ORIGINAL ARTICLE

# Beneficial effects of green coffee and green tea extract combination on metabolic syndrome improvement by affecting AMPK and PPAR-α gene expression

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## ABSTRACT

Effect of green coffee and green tea extract on metabolic syndrome. To explore green coffee and green tea extract combination effect on metabolic profile and blood pressure improvement through adenosine monophosphate-activated protein kinase (AMPK) and Peroxisome Proliferator-Activated Receptor α (PPARα) gene expression modulation. Experimental laboratory research with pre- and post-control group design. Twenty-five metabolic syndrome rats model were grouped into five groups ($n$ = 5): standard control (normal), metabolic syndrome (SM), green coffee extract (GC), green tea extract (GT), and combination green coffee and green tea extract (CM). The extract was given during 9 weeks. Serum glucose, triglyceride, high-density lipoprotein, and systolic blood pressure level were analyzed before and after the extract administration. At the end of the study, PPAR-α and AMPK-α2 gene were analyzed. Independent $t$-test. CM group had significantly higher PPAR-α, and AMPK-α2 gene expression compared to those of SM, GC, and GT group. Green coffee and green tea extract combination administration improved metabolic profile and blood pressure on metabolic syndrome through affecting PPAR-α and AMPK-α2 gene expression.

**Key words:** Adenosine monophosphate-activated protein kinase-α2, green coffee, green tea, metabolic syndrome, Peroxisome Proliferator-Activated Receptor α

## INTRODUCTION

Coffee and tea are widely consumed in the world. Chlorogenic acid (CGA), the most bioactive compound in the green coffee bean, exert as antidiabetic and anti-dyslipidemia.[1] CGA may modulate lipids and glucose metabolism, which related to Peroxisome Proliferator-Activated Receptor α (PPARα) function such as facilitating lipid clearance in the liver, improving insulin sensitivity, and affecting PPARα and adenosine monophosphate kinase (AMPK) gene expression.[2]

Epigallocatechin gallate (EGCG), known as EGCG, an essential flavonoid elements in green tea leaf, exert as anti-hyperglycemia and anti-dyslipidemia.[3] Many studies suggested the beneficial effect of tea consumption in alleviating hyperglycemia and dyslipidemia through some metabolic pathways. The previous study showed that EGCG

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improved AMPK phosphorylation, suppression of hepatic gluconeogenesis, and increased PPAR α gene expression in metabolic syndrome.[4]

The combination of green coffee and green tea extract administration showed better effects on metabolic profile improvement on metabolic syndrome rat model through hepatic PPARα and AMPK gene expression.

## SUBJECTS AND METHODS

### Animal care and experimental protocol

Twenty-five Sprague–Dawley rats were purchased from the department of drug and food control, Indonesia. They were placed in the standard cages with maintained temperature of 25° ± 1°C, standard humidity at 50% ± 1%, and 12 h light/dark cycle. All rats were acclimated during a week. They consumed a standard pellet diet and drank fresh water *ad libitum*. Metabolic syndrome rats obtained a diet with high sucrose and high fat during 8 weeks and injection of streptozotocin (30 mg/bw.t) through intraperitoneal in the 2nd week.[5]

The metabolic syndrome rats were defined regarding to the National Cholesterol Education Program Adult Treatment Panel III NCEP ATP III criteria as follows: (1) high blood glucose (>126 mg/dL), (2) high triglyceride (TG) (>150 mg/dL), (3) high systolic blood pressure (≥140 mmHg), and (4) low high-density lipoprotein (HDL) levels (<40 mg/dL).[5] Furthermore, the rats was subdivided into five groups ($n$ = 5): the standard group (normal), metabolic syndrome (SM), 200 mg/kg bw.t green coffee bean extract (GC), 300 mg/kg bw.t green tea extract (GT), and combination of 200 mg/kg bw.t green coffee bean extract and 300 mg/kg bw.t green tea extract (CM). The extract was given daily through the oral gavage. Extract dose was given using a formula based on the body weight. The intake of food and water were daily recorded. After 9 weeks, the animals were euthanized using diethyl ether following a 12 h period of fasting. The samples of blood were obtained from the heart and then transferred into a microcentrifuge tube, and serum samples were separated after the centrifugation.

