RESEARCH ARTICLE

Green Tea and Decaffeinated Light Roasted Green Coffee Extract Combination Improved Cardiac Insulin Resistance through Free Fatty Acids and Adiponectin/FAS Pathway Amelioration in Metabolic Syndrome Rat Model [version 1; peer review: 1 approved with reservations, 1 not approved]

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V1 First published: 30 Sep 2021, 10:990
<https://doi.org/10.12688/f1000research.55470.1>
 Latest published: 30 Sep 2021, 10:990
<https://doi.org/10.12688/f1000research.55470.1>

Abstract

Background: Insulin resistance has been independently associated with cardiac diseases. A free fatty acid is recently known to induce cardiac insulin resistance due to low-grade inflammation. Therefore, the improvement of free fatty acid levels can also improve cardiac insulin resistance. This study investigated the combination of green tea and decaffeinated-light roasted green coffee extract in improvement of free fatty acid-induced cardiac insulin resistance by improving the adiponectin/FAS pathway.

Methods: This study used 25 males Sprague-Dawley rats induced by a high-fat high sucrose diet and injection of low dose streptozotocin to make a metabolic syndrome (MS) rat model and standard chow as healthy control rats. The MS rats were treated with green tea (200 mg/ b. w.), decaffeinated-light roasted green coffee (300 mg/ b. w.), and the combination of both extracts in 9 weeks. Experimental groups in this study were divided into 5 groups: 1) MS (HFHS diet + STZ) group, 2) NC (normal chow) group, 3) GT (green tea extract) group, 4) GC (decaffeinated-light roasted green coffee extract), 5) CM (combination of both extracts) group. Adiponectin and HOMA-IR level was analysed using ELISA, and the gene expression of *Adipo-R1*, *FAS*, *PI3K*, *PDK1*, *Akt*, *GLUT4* was measured by RT-PCR.

Results: The combination of green tea and decaffeinated-light

Open Peer Review

Approval Status ? X

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30 Sep 2021	view	view
1. A. Vijaya Anand , Bharathiar University, Coimbatore, India		
2. Sylvia Riedel , South African Medical Research Council (MRC), Tygerberg, South Africa		

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roasted green coffee showed synergistic effects in improving FFA levels. The adiponectin/FAS pathway was attenuated in the CM group. Moreover, the combination also showed improvement in cardiac insulin resistance markers such as IRS1/2, PI3K, PDK1, Akt, and GLUT4.

Conclusions: The combination of green tea and decaffeinated-light roasted green coffee extract improved cardiac insulin resistance better than green tea and green coffee extract administration alone by reducing free fatty acids levels through adiponectin/FAS pathway modulation.

Keywords

metabolic syndrome; green tea; green coffee; cardiac insulin resistance

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Author roles: Lukitasari M: Conceptualization, Data Curation, Methodology, Writing – Review & Editing; Rohman MS: Conceptualization, Funding Acquisition, Supervision, Writing – Review & Editing; Nugroho DA: Conceptualization, Data Curation, Methodology, Resources; Nur Kholis M: Data Curation, Formal Analysis, Investigation, Writing – Original Draft Preparation; Wahyuni NA: Data Curation, Investigation, Writing – Original Draft Preparation; Widodo N: Project Administration, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This research was funded by the Research and Community Service Department Faculty of Medicine, Brawijaya University under Grant 3125/UN.10.F08/PN/2021

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Lukitasari M, Rohman MS, Nugroho DA *et al.* **Green Tea and Decaffeinated Light Roasted Green Coffee Extract Combination Improved Cardiac Insulin Resistance through Free Fatty Acids and Adiponectin/FAS Pathway Amelioration in Metabolic Syndrome Rat Model [version 1; peer review: 1 approved with reservations, 1 not approved]** F1000Research 2021, 10:990 <https://doi.org/10.12688/f1000research.55470.1>

First published: 30 Sep 2021, 10:990 <https://doi.org/10.12688/f1000research.55470.1>

Introduction

The incidence of metabolic syndrome (MS) features, such as obesity, hyperglycemia, and insulin resistance has increased and is associated with excessive food consumption.¹ MS prevalence has increased about 50–75% in the last decade.² A previous study showed that insulin resistance had been known to be a significant predictor of heart failure (HF)³ and the major feature in HF patients.⁴ Many studies have shown that insulin resistance might develop cardiac remodellings such as hypertrophy, fibrosis, and cardiac dysfunction without coronary artery disease or hypertension.^{5–9} Lipotoxicity by free fatty acids (FFAs) has been the primary cause of insulin resistance¹⁰ through low-grade inflammation in cardiac tissue.¹¹ Kim *et al.* (2009)¹² reported that FFAs might cause inflammation and suppress glucose uptake through inhibition IRS-1 in cardiac tissue. Meanwhile, reducing FFAs plasma levels could improve cardiac function in the high-fat diet-induced obese rat model.¹³ Therefore, improving the lipid profile could prevent the development of cardiac insulin resistance.

