RESEARCH ARTICLE

Decaffeinated light-roasted green coffee and green tea extract combination improved metabolic parameters and modulated inflammatory genes in metabolic syndrome rats [version 1; peer review: 2 approved with reservations]

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Abstract

Background: Individually, green tea and green coffee have been extensively studied for mitigation of metabolic syndrome (MS) in both rats and humans; however, their combined effect requires further investigation. Thus, we compared the metabolic effect of combining green tea and decaffeinated light roasted green coffee on MS in rats.

Methods: An MS animal model was constructed by feeding Sprague-Dawley rats with a high-fat-high-sucrose (HFHS) diet for eight weeks and a low dose of streptozotocin (STZ) injection at week 2. Rats fed with HFHS diets and injected with STZ successfully developed MS phenotypes, indicated by higher body weight, systolic blood pressure, plasma triglyceride level, plasma fasting blood glucose level, and lower plasma HDL-C level, compared to those fed with a normal chow diet. Subsequently, MS rats were continuously fed with HFHS and divided into four groups: MS rats, MS with 300 mg/bw.t green tea extract (GT), MS with 200 mg/bw.t green coffee extract (GC), and MS with combined green tea and green coffee extract (CM) for nine weeks.

Results: Combining green tea and green coffee have synergistic effects on reducing plasma fasting blood glucose and triglyceride level. Inflammatory markers both in plasma and liver tissue robustly

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decreased in CM group rats. However, the reduction of systolic blood pressure was observed only in GT and CM groups. Moreover, all treatment resulted in an increase in plasma HDL-C level in MS rats.

Conclusions: Our data highlighted that, in MS animal models, combined green tea and decaffeinated light roasted green coffee augment their several individual beneficial effects of improved metabolic parameters and modulated inflammatory genes.

Keywords

metabolic syndrome, green tea, green coffee, combined extracts

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Introduction

Metabolic syndrome is a global public health concern. The prevalence of this disease is estimated to be about one-quarter of the world population. Metabolic syndrome is a condition that consists of risk factors such as abdominal obesity, increase triglycerides (TG), hyperglycemia, hypertension, and low high-density lipoprotein cholesterol (HDL-C) levels. Individuals with metabolic syndrome have a two to fivefold risk of developing diabetes, cardiovascular disease, stroke, and death from all causes¹. The pathogenesis of metabolic syndrome involves the strong interaction of genetic and modifiable risk factors that constitute the metabolic syndrome. Previous studies have shown that nuclear factor-kappa B (NF- κ B) signalling is a crucial pathway in chronic low-grade inflammation process and has been studied extensively in the context of obesity and the metabolic syndrome^{2,3}. Management of this disease consists of a dual approach that combines lifestyle modification and pharmacological intervention on those risk factors. During the past decades, various natural compound derived from plant extracts showed a beneficial effect in the management of metabolic syndrome⁴.

Tea, dried leaves of *Camellia sinensis*, is classified into green, black, and Oolong tea according to its leaf treatment. It contains numerous catechins, mainly epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate (EGCG)^{5,6}. In recent years, green tea and its constituents have been massively studied for its beneficial health effects, including alleviation of metabolic syndrome. Most of these studies showed green tea extract and its isolated constituents lowered body weight, blood glucose levels, and increased insulin sensitivity in animal models with metabolic syndrome induced either by high-fat diets, insulin resistance, or genetic modification⁷. A meta-analysis by Zhong *et al.*⁸ also revealed that tea extract reduced body mass index (BMI) and body weight in metabolic syndrome patients. Comparable with green tea, green coffee has recently drawn attention due to its health benefit. Green coffee consists of phenolic compounds, such as chlorogenic acids (CGA), and cinnamic acids (caffeic, ferulic or coumaric acid) which are known as protective agents against metabolic syndrome and type 2 diabetes mellitus⁹. The meta-analysis by Roshan *et al.*¹⁰ and Nikpayam *et al.*¹¹ showed green coffee administration had an ameliorating effect on metabolic syndrome parameters, such as systolic blood pressure, fasting blood glucose, and homeostatic model of assessment of insulin resistance (HOMA-IR). In addition, both green tea and green coffee have anti-inflammatory action and has been used as functional food for some metabolic disease protection^{12,13}. However, some studies reported drawbacks of green coffee administration due to its caffeine content^{14–16}. Hence, our study used decaffeinated coffee extracts to avoid the disadvantage of caffeine from coffee intake. Moreover, the CGA antioxidant activity is determined by the coffee's roasting level^{17,18}. Light roasted coffee beans possessed the highest CGA antioxidant activity. Our previous study showed that light roasted green coffee had a beneficial effect on adiponectin and insulin resistance amelioration in metabolic syndrome rat models¹⁹. While many studies reported the beneficial effect of green tea or green coffee extract on several diseases, few reported