### Extraction of green coffee bean

*Coffea canephora* robusta was roasted by automatic coffee roaster at 180°–200°C until the first crack. Furthermore, the coffee bean was mashed with a coffee grinder and then macerated by ethanol 95% to obtain the raw extract. Furthermore, the raw extract was filtered through a filter cloth to separate the liquid phase from the solid phase. Moreover, the liquid phase was concentrated using a rotary evaporator on the temperature of ±40°C. Finally, column chromatography was completed using silica gel C18 11% to attract bioactive compounds and to separate them from other substances.

### Extraction of green tea

Green tea extracted from the young green tea leaf. Green tea leaf weighed 500 g was dried using a cabinet dryer (temperature of 50° C) for 8 h to obtain green tea with 8%–10% water content. The green tea was mashed with a blender and then boiled at 80°C for 30 min. The crude extract was filtered using a filter cloth to separate the liquid phase from the solid phase. The liquid phase was concentrated using a rotary evaporator on the temperature of ±40°C. Finally, column chromatography was completed using silica gel C18 11% to attract bioactive compounds and to separate them from other substances, and then, the filtered product was evaporated.

### High-performance liquid chromatography analysis

CGA in the extract of green coffee and EGCG in the green tea extract were analyzed by the high-performance liquid chromatography (HPLC) system (Shimadzu Corporation, Japan).

### Doses of determination

Green coffee extract and green tea extract doses were determined from the preliminary study in our laboratory. The optimum dose was 200 mg/bw.t for green coffee extract and 300 mg/bw.t for green tea extract.[6,7]

### Biochemistry analysis

The fasting blood glucose (FBG), TG, and HDL levels in serum were analyzed enzymatically by commercial kits (Biolabo, France).

### Measurements of systolic blood pressure

A tail-cuff sphygmomanometer technique was used to measure the rat systolic blood pressure at the baseline and at the end of the study. The measurements were taken consecutively three times, and the average was calculated and taken as a final reading for spontaneous bacterial peritonitis (SBP).

### Isolation of RNA and reverse transcription polymerase chain reaction

RNA was obtained from tissues of the liver and isolated using the easy-BLUE (Intron Biotechnology) according to the factory standard protocol. Reverse transcription reaction was converted by ReverTra Ace-α kit (Toyobo, Japan). Furthermore, the RNA expression levels were carried out using polymerase chain reaction (PCR) LightCycler 96 system (Takara, Japan) with a GoTaq PCR Kit (Promega, Madison, USA) based on the recommended protocols from the manufacturer. The sequences of primer were as follows: beta-actin, forward: 5'-CGAGTACAACCTTCTTGCAG-3' and reverse: 5'-CATTGTAGAAAGTGTGGTGC-3'; PPARα, forward: 5'-TATTTATTGCATCCAGTGGG-3' and reverse: 5'-ATGTTTCCCATCTCTTGTAA-3'; and AMPK-α2, forward: 5'-ATCAATTGACAGGCCATAAA-3' and reverse: 5'-AGATGTAGTCGAACAATTCA-3'.

### Statistical data analysis

The data were presented as mean values ± standard deviation (SD). Independent t-test with significant $P < 0.05$ was applied to the data.

## RESULTS

The metabolic syndrome rats in this study were characterized by obesity, high systolic blood pressure, high TG, hyperglycemia, and low HDL cholesterol, as shown in Table 1. These characteristics were similar to metabolic syndrome characteristics in humans, as stated by the NCEP ATP III criteria.

### The concentration of CGA and caffeine in green coffee extract and EGCG in green tea extract

The green coffee extract HPLC analysis showed that the CGA concentration was 27.134 μg/g green coffee extract. Furthermore, the epigallocatechin-3-gallate concentration was 74.126 μg/g in the extract of green tea.

### Green coffee and green tea extract combination effect on metabolic syndrome parameters improvement

Green coffee and green tea extract effects on metabolic syndrome improvements were presented in Table 2. The level of FBG, TG, and HDL was not statistically different among all groups at the baseline. The CM group showed a significant improvement in FBG and TG level compared to that of SM group at the end of the study. The systolic blood pressure was not statistically different among all groups at the baseline. The CM group showed a significant improvement in systolic blood pressure compared to that of the SM group in the end of the study.