Recently, natural compound derivatives revealed beneficial effects in metabolic syndrome improvement. One of the natural compounds that are widely consumed is epigallocatechin-3-gallate (EGCG) which is mainly derived from green tea leaves.¹⁴ Many studies have investigated the beneficial effect of green tea administration and its constituents in alleviating metabolic syndrome. Most of those studies revealed that green tea administration modulated insulin sensitivity, reduced blood glucose level, and improved lipid profile in metabolic syndrome animal models.^{15–17} Additionally, FFAs-induced insulin resistance and hyperglycemia-induced cardiac fibrosis could be attenuated by EGCG administration *in vivo*.¹⁸ Moreover, chlorogenic acid (CGA), a phenolic compound in green coffee beans, has been another natural compound derivative that could alleviate metabolic syndrome.^{19,20} CGA potentiates insulin activity similar to the therapeutic mechanism of metformin²¹ but does not induce obesity, unlike thiazolidinedione (TZD) or insulin therapy.²² A previous study also showed that CGA improved FFAs metabolism in rats hepatic tissue.²³ Nevertheless, green coffee extract administration has detrimental effects due to the caffeine content,^{24–26} and the CGA antioxidant activity depends on the roasted level of green coffee beans.^{27,28} Therefore, this study used decaffeinated light-roasted green coffee beans to avoid the detrimental effects of caffeine content and reach the highest antioxidant activity of CGA. Many studies showed that green tea or green coffee extract administration could treat metabolic syndrome. Still, analysis investigating the beneficial effect of these extract combinations in alleviating metabolic syndrome remains limited, whereas natural compounds have a more significant therapeutic effect when combined.²⁹ This study investigated the combination of green tea and decaffeinated, light roasted green coffee extract to improve free fatty acid-induced cardiac insulin resistance by improving the adiponectin/FAS pathway.

Methods

Ethics statement

All experimental procedures were approved by the ethical committee of Faculty of Medicine, Brawijaya University with registration number 405/EC/KEP/10/2016. All efforts were made to ameliorate harm to the animals by using the standard protocol from the Indonesian Ministry of Health ethical research guidelines for animal experimental research.

Animals and experimental design

This study is part of a larger study³⁰ which used 25 males Sprague–Dawley rats (age 9 weeks) obtained from the National Agency of Drug and Food Control, Indonesia. The ethical committee approved the experimental protocols of the Faculty of Medicine, Brawijaya University. Rats were maintained and acclimatized in the environmentally controlled standard cage as described in our previous study.³⁰ Animals were grouped as the previous study as follows: i) normal control group (NC) that were assigned to standard chow without STZ injection; metabolic syndrome rats were obtained after HFHS diet and low dose STZ (bioWORLD cat.41910012-4) injection intraperitoneally at the second week of the protocol. NCEP ATP III criteria confirmed metabolic syndrome features. The metabolic syndrome rats were assigned to four groups ii) metabolic syndrome group (MS); iii) green tea group (GT) that were given the HFHS diet, STZ injection, and green tea extract (300 mg/kg b.w.) administration per oral; iv) green coffee group (GC) that were assigned to HFHS diet, STZ injection, and green coffee extract (200 mg/kg b.w.) administration; v) combination group (CM) that were assigned to HFHS diet, STZ injection, and combination of green tea (300 mg/kg b. w.) and green coffee (200 mg/kg b. w.) extract administration, respectively.³¹ Green tea and coffee extract were administered in millilitres *via* oral gavage daily based on weekly measured body weight.³¹ After 9 weeks of exact treatment, animals were euthanized by cervical decapitation previously anaesthetized using diethyl ether after a 12-hour fast. As soon as the rats died, the blood samples were obtained from the heart and transferred into a microcentrifuge tube to get serum samples by centrifugation (4,000 ×g for 15 minutes at 4°C).³¹

Extraction procedures of green tea leaves

The green tea leaves were obtained from Sukawana, Bandung, Indonesia (1550 MAMSL). Protocol of green tea extraction followed the procedure of Saifur Rohman *et al.* (2021).³¹

Extraction procedures of decaffeinated, light roasted green coffee beans

The coffee beans used in this experiment were *Coffea canephora/robusta*. The coffee beans were obtained from Dampit, Malang, Indonesia (800 MAMSL). The protocol of green coffee extraction followed the procedure of Saifur Rohman *et al.* (2021).³¹

High-performance liquid chromatography analysis

Bioactive compound level in green tea extract (EGCG) and green coffee bean extract (caffeine and CGA) were analyzed by high-performance liquid chromatography (HPLC) system using a Shimadzu Brand chromatograph (model SCL10AVP, Japan) that set up with a C-18 reverse-phase column (Shim-pack VP ODS 5µm 150 × 4.6 mm). Details of the method were explained in the previous study.³¹

Dose determination

Our previous study determined the exact doses based on the optimal doses for each extract.³¹

Physiological measurements

Daily food intake and fluid intake were measured each day, and body weight every week. Foods and fluids intake were measured by subtracting the amount provided by the remaining. More information was written in the previous study.³¹

