

Turmeric and Mangosteen Extract Modulate Autophagy Gene Expression in High-Fat Diet-Induced Rats

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Abstract

Background: High-fat diet (HFD) increases the risk of obesity, metabolic syndrome, coronary artery disease, and chronic kidney disease, resulting in lipotoxicity. Turmeric and mangosteen are two ingredients mostly used in Indonesian food and are known for their antihyperlipidemic and antioxidant effects. The aim of this study was to explore the effect of turmeric and mangosteen on histopathology and autophagy gene expression in HFD-induced kidneys in rats model.

Methods: The study was an experimental study, including 25 male Wistar rats aged 8 weeks, divided into 5 groups with a completely randomized design; a group with a standard diet was the negative control group, the group with a high-fat diet was a positive control group and the HFD groups with turmeric or mangosteen or fenofibrate. The study was conducted in Maranatha Biomedical Research Laboratory from January to November 2022. Autophagy gene expression (LC3, p62) was measured along with the histopathological scoring to observe necrosis, inflammation, and fat degeneration state. Data was analyzed using One Way ANOVA or Kruskal Wallis and post hoc Least Significant Difference or Mann Whitney.

Results: There were significant differences in inflammation in groups treated with mangosteen ($p=0.007$); and in fat degeneration in groups treated with mangosteen and fenofibrate ($p=0.007$). Furthermore, the LC3 gene expression was increased in all HFD groups accompanied by the decrease of the p62 gene expression in groups treated with turmeric ($p=0.020$) and fenofibrate ($p=0.005$).

Conclusion: Mangosteen decreases inflammation and fat degeneration scoring, while turmeric increases autophagy in the kidneys of HFD-induced Wistar rats.

Keywords: Autophagy, fat degeneration, HFD, inflammation, kidney

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Introduction

The worldwide increase in high-fat diets (HFD) has contributed to the incidence of obesity, diabetes, coronary artery disease, chronic kidney disease (CKD), and others.^{1,2} A study in West Asia has found a significant increase in CKD incidence after high-fat and high-sugar dietary patterns.² Lipid accumulation after

consuming HFD is correlated with ectopic accumulation which has a deleterious effect on renal cells and tissue, leading to a decrease in renal function.³ Previous studies have shown the correlation between lipid accumulation and kidney injury in mice and rats kidney induced by an HFD.⁴⁻⁶ Moreover, consumption of an HFD has proven to increase renal lipid accumulation, inflammatory cytokines,

oxidative stress, mitochondrial dysfunction, and tubular injury.⁵⁻⁷ Proximal tubular inflammatory injuries, as a result of the HFD, are proved by functional impairment such as oxidative stress, mitochondrial dysfunction, and lysosomal acidification, whereas structural lesions such as epithelial cell detachment, tubular dilatation, lipid accumulation, vacuolar degeneration, and tubulointerstitial extracellular matrix accumulation have also been affected.⁷ Overall, those negative effects could be summarized as a lipotoxicity effect in the kidney caused by excessive fat intake.⁸

Lysosomal dysfunction and impaired autophagy flux also have roles in inducing lipotoxicity in the kidney.⁹ Autophagy is a major mechanism for cellular degradation and is significantly involved in sequestering lipids through lipophagy.^{10,11} Prevention of kidney injury induced by lipotoxicity could be made by modulating autophagy in the kidney.¹² Lipid overload can stimulate autophagy that counteracts lipotoxicity in kidney tubules and also induces lysosomal dysfunction, stagnating the flux of autophagy, and causing kidney injury.⁹

Supplementation of natural ingredients such as turmeric and mangosteen have a protective role on renal oxidative stress and lipid metabolism in animal models induced by HFD.¹³⁻¹⁵ Curcumin, an active component of turmeric rhizomes (*Curcuma Longa*), is a renoprotective agent through AMPK and Nrf2 pathways which inhibits oxidative stress and lipid accumulation in the kidney.¹⁵ A xanthone chemical structure, α -mangostin, is a major constituent in mangosteen peel extract (*Garcinia mangostana*) that has been proven to have antioxidant properties in the kidney by increasing GSH (glutathione), GPx (glutathione peroxidase), CAT (catalase), eNOS/NO (endothelial nitric oxide synthase/nitric oxide), leading to decrease of ROS (reactive oxygen species) and MDA (malondialdehyde) levels.¹⁶ However, research on the effect of ethanolic extract of turmeric and mangosteen peel on autophagy in kidney rats induced by an HFD is still limited. Therefore, this study aimed to explore the effect of turmeric and mangosteen peel ethanol extract on histopathology and autophagy gene expression in the kidneys of male Wistar rats induced by an HFD.