**Table 1: Baseline characteristics**

| Variables | Normal | SM | *P* |
|---|---|---|---|
| Body weight | 295.80±5.11 | 352.95±35.23 | 0.000 |
| FBG | 94.50±18.59 | 244.90±28.59 | 0.004 |
| TG | 84.00±2.94 | 278.40±58.20 | 0.000 |
| HDL cholesterol | 42.75±2.36 | 32.37±5.76 | 0.002 |
| SBP | 123.25±5.31 | 151.35±6.53 | 0.000 |

Values are presented as mean±SD, $n$=5. SBP: Systolic blood pressure, FBG: Fasting blood glucose, TG: Triglyceride, HDL: High-density lipoprotein, SD: Standard deviation, SM: Metabolic syndrome

### Green coffee bean and green tea extract combination effect on gene expression in the liver

The effects of green coffee bean and green tea extract combination on PPARα and AMPKα2 showed in Figure 1. In the liver tissue, the GC, GT, and CM group showed a significantly higher expression of AMPKα2 and PPARα compared to those of the SM group. Furthermore, the CM group showed significantly higher PPARα and AMPKα2 gene expression compared to that of GC and GT group ($P < 0.05$).

## DISCUSSION

This is the first study that showed the synergistic effects of green coffee bean and green tea extract combination on metabolic syndrome improvement by affecting AMPK and PPARα gene expression. This study revealed that administration of 200 mg/kg bw.t green coffee bean extract and 300 mg/kg bw.t green tea extract for 9 weeks improved metabolic profile in the metabolic syndrome rat model. This study showed that green tea and green coffee extract combination increased the hepatic tissue of AMPK-α2 and PPARα gene expression.

AMPK is the primary sensor and modulator of energy homeostasis in cells. AMPK activation induces liver and heart fatty acid oxidation; suppresses cholesterol, fatty acid, TG synthesis; activates fatty acyl-CoA beta-oxidation; and increases glucose uptake.[8] Moreover, the administration of green tea and green coffee extract combination resulted in a more considerable effect on AMPK gene expression. EGCG, as the primary polyphenol in green tea extract, increased not only the AMPK gene expression but also its activation through LKB1 in the hepatic tissue.[9] Furthermore, CGA, as the primary polyphenol in the green coffee extract, increased expression and activation of all AMPK gene.[10] The improvement of AMPK gene expression and activation resulted in better improvement of insulin resistance.

Energy balance regulation is modulated by PPARα through energy expenditure regulation.[11] PPARα plays an essential role in the homeostasis of lipid, fatty acid oxidation, and glucose metabolism.[12] Activation of PPARα rises HDL levels in plasma, transports HDL particles from

**Table 2: Green coffee extract and green tea extract combination effects on metabolic profile and systolic blood pressure**

| Variables | SM | GC | GT | CM |
|---|---|---|---|---|
| FBG | 283.2±31.92 | 218±15.73 | 213.4±8.70 | 186.20±13.99[a,b,c] |
| TG | 317.80±6.80 | 209.40±2.51 | 190.06±2.73 | 160.80±14.04[a,b,c] |
| HDL cholesterol | 30.80±3.11 | 44.20±3.49 | 46.20±8.01 | 47.00±3.67[a] |
| SBP | 163±11.11 | 142.2±3.56 | 144.2±8.52 | 146.56±13.41[a] |

Values are mean ± SD, n = 5. [a]$P$< 0.05, compared to the SM group. [b]$P$< 0.05, compared to the GC 200 group. [c]$P$< 0.05, compared to the GT group SM, Metabolic syndrome Induces; GC, Metabolic syndrome with green coffee extract 200 mg/kg.bw.t; GT, Metabolic syndrome with green tea extract 300 mg/kg.bw.t CM, Metabolic syndrome with green coffee extract 200 mg/kg.bw.t and green tea extract 300 mg/kg.bw.t. SBP: Systolic blood pressure, FBG: Fasting blood glucose, TG: Triglyceride, HDL: High-density lipoprotein, SD: Standard deviation