Biochemistry analysis

The fasting blood glucose (BIOLABO, cat no. 80009), triglycerides (TG) (cat no. 80019), and HDL-Cholesterol (BIOLABO, cat no. 86516) were analyzed with commercial kits (Biolabs, France) enzymatically as in the previous study.³¹

Enzyme-linked immunosorbent assay (ELISA)

Rat serum was collected at the end of the study and stored at –80°C. ELISA analysis was conducted for measuring non-esterified fatty acid (NEFA) (Ref E-BC-K014, Elabscience, United States), (Ref E-EL-R2466, Elabscience, United States), and adiponectin level (Ref E-EL-R3012, Elabscience, United States). The measurements were conducted using the manual protocol by the manufacturer and read using ELx808 Absorbance Microplate Reader (BioTek, China) to get the result as ng/mL.

Homeostatic model assessment for insulin resistance (HOMA-IR)

This study measured HOMA-IR using the Commercial ELISA Kit (Ref E-EL-R2466, Elabscience, United States). The measurement was conducted using the manual protocol by the manufacturer and read using ELx808 Absorbance Microplate Reader (BioTek, China) to get the result as ng/mL. HOMA-IR was used to calculate an index from the product of the fasting concentrations of blood glucose (mg/dL) and insulin (microU/L) levels divided by 14.1. Lower levels of HOMA-IR indicate greater insulin sensitivity, and higher levels indicate insulin resistance (Van Dijk *et al.*, 2013).

Systolic blood pressure measurements

Blood pressure was measured three times by a tail-cuff sphygmomanometer technique (Ugo Basile 58500) at the baseline and the end of the study. The average of those three measurements was calculated and shown as the final reading for SBP.

Isolation of total RNA and reverse transcription-polymerase chain reaction

Total RNA was obtained from liver and heart tissues and isolated using the easy-BLUE (Intron Biotechnology, cat no. 17061). Reverse transcription reaction was converted by a ReverTra Ace-α kit (Toyobo, FSK-101). The mRNA expression levels were measured using the touchdown PCR protocol with polymerase chain reaction (PCR) LightCycler 96 system (Takara, cat no. TP600). The PCR process was performed using a GoTaq Green Master PCR Kit (Promega, cat no. M7822) according to the manufacturer's protocols with one of the target gene primers. The primer sequences were as follows: β-actin forward 5'-CGA GTA CAA CCT TCT TGC AG-3', reverse 5'-CAT TGT AGA AAG TGT GGT GC-3'; FAS forward 5'-TGG AGA AGC CCA GGA ACA ACT CAT-3', reverse 5'-ACC GAG TAA TGC CGT TCA GTT CCT-3'; Adipo-R1: forward 5'-GAC AGG CCT AGG TGT CCA TCA-3', reverse 5'-TCG TAT GGG ATG ACC CTC CA-3'; PI3K forward 5'-CCT CTC CTT ATA AAG CTC CTG GAA-3', reverse 5'-GAT CAC AAT CAA GAA GCT GTC GTA A-3'; IRS1 forward 5'-AAG CAC CTG GTG GCT CTC TA-3', reverse 5'-TCA GGA TAA CCT GCC AGA CC-3'; IRS2 forward 5'-ATA CCG CCT ATG CCT GTC TG-3', reverse 5'-AGA AGA AGC TGT CCG AGT GG-3'; PDK1 forward 5'-CGT CCC GCA CGT AGA G-3', reverse 5'-TCC TCA GCA CTC TTG TCC TTA-3'; AKT forward 5'-TCA CCT CTG AGA CCG ACA CC-3', reverse 5'-ACT GGC TAG TAG GAG AAC TGG-3'; GLUT4 forward 5'-CTT CCT TCT ATT TGC CGT CCT C-3', reverse 5'-GCT GCT TTG TCC TTC ATC CTG-3'. Gene expression was defined as the relative expression level after being compared with the housekeeping gene (β-actin).

Data and statistical analysis

Data were presented as mean SD and statistically analyzed using an independent t-test with a confidence level of 95% as significant at $p \leq 0.05$.

Results

The concentration of bioactive compounds in green tea and green coffee extract on HPLC analysis

HPLC analysis showed that EGCG concentration in green tea extract was 74.126 $\mu\text{g/g}$. Meanwhile, CGA, caffeine, and polyphenol concentrations in the green coffee extract was 27.134 $\mu\text{g/g}$ and 43,473 $\mu\text{g/g}$, respectively.³¹

Biochemistry characterization in experimental animals

Our previous study reported that rats with the HFHS diet and low-dose STZ injection had metabolic syndrome. It was proved by the measurement of systolic blood pressure (SBP), fasting blood glucose (FBG) level, triglyceride (TG), and HDL cholesterol (HDL) plasma level in rats induced HFHS diet with STZ injection (MS) group met the metabolic syndrome characteristic in accordance to NCEP-ATP III criteria in 8 weeks of duration. Significant differences in SBP, FBG, TG, and HDL levels were observed between the normal control group (NC) and the metabolic syndrome group (MS) ($p < 0.05$).³² The pre- and post-test in all extract-intervention groups showed improvement in SBP, FBG, TG, and HDL levels. Meanwhile, the GC group had no statistically significant difference between pre- and post-test, except the HDL level (data were available in <https://doi.org/10.6084/m9.figshare.13249163.v3>).

