acid binding protein 1 (FABP1), proved its crucial role in fatty acid clearance and catabolism in the liver.[7] Furthermore, PPARα is also involved in sphingolipid synthesis through modulating serine palmitoyltransferase (SPT) enzyme in mouse liver.[8]

Modulators of PPARα could be found in herbal ingredients and synthetic compounds such as the fibrate class of drugs to treat hyperlipidemia.[9] Natural compounds such as terpenes, carotenoids, phenylpropanoids, and polyphenols have been investigated for their role in modulating PPARα.[9] As primary polyphenols isolated from *Curcuma longa*, curcumin in turmeric has a potential

Received: 20 December 2022; Revised: 28 August 2023; Accepted: 11 December 2023; Published: 19 December 2023

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role as a PPARα modulator.[10] Phenolic acids and flavonoids in mangosteen peel ethanol extract also have a potential role in modulating PPARα.[11] The investigation of the role of turmeric and mangosteen peel in modulating PPARα expression in the liver still needs to be completed. Therefore, the purpose of this study was to assess the effect of turmeric and mangosteen peel on the expression of the PPARα gene in the rat liver induced by a high-fat diet.

Methods

Eight-week-old male Wistar rats weighing 200± 20 g were divided into five groups (n=5): negative control groups, positive control groups, turmeric, mangosteen peel, and fenofibrate groups. The negative control group was given no treatment, and the positive control was given a high-fat diet. The turmeric group was assigned a high-fat diet continued by ethanol extract of turmeric 270 mg/kgBW/day orally. The mangosteen peel group was given a high-fat diet, continued by ethanol extract of mangosteen peel 270 mg/kgBW/day orally. In contrast, the fenofibrate group was assigned a high-fat diet, continued by fenofibrate 15 mg/kgBW/day orally. All Wistar rats were housed at room temperature with 12 hours of light and dark cycles daily in Maranatha Biomedical Research Laboratory from February to October 2021. All procedures were based on the use and care of laboratory guidelines. After 2 weeks of the adaptation period, the animals were given seven weeks of a high-fat diet, followed by seven weeks of turmeric, mangosteen peel, and fenofibrate treatment. At the end of the study, the animals were terminated, and the liver was extracted for RNA extraction and semi-quantitative PCR. Approval of all protocols was based on the Research Ethics Committee of the Faculty of Medicine, Universitas Kristen Maranatha No: 131/KEP/IX/2022. The high-fat diet used in this study was obtained from PT Prospet consists of 34.9 gm% fat, 26.2 gm% protein, and 26.3 gm% carbohydrate.[12]

Extract ethanol of turmeric and mangosteen was obtained from PT Sidomuncul. Every turmeric capsule was composed of 500 mg *Curcuma domesticate rhizoma*, equivalent to 100 mg curcuminoid, and this dose was equal to 40 grams of fresh turmeric. While every mangosteen peel capsule was composed of 400 mg *Garcinia pericarpium*, and this dose was equivalent to 10 grams of fresh mangosteen peel. Fenofibrate dose 15 mg/kgBW/day obtained from fenofibrate capsules 100 mg. All treatments were given for 7 weeks.

Frozen liver tissues from the rats were extracted to obtain a good quality of RNA, using Trisure reagent (Bioline, United Kingdom), following its manufacturer's instruction. The quality (purity and concentration) of the RNA was measured using spectrophotometry at 268/280 nm absorbance (Multiscan Go). Then, the procedure was followed by semi-quantitative PCR, where we used The One Step RT PCR Kit (Bioline, United Kingdom). GAPDH was used to normalize the data in this study as a housekeeping gene. The process was then followed by electrophoresis, and in order to visualize the electrophoresis gels, we used the BluePad Detection System. This process was followed by quantifying each PCR band using Image J software.

The list of primer sequences used in the study was provided in Table. The design of the study was randomized design for grouping the animals, and the assessment was done at the end of the experiment. Statistical analysis was done using SPS 26.0, and normality and homogeneity tests were done before comparing the differences between groups using one-way ANOVA followed by post hoc LSD.

Table Primers Used for Semi-Quantitative-PCR Analysis

Gene Symbol	Primer Sequence (5' to 3') Upper Strand: Sense Lower Strand: Antisense	Product Size (bp)
PPARα	ACGATGCTGTCCTCCTTGATG	407
	GCGTCTGACTCGGTCTTCTTG	
GAPDH	GTTACCAGGGCTGCCTTCTC	177
	GATGGTGATGGGTTTCCCGT	

Results

In this study, we found a significant result of the ANOVA test (p=0.029) when comparing the relative ratio of PPARα gene expression between groups after induction of a high-fat diet. A post hoc LSD test was used to compare the differences between groups. The result showed significant increases of PPARα gene expression in turmeric (a, p=0.034) and fenofibrate (b, p=0.004) groups compared to positive control groups and between fenofibrate group and negative control group (c, p=0.015). The PCR band expression of PPARα is shown in Figure 1, while the graphical result of the relative ratio is shown in Figure 2.