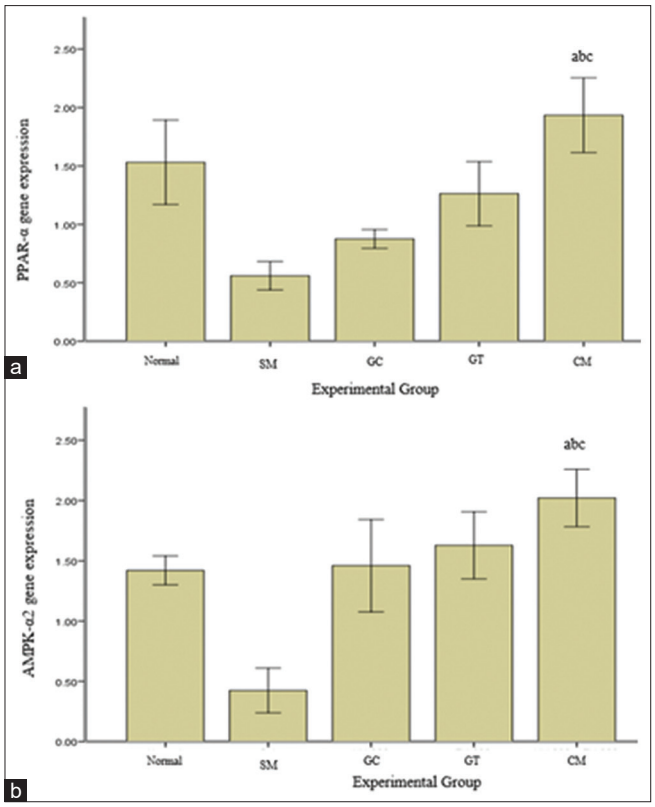


**Figure 1:** Green tea and green coffee bean extract combination effect on Peroxisome Proliferator-Activated Receptor α (a) and adenosine monophosphate-activated protein kinase α2 (b). Values are presented as mean ± standard deviation, $n = 5$. a: $P < 0.05$ compared to the SM group. b: $P < 0.05$ compared to the GC group. c: $P < 0.05$ compared to the GT group. SM: Metabolic syndrome, GC: Metabolic syndrome with green coffee extract 200 mg/kg. bw.t, GT: Metabolic syndrome with green tea extract 300 mg/kg. bw.t, CM: Metabolic syndrome with green coffee extract 200 mg/kg. bw.t and green tea extract 300 mg/kg. bw.t

peripheral tissues to the liver, and decreases TG level in plasma. Therefore, PPARα agonists inhibits dyslipidemia in metabolic syndrome.[13]

Recently, this study showed that green coffee and green tea extract combination administration resulted in more considerable expression of PPARα gene compared to that of green tea or green coffee alone. It was revealed that green tea and green coffee combination work synergistically in PPARα gene improvement.

Recently, this study showed that a combination of 200 mg/kg bw.t of green coffee bean extract and 300 mg/kg bw.t green tea extract was the effective dose in improving fasting blood glucose and TG level in a rat model of metabolic syndrome. However, no significant differences observed in HDL-cholesterol among all interventional groups. This study showed that AMPK and PPARα pathway mediated the synergistic effect of green coffee and green tea extract combination as anti-hyperglycemia and anti-dyslipidemia.

EGCG in green tea extract had hypolipidemic effects that modulate PPARα gene expression. EGCG effect on PPARα and its target gene expression resulted in reduced plasma TG, hepatic lipid accumulation, LDL cholesterol levels, and body fat mass.[14,15] It suggested that EGCG may act as key modulator of PPARα in hepatic fatty acid oxidation that alleviates hyperlipidemia.

CGA from green coffee extract also had hypoglycemic and hypolipidemic effects. The previous study reported that green coffee extract administration in C57BL mice reduced serum glucose, TG level after 6 weeks that significantly lower compared to that of the HFD group.[16] It was showed that CGA might regulate glucose and lipid metabolism through PPARs activation in rats.[17]

This study also demonstrated that green coffee and green tea combination administration had a slight blood pressure-lowering effect. The blood pressure-reducing effect of green coffee extracts was first reported in spontaneously hypertensive rats (SHR).[18] The previous study by Panchal *et al*. demonstrated oral administration of 5% aqueous coffee extract during 8 weeks reduced the SBP by 10–15 mmHg from the baseline.[19] Hypotensive effect of green tea extract rich EGCG improved endothelial in SHR. Therefore, green tea extract administration improved the systolic blood pressure.[20]

## CONCLUSIONS

Green coffee and green tea extract combination administration improved the metabolic profile and blood pressure on metabolic syndrome through affecting PPAR-α and AMPK-α2 gene expression.

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### Conflicts of interest

There are no conflicts of interest.

## REFERENCES

1. Meng S, Cao J, Feng Q, Peng J, Hu Y. Roles of chlorogenic acid on regulating glucose and lipids metabolism: A review. Evid Based Complement Alternat Med 2013;2013:801457.
2. Ong KW, Hsu A, Tan BK. Anti-diabetic and anti-lipidemic effects of chlorogenic acid are mediated by ampk activation. Biochem

Pharmacol 2013;85:1341-51.