Combination green tea and decaffeinated, light roasted green coffee extract lowered plasma non-esterified free fatty acids (NEFA) levels

A significant increase was observed in plasma NEFA levels in the MS group compared to the NC group ($p < 0.05$). After extract intervention, the plasma NEFA levels in all extract treated rats were significantly lower ($p < 0.05$) compared to that of the MS group. Interestingly, the CM group had the least plasma NEFA levels and a significant difference than GT ($p < 0.05$) and GC ($p < 0.01$) was observed in plasma NEFA levels (Figure 1A). It suggested that combining the green tea extract and decaffeinated light roasted green coffee was more effective in reducing FFAs than green tea or green coffee extract single administration (Figure 1A).

Figure 1. Free fatty acid levels and insulin resistance status. A. Serum level of non-esterified free fatty acids; B. HOMA-IR score index was used as representation of insulin resistance status. Data are expressed as mean $\pm$ SEM (N = 4-5). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared with MS. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$ compared with GT. \$ $p < 0.05$, \$\$ $p < 0.01$, \$\$\$ $p < 0.001$ compared with GC.

Combination green tea and decaffeinated light roasted green coffee extract improved adiponectin levels and adipo-R1 gene expression

Adiponectin levels were significantly lower in the MS group than in the NC group ($p < 0.05$). The GT group showed lower levels of adiponectin than the GT group. Still, the combination extract group showed a better effect in improving adiponectin levels significantly compared to that of the GT and GC groups ($p < 0.05$) (Figure 2A). Moreover, the relative mRNA expression levels of adiponectin-receptor 1 (Adipo-R1) in this study also significantly lower in the MS group compared to that of the NC group ($p < 0.001$) (Figure 2B). All extract treated groups, either GT, GC, or CM, revealed a significantly higher of relative mRNA expression in Adipo-R1 gene expression compared to that of the MS group ($p < 0.001$) (Figure 2B). Moreover, the CM group showed the highest Adipo-R1 mRNA expression levels among other extract treated groups ($p < 0.01$) (Figure 2B).

Combination green tea and decaffeinated light roasted green coffee extract improved free fatty acids levels and FAS gene expressions

This study revealed the effect of green tea and green coffee extract administration on the free fatty acid synthesis pathway by analyzing the NEFA levels and adiponectin/Adipo-R1/AMPK/FAS pathway involvement.

There was an increase in fatty acid synthase (FAS) gene expression in the liver. The expression of relative mRNA level of FAS was significantly higher in the MS group compared to that of the NC group ($p < 0.001$) (Figure 2C). All extract-treated groups showed a lower of relative mRNA level of FAS gene in the GT, GC, or CM groups and significantly different with the MS group ($p < 0.05$) (Figure 2C). However, there was no significant difference in FAS gene expression between the GT and GC groups ($p > 0.05$). Still, the FAS gene expression in the CM group showed a significant difference compared to the GT and GC group ($p < 0.01$) (Figure 2C). These results were linear with our previous study; we reported that HFHS diet and low dose STZ injection induced metabolic syndrome rats had lower AMPK- $\alpha 2$ gene expression ($p < 0.05$). Meanwhile, the combination of green tea and green coffee extract showed substantially higher AMPK- $\alpha 2$

Figure 2. Adiponectin/FAS Measurement. A. Adiponectin serum levels; B. Gene expression of Adipo-R1; C. Gene expression of FAS. Data are expressed as mean $\pm$ SEM (N = 4-5). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared with MS. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$ compared with GT. \$ $p < 0.05$, \$\$ $p < 0.01$, \$\$\$ $p < 0.001$ compared with GC.

expression compared to that of single extract either green tea or green coffee extract administration ($p < 0.05$).³² Therefore, this study revealed that green tea or green coffee extract administration could ameliorate the FFAs levels by improving the adiponectin/adipo-R1/AMPK/FAS pathway in liver tissue rats. However, the combined extract was more effective.

Combination green tea and decaffeinated light roasted green coffee extract improved HOMA-IR

HFHS diet with STZ injection rats showed a higher homeostatic model assessment for insulin resistance (HOMA-IR) index compared to that of the NC group ($p < 0.001$) (Figure 1B). All of the extract treated groups revealed a lower HOMA-IR index compared to the MS group ($p < 0.05$) (Figure 1B). Moreover, the CM group showed the lowest level in the HOMA-IR index among other extract treated groups ($p < 0.001$). This indicated that the combination revealed a better effect in improving insulin resistance.