Discussion

The results showed that there was an increase in PPARα gene expression in the turmeric group and fenofibrate group which were significantly different from the positive control group that received high-fat feed (p<0.05), there was also an increase in PPARα gene expression in the fenofibrate group compared to negative controls that received standard feed (p<0.01). It means that turmeric and fenofibrate increased the expression of the PPARα gene, thus the mechanism of decreasing blood fat levels in this study through increased expression of the PPARα gene. PPAR is a group of receptors involved in metabolic diseases such as hyperlipidemia, diabetes, and obesity.[13] PPAR is a receptor found in vertebrate creatures containing Zn. The PPAR family consists of three members: PPARα, PPARδ (PPARβ), and PPARγ. PPARα can be activated by natural and synthetic agents, such as PUFAs, eicosanoids, and hypolipidemic drugs (fibrates). Activating PPARα by various ligands can modify critical biological processes,

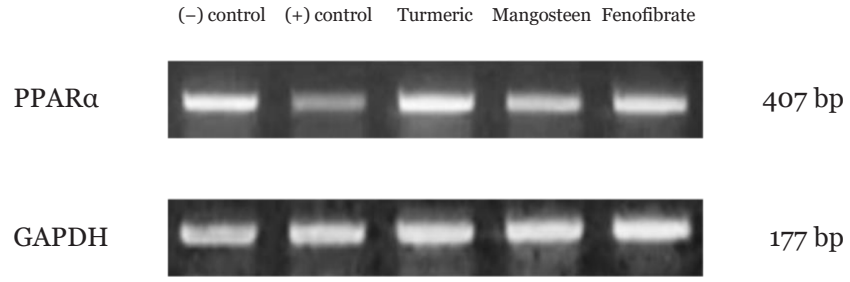

Figure 1 PCR Band of PPARα Gene Expression

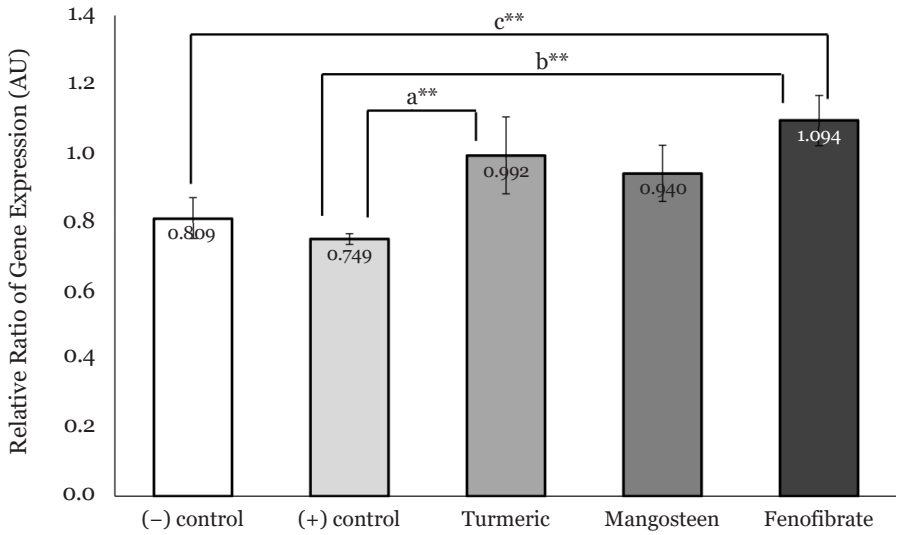

Figure 2 Relative Ratio of PPARα Gene Expression
Note: *significant (p<0.05), **very significant (p<0.01)

especially those related to the body's energy production mechanisms and inflammatory responses.[14] PPARα inside the liver organs are activated by fasting. PPARα is a molecular target for hypolipidemic drug fibrate, which is used in treating dyslipidemia.[15,16] Control of metabolic syndrome and dyslipidemia that can cause atherosclerosis is carried out by the peroxisome proliferator-activator receptors family (PPARs). PPARα is expressed in high concentrations in liver cells, intestinal cells, monocytes/ macrophages, smooth muscle cells, endothelial cells, lymphocytes, microglia, and astroglia. PPARα activation affects lipoprotein metabolism and reduces dyslipidemia associated with metabolic syndrome and hypertension.[17] PPARα activation also causes inhibition of expression of nuclear factor κB (NF-κB) or inflammatory protein activator 1.[18,19] Fibrate a PPARα agonist and have been used to treat dyslipidemia, studies have also proven PPARα agonists have anti-inflammatory that suppresses pro-inflammatory cytokines interleukin-1 beta, TNFα, ICAM-1 and anti-thrombotic effects.[20–22] PPARα receptors are commonly found in liver cells and play a role in fat metabolism, including the degradation of fatty acids.[23] PPARα is a transcription factor that regulates the transcription of mitochondrial and peroxisomal genes β-oxidation and inflammatory reactions. Research shows that PPARα expression is significantly reduced in nonalcoholic steatohepatitis liver disease patients (NASH).[24] Agents that increase the expression of the PPARα gene are expected to prevent nonalcoholic steatohepatitis liver disease patients.[25,26]

Conclusion

The conclusion of this research is turmeric and fenofibrate are suggested to increase the expression of the PPARα gene in Wistar rat liver induced by a high-fat diet.

Conflict of Interest

The authors declared no conflict of interest.

Acknowledgment

This study is supported by Hibah Internal from Maranatha Christian University to DKJ and HY with 034/SK/ADD/UKM/2021.

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