the impact of the combination of these two compounds. A study by Tulp *et al.*²⁰ showed that natural compounds have shown greater activity when they are present in a mixture. Considering this, our study aimed to investigate the potential effects of green tea and decaffeinated light roasted green coffee combination in metabolic syndrome rats.

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.

Animal care and experimental protocol

A total of 25 male Sprague Dawley rats aged nine weeks old were obtained from the National Agency of Drug and Food Control, Indonesia. The number of animals was calculated using the Festing formulation²¹. The study was performed in the Animal Physiology Laboratory, Faculty of Mathematics and Natural Sciences, from March to July 2017. Rats were housed individually in a metabolic cage sized 50 cm x 30 cm x 15 cm at a temperature of 25°C and 40%–70% relative humidity. They were maintained with food and drink *ad libitum* with 12:12 hour light-dark cycle. After one week of acclimatization, the healthy rats were randomized using Excel (=RAND function) to determine their diet - either normal chow, high fat, or high sucrose (HFHS) diet - for the 17 weeks duration of the experimental protocol. On the 2nd week of the protocol, the HFHS fed rats were induced by a single intraperitoneal injection of streptozotocin (STZ) (bioWORLD cat.41910012-4) (30 mg/ kg B.W.). These procedures were performed in order to get experimental an animal model that represented the features of human metabolic syndrome. The experimental animals were not blinded during this study. The animals were fasted for 8-10 hours prior to blood drawing. The blood (0.5 ml) was obtained from the rats' tail and then centrifuged with 4500 rpm for 15 minutes to get the serum. The fasting blood glucose, triglyceride, and HDL level were determined by GOD-PAP, GPO, and indirect methods, respectively (BIOLABO cat LP80209 for glucose level determination, cat 80019 for triglyceride level determination, cat 86536 and cat 80106 for HDL level determination). Animals with fasting blood glucose levels over 126 mg/dl, triglyceride level over 150 mg/dl, systolic blood pressure over 140 mmHg, and HDL cholesterol lower than 40 mg/dl were considered to have metabolic syndrome, based on NCEP-ATP III criteria²². Five rats were assigned to normal chow without STZ injection (NC). The metabolic syndrome rats were weight-matched distributed into four experimental groups (five rats/group) (i) HFHS and STZ injection (MS), (ii) HFHS, STZ injection, and green tea extract at 300 mg/kg B.W. (GT), (iii) HFHS, STZ injection, and green coffee extract at 200 mg/kg B.W. (GC), and (iv) HFHS, STZ injection, and combination extract of green tea and green coffee at 300 mg/kg and 200 mg/kg B.W, respectively²³. The doses of the extract were obtained from our

previous studies^{19,24,25}. The dose of extract was given in millilitres based on weekly measured body weight and given via oral gavage daily. The food and water intake were recorded daily. The sacrifice procedures were explained as follows: (1) The rats were fasted for 12 hours; (2) After that, the rats were anesthetized by diethyl ether prior to the euthanasia in order to prevent painful euthanasia; (3) After the rats being anesthetized, they were euthanized by cervical decapitation. (4) Immediately after the rats died the blood was drawn from the heart into a microcentrifuge tube; (5) The hepatic tissues were obtained from the right lobe. Serum samples were obtained by centrifugation at 4,000 x g for 15 minutes at 4°C.

Extraction and decaffeination of green coffee bean

The coffee bean was obtained from Dampit coffee plantation, Malang, Indonesia (800 MAMSL). An automatic coffee roaster (N500i) light roasted *Coffea canephora* var *robusta* 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 produce the crude extract. The ethanol solvent is used due to its polar feature that could attract the active compounds contained in the green coffee bean. Furthermore, the crude extract was filtered using a filter cloth to separate the liquid phase from the solid phase. Moreover, the liquid phase was concentrated using a rotary evaporator (RV10 autoV, IKA) at $\pm 40^\circ\text{C}$. Finally, column chromatography completed using silica gel C18 17% (SiliaBond® C18, SiliCycle Inc) as a static phase to reduce the caffeine content, to attract more CGA, and to separate them from other substances, and then the filtered product was evaporated^{18,26}.