Combination green tea and decaffeinated light roasted green coffee extract ameliorated cardiac insulin resistance marker expressions

The higher HOMA-IR in HFHS diet with low dose STZ injection induced rats was accompanied by a decrease of cardiac insulin signalling protein gene expression compared to that of the NC group ($p < 0.05$) (Figure 1B). It was illustrated by the lower expression of IRS1/2, PI3K, PDK1, Akt, and GLUT4. A higher cardiac insulin signalling protein gene expression was observed in all extract treated groups. Nevertheless, the GT group showed higher IRS2, AKT, and GLUT4 gene expressions compared to that of the GC group (Figure 3B, E, F). However, those markers' relative mRNA expression levels were higher in the CM group than in the GT or GC group ($p < 0.001$) (Figure 3).

Figure 3. Gene expression measurement of cardiac insulin signalling. A. Relative mRNA expression level of IRS1; B. Relative mRNA expression level of IRS2; C. Relative mRNA expression level of PI3K; D. Relative mRNA expression level of PDK1; E. Relative mRNA expression level of Akt; F. Relative mRNA expression level of GLUT4. Data are expressed as mean $\pm$ SEM (N = 4-5). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared with MS. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$ compared with GT. \$ $p < 0.05$, \$\$ $p < 0.01$, \$\$\$ $p < 0.001$ compared with GC.

Discussion

Metabolic syndrome is a metabolic disturbance characterized by insulin resistance and a decrease of adiponectin as a biomarker. Adiponectin has essential roles in metabolic profile, such as improved glucose and lipid metabolism and insulin resistance.³³ Adiponectin, an inducer of AMP-activated protein kinase (AMPK) has a regulatory effect on glucose and lipid metabolism that had an essential role in free fatty acid production through inhibition of fatty acid synthetase (FAS).³⁴ The present study showed lower adiponectin and higher FFA levels in MS rats than the normal rats and intervention rat groups. The administration of green coffee and green tea in MS rats improved adiponectin and FFA serum levels. The improvement of adiponectin and FFA levels were related to the modulation of adipo-R1 and AMPK gene expression in hepatic tissue.^{35–37} This study showed improvement in adipoR1 in the extract treated groups. Additionally, FAS gene expression in hepatic tissue was lower in either the green tea or green coffee group. Furthermore, our previous study showed that hepatic AMPK expression was improved in all intervention groups, and the highest level showed in the combination extract group.³²

Lower adiponectin levels and higher FFA levels are related to insulin resistance in hepatic, skeletal, and cardiac tissue.^{33,38} This study showed that higher HOMA-IR levels reflected insulin resistance conditions. The highest HOMA-IR was shown in MS group rats compared to that of intervention group rats. Moreover, green tea and green coffee extract administration had significantly lower HOMA-IR levels compared to that of MS group rats. Lower HOMA-IR in the extract treated group was accompanied by improvement of insulin signalling in cardiac tissue. Our study showed cardiac tissue gene expression improvement related to insulin signalling, such as through IRS, Akt, PI3K, PDK1, and GLUT4 in MS rat with green tea and green coffee administration. The results showed that insulin signaling gene expressions were significantly higher in the intervention group compared to MS group rats. Moreover, the combination of green tea and green coffee had the highest expressions.

The present study aimed to investigate the effect of combining green tea and green coffee on cardiac insulin resistance caused by the elevation of FFA serum and lower adiponectin levels. This study showed that the administration of green tea and green coffee improved cardiac insulin resistance by reducing serum free fatty acids levels and elevating serum adiponectin levels by modulation of adipo-R1, AMPK, and FAS gene expressions. These results were similar to previous studies that improving serum FFA and adiponectin signalling pathway could attenuate insulin resistance.^{39–42} Although animal and human studies have shown that lowering serum FFAs directly improved insulin resistance in some tissues such as muscles, adipose, hepatic, and endothelial tissues, few studies showed the effect on cardiac tissues. Moreover, green coffee administration could improve blood lipids and increase metabolic rates, fatty acid oxidation, and hepatic triglyceride in obese animal rat or mice models using diet or genetic modification.^{12,43–45} In addition, a chlorogenic acid compound in the green coffee extract was associated with the improvement of adiponectin levels and had an important role in insulin sensitivity and inflammation.⁴⁶ Besides, green tea administration was known to have similar effects.⁴⁷ Li *et al.* (2018) study showed that the EGCG compound in green tea extract inhibited gene expression involved in synthesizing de novo fatty acids, such as FAS, ACC, and SC.⁴⁸ Other studies showed that EGCG had a beneficial effect on insulin resistance by improving the AMPK pathway.¹⁸

Administration of a combination of green coffee and green tea extract in this study showed a higher adiponectin level compared to that of green coffee or green tea single extract administration. Additionally, the Adipo-R1 gene expression was higher in the CM group. Previous studies showed that green tea extract had a beneficial effect on the adiponectin signalling pathway through improving adiponectin levels and modulating adipo-R1 expressions.^{35,36} On the other hand, few studies have revealed the effect of green coffee on the improvement of FFA levels.^{49,50} Our study also showed that green tea and green coffee administration were adequate to reduce serum FFA levels. This study was in accordance with previous studies that revealed the improvement of adiponectin and adipo-R1 is involved in lower serum FFA levels.^{18,39,43–45} Interestingly, the combination of both extracts exhibited lower serum FFA levels than green tea or decaffeinated-light roasted green coffee alone.