Methods

This study was an animal experimental study with a completely randomized design for grouping the animals. The design of the study

was a post-test design, with assessments conducted only at the end of the experiment. This study included male Wistar rats, 8 weeks of age, with a body weighed of 220 ± 20 g, that were divided into 5 groups each consisting of 5 rats; a negative control group, a positive control group, and three groups that were given turmeric, mangosteen, and fenofibrate. The negative control group was given a normal chow diet for 14 weeks, while the positive control was given a high-fat diet for 14 weeks. The other 3 groups were given only HFD for 7 weeks, then continued with an extra turmeric ethanol extract, or mangosteen ethanol extract, or fenofibrate, respectively. HFD itself contained 71% fat, 13% carbohydrate, and 18% protein.¹⁷ Previous studies had proven that 12, 14, and 16 weeks of HFD would induce kidney injury by the induction of oxidative stress, mitochondrial dysfunction, pro-inflammatory cytokines, and podocyte injury.^{5,18,19} In our study, 14 weeks of HFD had been chosen to serve the study aim of preventing kidney injury after HFD induction.¹⁸ Male Wistar rats were included to reduce confounding factors such as hormones. Each group of rats was placed in one cage, in a room with a stable temperature and a 12-hour cycle of light and dark. All procedures were taken care of based on laboratory guidelines, with ethics approval from the Ethics Committee of the Faculty of Medicine, Universitas Kristen Maranatha, no. 039/KEP/VI/2022. At the end of the study, the kidneys were extracted and then divided into 2 parts, one was for RNA extraction which was stored in -80°C refrigerator for further use, and the other part was stored in formalin for hematoxylin-eosin staining, for further histopathology findings.

The extract of turmeric and mangosteen peel were attained from PT Sidomuncul in the form of capsules. The dose was 270 mg/kg each day for turmeric and mangosteen, and 15 mg/kg each day for fenofibrate.^{20,21} The study was conducted in Maranatha Biomedical Research Laboratory in Maranatha Christian University, from January to November 2022. Kidney sections with $2\mu\text{m}$ thickness were stained with hematoxylin and eosin (HE) for histopathology scoring examination using a microscope (Optilab) with 400x magnification. Analysis for histopathological scoring was conducted blindly by an expert pathologist. The scoring system used was a modification from the previous study.²² The level of necrosis, inflammation, and fat degeneration were examined for each slide, and the scoring system was as

follows: (1) Necrosis: 0=no necrosis, 1=focal necrosis, 2=multifocal/diffuse necrosis; (2) Inflammation: 0=no inflammation, 1=mild inflammation, 2=moderate/severe inflammation; (3) Fat degeneration on tubules: 0=no fat degeneration, 1=focal degeneration, 2=multifocal/diffuse degeneration.

The stored kidney tissues were extracted to obtain pure RNA, using TRIsure reagent (Bioline, United Kingdom), with procedures following the manufacturer's instructions. Measurement of RNA concentration and purity were conducted using spectrophotometry at 260/280 nm absorbance (Multiscan Go, Thermo Scientific). The procedure then continued with semiquantitative PCR using The One Step RT PCR Kit (Bioline, United Kingdom). GAPDH served as a housekeeping gene. Visualization of the gels was conducted using BluePad Detection systems and continued with quantification using Image J. The primer sequence used were as follows: LC3, 5'-GTGTGTGGGTTGTGTACGTCG-3' and 5'-CTAGGCATCGAGGTTGACATT-3'; p62, 5'-CTTGGCTGAGTACCACTCTTATC-3' and 5'-GCTCCAGGTCTCAGGGCTTCACA-3'; GAPDH, 5'-GTTACCAGGGCTGCCTTCTC-3' and 5'-GATGGTGATGGGTTTCCCGT-3'.

The data was analyzed using SPSS 26.0, by

conducting a normality and homogeneity test before comparing the differences between groups using One Way ANOVA or Kruskal Wallis, followed by post hoc LSD or Mann Whitney.

Results

There was no difference in kidney weight among groups after 7 weeks of HFD ($p=0.418$), with the average \pm SEM for each group as follows: negative control 0.874 ± 0.06 , positive control 0.782 ± 0.04 , turmeric 0.862 ± 0.03 , mangosteen 0.838 ± 0.04 , and 0.798 ± 0.02 .

The scoring system was used to examine the histopathology of the kidney after 7 weeks of HFD. Necrosis, inflammation, and fat degeneration were examined from the slides (Figure 1).