3. Singh BN, Shankar S, Srivastava RK. Green tea catechin, epigallocatechin-3-gallate (EGCG): Mechanisms, perspectives and clinical applications. Biochem Pharmacol 2011;82:1807-21.
4. Kim J, Yang G, Kim Y, Kim J, Ha J. AMPK activators: Mechanisms of action and physiological activities. Exp Mol Med 2016;48:e224.
5. Rohman MS, Lukitasari M, Nugroho DA, Nashi W, Nugraheini NI, Teguh WS. Development of an experimental model of metabolic syndrome in Sprague Dawley rat. Res J Life Sci 2017;4:76-86.
6. Lukitasari M, Nugroho DA, Rohman MS, Nugrahini NI, Sardjono TW. Light-roasted green coffee extract improved adiponectin, insulin resistance, and metabolic profile of metabolic syndrome rat model. Asian J Pharm Clin Res 2017;10:279.
7. Lukitasari M, Nugroho DA, Rohman MS. Green tea extract administration had a beneficial effect on PPAR Alpha and PPAR gamma gene expression in metabolic syndrome rat model. J Hypertens 2018;36:e9.
8. Ruderman NB, Carling D, Prentki M, Cacicedo JM. AMPK, insulin resistance, and the metabolic syndrome. J Clin Invest 2013;123:2764-72.
9. Santamarina AB, Oliveira JL, Silva FP, Carnier J, Mennitti LV, Santana AA, *et al*. Green tea extract rich in epigallocatechin-3-gallate prevents fatty liver by AMPK activation via LKB1 in mice fed a high-fat diet. PLoS One 2015;10:e0141227.
10. Ong KW, Hsu A, Tan BK. Chlorogenic acid stimulates glucose transport in skeletal muscle via AMPK activation: A contributor to the beneficial effects of coffee on diabetes. PLoS One 2012;7:e32718.
11. Burkart EM, Sambandam N, Han X, Gross RW, Courtois M, Gierasch CM, *et al*. Nuclear receptors PPARbeta/delta and PPARalpha direct distinct metabolic regulatory programs in the mouse heart. J Clin Invest 2007;117:3930-9.
12. Berger J, Moller DE. The mechanisms of action of PPARs. Annu Rev Med 2002;53:409-35.
13. Jay MA, Ren J. Peroxisome proliferator-activated receptor (PPAR) in metabolic syndrome and type 2 diabetes mellitus. Curr Diabetes Rev 2007;3:33-9.
14. Collins QF, Liu HY, Pi J, Liu Z, Quon MJ, Cao W. Epigallocatechin-3-gallate (EGCG), a green tea polyphenol, suppresses hepatic gluconeogenesis through 5'-AMP-activated protein kinase. J Biol Chem 2007;282:30143-9.
15. Bose M, Lambert JD, Ju J, Reuhl KR, Shapses SA, Yang CS. The major green tea polyphenol, (-)-epigallocatechin-3-gallate, inhibits obesity, metabolic syndrome, and fatty liver disease in high-fat-fed mice. J Nutr 2008;138:1677-83.
16. Choi BK, Park SB, Lee DR, Lee HJ, Jin YY, Yang SH, *et al*. Green coffee bean extract improves obesity by decreasing body fat in high-fat diet-induced obese mice. Asian Pac J Trop Med 2016;9:635-43.
17. Wan CW, Wong CN, Pin WK, Wong MH, Kwok CY, Chan RY, *et al*. Chlorogenic acid exhibits cholesterol lowering and fatty liver attenuating properties by up-regulating the gene expression of PPAR-α in hypercholesterolemic rats induced with a high-cholesterol diet. Phytother Res 2013;27:545-51.
18. Suzuki A, Kagawa D, Ochiai R, Tokimitsu I, Saito I. Green coffee bean extract and its metabolites have a hypotensive effect in spontaneously hypertensive rats. Hypertens Res 2002;25:99-107.
19. Panchal SK, Poudyal H, Waanders J, Brown L. Coffee extract attenuates changes in cardiovascular and hepatic structure and function without decreasing obesity in high-carbohydrate, high-fat diet-fed male rats. J Nutr 2012;142:690-7.
20. Potenza MA, Marasciulo FL, Tarquinio M, Tiravanti E, Colantuono G, Federici A, *et al*. EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR. Am J Physiol Endocrinol Metab 2007;292:E1378-87.