Elevation of serum FFA levels is caused by excess nutrition intake, adipose lipolysis, and de novo fatty acid synthesis (glucose utilization), mainly in the hepatic tissues.³⁷ The present study showed that hepatic de novo fatty acid synthesis was increased in metabolic syndrome rats. It was illustrated by increasing FAS mRNA expressions in the MS group ($P < 0.01$ vs. NC group). This finding was linear with our previous study that AMPK mRNA expressions were reduced in MS rats.³² AMPK has an inhibitory effect on FAS⁵¹; thus, the reduction of AMPK showed a decrease in inhibitory regulation effect. Nevertheless, green tea or green coffee intervention in the present study showed a decrease in FAS expressions, and these were similar to other previous studies^{43,44,49,52,53}; however, our findings showed no significant difference between green tea and green coffee extract rat groups. Meanwhile, the combination of light roasted decaffeinated green coffee and green tea extract significantly reduced FAS mRNA expression compared to that of single

extract groups. These findings showed that green tea and green coffee had more practical effects on improving hepatic de novo fatty acid synthesis, thus reducing serum FFAs levels.

It was known that FFA induced insulin resistance.^{42,54} This study revealed HOMA-IR accompanied the increase of serum FFA levels in MS rats. Compared with previous studies,^{55–57} we had similar findings that administration of green tea decaffeinated-light roasted green coffee had beneficial effects on improving insulin resistance. It was illustrated by a lowering of HOMA-IR in all intervention groups. Meanwhile, the administration of the combination of both extracts showed a lower level of HOMA-IR than green tea or green coffee administration alone. A previous study showed that the administration of EGCG^{52,58} attenuated insulin sensitivity in skeletal and adipose tissue. Moreover, CGA and other polyphenols in green coffee improved insulin sensitivity in HFD Mice.⁴⁹ HOMA-IR reflects insulin resistance conditions from multi organs such as adipose, heart, and muscle, including skeletal and cardiac muscle.^{59,60}

Many reviews have revealed that FFA could induce alteration of cardiac structure and function without coronary disease or hypertension,⁷ which is called diabetic cardiomyopathy, and insulin resistance is the early step in the development of the disease.⁶¹ Many studies suggest that an increase of serum FFA levels can induce insulin resistance by inhibiting IRS1/PI3K/Akt in cardiac muscle,^{62–64} and associated with cardiac remodelling and dysfunction.^{65,66} It was recently known that FFAs could interact with toll-like receptors (TLR) and induce insulin resistance through the disturbance of insulin signalling pathways in cardiac tissues.^{11,67} Our findings observed that improvement of serum FFA levels was accompanied by improved cardiac insulin signalling pathway. All treatment groups showed a high of IRS1/2, PI3K, PDK1, Akt, and GLUT4 mRNA expression, but the GT group showed higher IRS2, Akt, and GLUT4 than the GC group. Rebollo-Hernanz *et al.* (2019) recently showed that phenolic compounds in green coffee, especially CGA, modulate adipogenesis and insulin resistance via PI3K/Akt signalling pathway in adipocytes.⁶⁸ Another study showed that chlorogenic acid stimulates glucose transport in skeletal muscle *via* AMPK activation in db/db mice.⁵⁶ Besides, the green tea administration showed the ameliorating mechanism of hyperglycemia by promoting GLUT4 translocation in skeletal muscle of diabetic rodents.⁵⁸ Moreover, another study revealed that EGCG administration induced GLUT4 translocation in skeletal muscle through PI3K- and AMPK-dependent pathways.¹⁶ Nevertheless, the combination of green tea and decaffeinated-light roasted green coffee extract had the highest expressions. It showed that green tea and green coffee extract had synergistic interaction that consequently improved insulin sensitivity.

This study had several limitations. Firstly, we did not investigate the biomarker in adipose tissues, which might give a comprehensive understanding of green tea and green coffee administration's effect on lipid metabolism. Secondly, we could not exhibit the molecular mechanisms for the effects of green tea and green coffee combination administration.

Conclusion

Our study revealed clear evidence that a combination of green tea and decaffeinated light roasted green coffee extracts improved cardiac insulin resistance better than single extract administration by ameliorating free fatty acids through adiponectin/FAS pathway modulation.

Data availability

Figshare: Underlying data for 'Green Tea and decaffeinated light roasted green coffee extract combination improved cardiac insulin resistance through free fatty acids and adiponectin/FAS pathway amelioration in metabolic syndrome rat model' <https://doi.org/10.6084/m9.figshare.13249163.v3>.