This study found no necrosis in all groups. Interestingly, there was a significant difference in inflammation ($p = 0.02$) and fat degeneration ($p=0.09$).

Post hoc LSD showed there were significant differences between negative control and positive control ($p = 0.0015$) in inflammation histopathological findings. Furthermore, there was also a significant difference between negative control and turmeric ($p=0.025$);

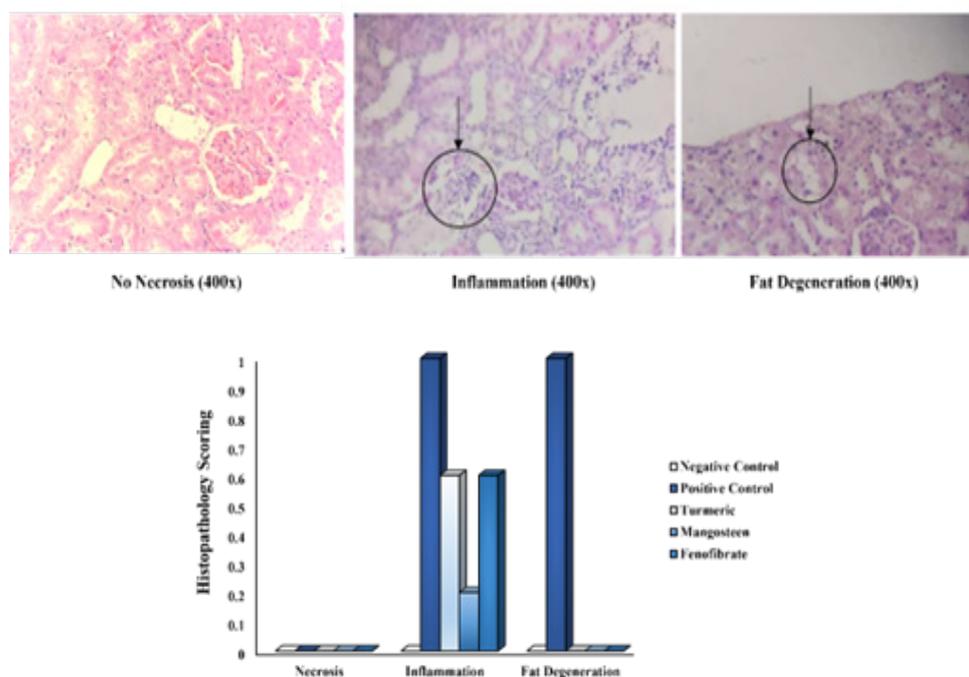


Figure 1 Histopathology Appearance (Above) and Scoring (Below) After 7 weeks of Treatment

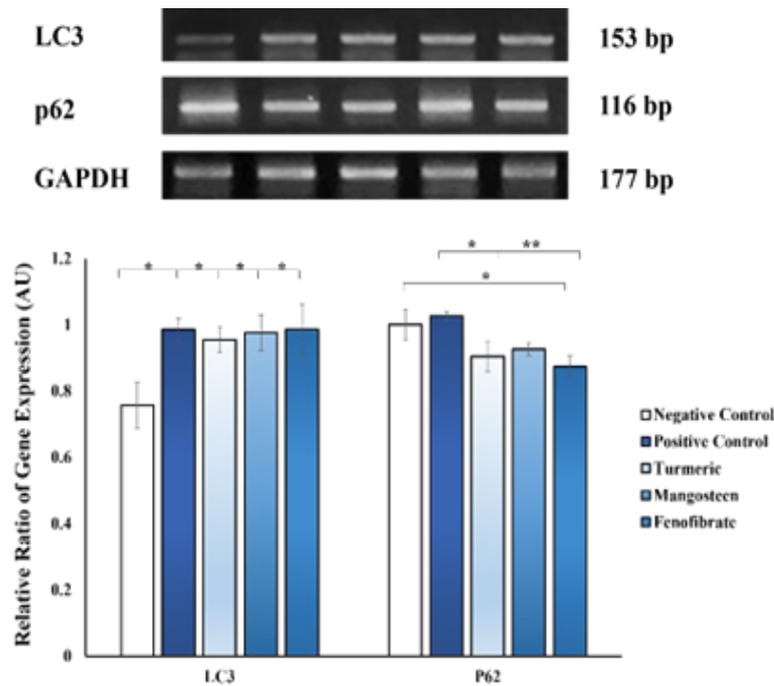


Figure 2 Autophagy Gene Expression Band (Above) and Relative Ratio (Below) After 7 weeks of Treatment

and fenofibrate (0.025); as well as between positive control and mangosteen (p=0.007).