This project contains the following underlying data:

- Data of Metabolic Syndrome Rat Model.xlsx (This file contains the analyzed data)
- RAW data metabolic syndrome.xlsx (This file contains the actual observed values of the variables)
- Chlorogenic acid Coffee HPLC.pdf (This file contains the value of coffee chlorogenic acid levels as measured by HPLC)
- Caffeine coffee HPLC.pdf (This file contains the value of coffee caffeine levels as measured by HPLC)
- Green Tea Catechin HPLC.pdf (This file contains the value of the catechin levels in the tea)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/) (CC-BY 4.0).

Author contributions

M.L: Conceptualization, Data Curation, Methodology, Writing – Review & Editing

M.S.R: Conceptualization, Funding acquisition, Supervision, Writing – Review & Editing

D.A.N: Conceptualization, Data Curation, Methodology, Resources

M.N.R: Data Curation, Formal Analysis, Investigation, Writing – Original Draft Preparation

N.A.W: Data Curation, Investigation, Writing – Original Draft Preparation

N.W: Project Administration, Supervision, Writing – Review & Editing

Acknowledgements

Thanks to the Medical Faculty of Brawijaya University, the Molecular Biology Laboratory of the Department of Biology Mathematics and Natural Sciences of Brawijaya University, and the Ministry of Education, Culture, Research, and Technology.

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Version 1

Reviewer Report 18 July 2022

<https://doi.org/10.5256/f1000research.59048.r138844>

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Sylvia Riedel

Biomedical Research and Innovation Platform, South African Medical Research Council (MRC), Tygerberg, South Africa

General impression: The authors determined whether green tea, decaffeinated green coffee, and a combination thereof can reduce features of metabolic syndrome in rats, with a specific focus on insulin resistance. The manuscript in its current form lacks important information as well as clarity. Grammar and sentence construction need to be reviewed critically throughout the manuscript. The work has merit, but there are too many errors and omissions in this version, the authors should rethink and revise the manuscript thoroughly. The referencing requires attention. Specific comments are highlighted below:

Abstract

- Second sentence - grammar. What does “improvement of free fatty acid” mean?
- Abbreviations need to be explained when first used (e.g. FAS).
- It is unclear how many rats were used per group, is it 25 for all groups or per group?
- From the abstract, it is not clear whether groups 3, 4, and 5 were given the high-fat high-sucrose (HFHS) diet and streptozotocin (STZ).
- What is the rationale for the combination group of green tea and green coffee?
- Was circulating adiponectin measured and in which tissues were the mentioned genes assessed?
- The results should be stated clearly and vague statements such as “showed synergistic effects” should be avoided.
- The p values should be included. A clear description of which parameters increased and which decreased in which groups should be included.

Introduction

- There is a specific set of diagnostic criteria for metabolic syndrome which should be detailed and referenced in the introduction. Reference 10 describes non-alcoholic fatty liver disease (NAFLD) and not insulin resistance (IR) as such, it seems inappropriate to cite here. There are a few sentences that do not make sense. The authors should draw a distinction between cardiac dysfunction and cardiac insulin resistance and define what cardiac insulin resistance entails.

Methods

- The study outline is not clear enough from the description, an illustration would be helpful. Critical details regarding the animal work are missing, such as the number of animals per group and dosage of STZ applied. What are the “NCEP ATP III” criteria? These need to be explained and referenced and how the rats used for this study meet these criteria needs to be presented with data (can be supplementary data).
- What is the composition of the HFHS diet?
- It seems that this study and the study by Rohman *et al.* 2021 (reference 31) are using the same animals. If this is the case, then this should be stated clearly. Both manuscripts could benefit from combining the results to provide a more substantial and comprehensive overview of the study in one single paper.
- It would be preferable if critical references such as 31 were already published/accepted articles, rather than cited while awaiting peer review.
- If the present study and reference 31 are different experiments, then critical data on the animal's metabolic status (fasting blood glucose, body weights, and data showing that metabolic syndrome was induced, etc.) needs to be provided in the manuscript (the data is shared, but clear visuals would be preferable) and all paragraphs in the methods including extract preparation and the animal study need to clearly reflect this.
- There is also mention that the study is part of a study published previously (reference 30) – it needs to be clarified in which way. It seems that some of the confusion derives from an unclear formulation of sentences.
- The preparation of the extracts can be referenced if the same method was followed as in reference 31, however, it is not clear what was used to gavage the animals and how the solutions were prepared. Were the animals in the normal control group and the metabolic syndrome control also gavaged and if so, with what? For future studies, alternative methods such as administering the extracts in the diet should be considered to replace gavaging. Extracts can be freeze-dried and mixed into the diet.
- It is not clear how the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was analyzed using a kit. How was fasting blood glucose determined? This section requires clarification.
- Statistical analysis using a t-test is inappropriate. To assess group differences at least a one-

way analysis of variance (ANOVA) or better yet a two-way ANOVA should be used for the MS-induced treatment groups. The MS treatment groups fit a 2x2 factorial design, and a two-way ANOVA will give more appropriate insight into the data, especially the combination of treatments. The negative control and positive control can be compared using a t-test. It is unclear whether the authors report standard deviations (SD) as stated in the statistics paragraph or standard error of the mean (SEM) as stated in the graph legends. The distinction is important and requires clarification.