As for fat degeneration, post hoc LSD showed significant differences between negative control and positive control (p=0.007), as well as between positive control and mangosteen (p=0.007) or fenofibrate (p=0.007).

This study found significant differences in LC3 gene expression (p=0.044) and p62 gene expression (p=0.022) from statistical analysis using One Way ANOVA. The autophagy gene expression band and the relative ratio were showed in Figure 2.

The mean±SEM of LC3 gene expression for each group as follows: negative control 0.758±0.068, positive control 0.986±0.034, turmeric 0.955±0.04, mangosteen 0.976±0.055, and 0.987±0.076; and p62 gene expression for each group was as follows: negative control 1.002±0.05, positive control 1.026±0.01, turmeric 0.905±0.04, mangosteen 0.927±0.02, and 0.874±0.03.

Post hoc LSD test showed significant differences in LC3 gene expression between negative control and positive control (p=0.01), turmeric (p=0.024), mangosteen (p=0.014), and fenofibrate (p=0.010); p62 gene expression between negative control and fenofibrate (p=0.015); and between positive control and turmeric (p=0.020) and fenofibrate (p=0.005)

Autophagy gene expression was significantly increased in the turmeric and fenofibrate group, proved by increased LC3 gene expression and decreased p62 gene expression. In positive groups, there was an increase of LC3 gene expression and increased p62 gene expression, showing inhibition of autophagy, whereas in mangosteen there was an increase of LC3 gene expression accompanied by decreased p62 gene expression although it was not statistically significant.

Discussion

The kidney is an organ with a complex architecture and is easily affected by circulating free fatty acid, considering its function for filtering blood.²³ Tubular lipotoxicity is influenced by either increased uptake or diminished β-oxidation of fatty acid.⁸ Lipotoxicity affects tubular cells, interstitial cells, and podocytes of the kidney, inducing endoplasmic reticulum stress, and leading to kidney dysfunction at its endpoint.^{5,6,8} As a major regulator of homeostasis, autophagy has an important role in restoring lipid sequestration via lipophagy and balancing ROS and antioxidant properties in the kidney induced by HFD.^{10,11}

This study found an alteration in

histopathology appearance and autophagy gene expression and no necrosis in all groups as well, indicating that HFD that was given was not causing a nephrotoxic effect in the treatment groups. Previous studies have shown that long-term HFD (23 months) promoted kidney lipid deposition and renal apoptosis in Bama Minipigs, and 12 weeks HFD induced renal cell apoptosis and oxidative stress in spontaneously hypertensive rats.^{24,25} As for the inflammation, increased inflammation was found in positive control compared to other treatment groups (turmeric, mangosteen, and fenofibrate). This inflammation might be caused by lipid peroxidation that induces oxidative stress in the kidney.^{5,7,25} This study also found an increase in fat degeneration in positive control compared to all other groups. This finding is supported by previous studies that found an increase in lipid accumulation in the kidney after HFD in humans and mice.^{9,26}

The mechanism involved in the effect of turmeric, mangosteen, and fenofibrate in decreasing fat degeneration might be correlated with autophagy. In this study, we found an increase of LC3 gene expression in the positive control, turmeric, mangosteen, and fenofibrate, followed by an increase of p62 gene expression in the positive group and a decrease of p62 gene expression in all treatment groups (turmeric, mangosteen, and fenofibrate). This result shows that autophagy might be inhibited in rats given HFD, and activated by turmeric and fenofibrate supplementation. As far as we know, autophagy has been an important strategy in maintaining homeostasis by catabolizing impaired organelles, lipids, and other cellular components to increase survival.¹⁰ In this research, the increase of autophagy in turmeric and fenofibrate might have been correlated with the decrease of inflammation and fat degeneration in the kidney.

A limitation of this study is that this study did not carry out a stereological examination of the kidney, kidney lipid content, lipid peroxidation, or other mechanism related to renal function such as Na,K-ATPase activity and facultative urine concentration. In other words, this study did not conduct examinations related to the accumulation of lipids in the kidney which might induce kidney damage, leading to disruption of kidney function.

In conclusion, turmeric and mangosteen have potential roles in reducing the effect of a high-fat diet on inflammation, fat degeneration, and modulating autophagy in the kidney of Wistar rats. Mangosteen and fenofibrate have

better potential in reducing inflammation and fat degeneration scoring, while turmeric and fenofibrate have better potential in modulating autophagy in the kidney induced by a high-fat diet.

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