Results

- Legends to the graphs require more details, such as an explanation of abbreviations and a clear description in which tissues gene expression was analyzed. In general, graph legends should provide enough information to understand the data presented without reading the text.
- It was stated that decaffeinated green coffee was used. An explanation is required as to why the extract seems to contain caffeine. There is a chromatogram for caffeine in the data section. The second sentence in the HPLC results section is not clear, it mentions CGA, caffeine, and polyphenols but only two values are given. These values should also be presented with standard deviations of replicate measurements. This needs clarification.
- Graph numbers do not align with the references in the text. For example, on page 6 the first paragraph refers to Fig 2, but the data is displayed in Fig 3. In the last paragraph on page 10, the authors also refer to Fig 2 which should be Fig 3.
- There was no protein expression included or reported. This is an important aspect to add as gene expression does not necessarily correlate with protein levels and function.

Discussion

- Once the manuscript structure has been clarified and revised, the discussion should be refocused and written concisely to clearly work out what new knowledge this study brings to the body of literature. Grammar should be checked.
- The last sentence in the limitations section "...we could not exhibit the molecular mechanisms for the effects of green tea and green coffee combination administration...", is unclear but seems to contradict the authors' conclusion.

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Metabolic diseases, inflammation, gastrointestinal physiology, food chemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 10 November 2021

<https://doi.org/10.5256/f1000research.59048.r98201>

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A. Vijaya Anand

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The authors intended to evaluate the effects of Green Tea and Decaffeinated Light Roasted Green Coffee Extract metabolic syndrome. It is an interesting and useful study. Firstly, this manuscript is well-organised and well-structured. Secondly, this study observed the differentially expressed genes and provided potential target candidates for the treatment of the metabolic syndrome.

1. There are some grammatical and syntax errors in the manuscript. For example, the words "A free fatty acid is" may be as "Free fatty acids are"; "improvement" as "the improvement" (in the 7th line of the abstract); "IRS-1" as "of IRS-1"; "Combination green" as "A combination of green"; "study also" as "study were also"; "higher of" as "higher"; "lower of" as "lower"; "different with" as "different from". The grammar mistakes which are not mentioned here also to be checked and corrected properly.
2. There are some typing mistakes as well, and authors are advised to carefully proof-read the text. For example, the word "prodcedure" may be as "procedure"; "compound level" as "compound levels"; "tothe" as "to the"; "extract treated" as "extract-treated"; "theMSgroup" as "the MS group"; "theNCgroup" as "the NC group"; "GTgroup" as "GT group"; "theGTandGCgroups" as "the GT and GC groups"; "theCMgroup" as "the CM group"; "injection induced" as "injection-induced"; "pathway" as "pathways"; "tissue.." as "tissue."; "decaffeinatedlight" as "decaffeinated light". The typos not mentioned here also to be checked and corrected properly.
3. The keywords should not be from the title and also the remaining part of the manuscript. The

keywords should assist computer searches to find your specific article.

4. Check the abbreviations throughout the manuscript and introduce the abbreviation when the full word appears the first time in the text and then use only the abbreviation (For example, high-fat high sucrose (HFHS), streptozotocin (STZ), metabolic syndrome (MS), chlorogenic acid (CGA), high-fat diet (HFD), etc.). And it should be in both abstract as well as in the remaining part of the manuscript. Make a term abbreviated in the article when it is repeated at least three times in the text, not all of them need to be.

6. The weight of the animals should be included in the materials and methods under the heading "Animals and experimental design". The composition of the standard diet may be included in the materials and methods and also where it has been obtained.

7. In the statistical analysis the authors should include the software and version of the software used in the present findings under the heading "Data and statistical analysis".

8. The referencing in this paper is inconsistent. The authors have not given the references as per the journal format. In many places, the authors used numbers and authors with year and in few places used only numbers and in some places the authors' used names and years only. For example, "Van Dijk *et al.*, 2013" and it should be corrected properly.

9. The figures should be improved and a proper footnote should be given. All legends should have enough description for a reader to understand the figures without having to refer back of the main text of the manuscript. The authors have used the abbreviations in the axis and expansions that are not given with the graph. The quality of the image is not to be changed since it seems good. The only suggestion here is the authors may include the expansion of the terms used like 1) MS (metabolic syndrome), 2) NC (normal chow) 3) GT (green tea extract) 4) GC (decaffeinated-light roasted green coffee extract), 5) CM (combination of both extracts) as legends.

10. The author should justify how the dose fixation is done (is it based on toxicity test or previous reference, if previous should be included in the reference section) in materials and methods or discussion section of the manuscript.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Phytothreapeutics, Clinical Biochemistry, Medical Genetics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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