Extraction of green tea

The green tea was obtained from Sukawana green tea plantation, Bandung, Indonesia (1550 MAMSL). The second and third uppermost young green tea leaves were extracted. Green tea leaf weighing 500 grams was dried using a cabinet dryer (temperature of 50°C) for 8 hours to obtain green tea with 8 – 10% water content was measured using gravimetric analysis. The green tea was mashed with a blender and then boiled at 80°C for 30 minutes. 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 at the temperature of $\pm 40^\circ\text{C}$. Finally, column chromatography completed using silica gel C18 17% (SiliaBond® C18, SiliCycle Inc) as a static phase to attract bioactive compounds and to separate them from other substances, and then the filtered product was evaporated^{27,28}.

HPLC analysis

For the determination of caffeine and chlorogenic acid levels in the coffee, and epigallocatechin gallate level in the green tea, 1 gram of extract was diluted with 100 mL of distilled water. The HPLC analysis was performed using a Shimadzu Brand chromatograph (model SCL10AVP, Japan) that set up with a C-18 reverse-phase column (Shim-pack VP ODS 5 μm 150 x 4,6 mm). The HPLC was paired to a UV/visible spectrophotometric detector (SPD-20-A UV Vis Detector) then connected by an interface (CBM-101) to a microcomputer for data

processing. The protocol for analysis were as follows: (1) flow (1 mL/ min for caffeine and chlorogenic acid and 0,45 ml/min for epigallocatechin); (2) mobile phase method using isocratic method with 25% methanol for caffeine and chlorogenic acid and 0,1% TFA in acetonitrile for epigallocatechin; (3) static phase silica C18 with column temperature 25°C; (4) 10 μL of injection volume for caffeine and chlorogenic acid and 1 μL for epigallocatechin; (5) the wavelength detection at 272 nm for caffeine, 324 nm for chlorogenic acid, and 280 nm for epigallocatechin; (6) run time 15 minutes for caffeine, 30 minutes for chlorogenic acid, and 20 minutes for epigallocatechin. The concentrations of the compounds were determined with standard concentration curves.

Dose determination

Decaffeinated light roasted Green coffee extract and green tea extract doses were determined from the previous study in our laboratory. The optimum dose was 300 mg/bw.t for green tea extract and 200 mg/bw.t for green coffee extract^{19,24,25}. Daily food intake and fluid intake was measured every day, and body weight was measured every week. The food and fluid intake for each rat was measured by subtracting the measured amount provided by the remaining amounts in the cage.

Biochemical analysis

The serum concentrations of fasting glucose (BIOLABO, cat no. 80009), triglycerides (TG) (cat no. 80019), and HDL-Cholesterol (BIOLABO, cat no. 86516) were measured enzymatically using commercial kits. C-Reactive Protein (Elabscience cat no. E-EL-R0506) and TNF- α (Elabscience cat no. E-EL-R0019) were analyzed by enzyme-linked immunosorbent assay (ELISA).

Blood pressure measurements

Blood pressure was measured using the tail-cuff method with a sphygmomanometer technique (Ugo Basile 58500) at the baseline and the end of the experiment. Three readings are taken consecutively, and the average was then calculated and taken as a final reading for SBP.

Isolation of total RNA and RT-PCR

Total RNA of liver tissues was isolated using the Easy Blue (Intron Biotechnology, cat no. 17061) according to the manufacturer's protocol. Reverse transcription reaction was performed using a ReverTra Ace- α kit (Toyobo, FSK-101). Then the RNA expression levels were carried out using PCR Light Cycler 96 system (Takara, cat no. TP600) using a GoTaq Green Master PCR Kit (Promega, cat no, M7822) according to the manufacturer's protocols. Primer sequences were as follows: B-actin, forward: 5'-TGA GAG GGA AAT CGT GCG TGA CAT-3' and reverse: 5'-ACC GCT CAT TGC CGA TAG TGA TGA-3'; NF-KB, forward: 5'-AAC GCA TCC CAA GGT GCT GGA A-3' and reverse: 5'-GCA GCT GGA AAA GCT CAA GCC A-3'; TNF- α , forward: 5'-CGT CAG CCG ATT TGC CAT TTC-3' and reverse: 5'-TGG GCT CAT ACC AGG GCT TG-3'; IL-6, forward: 5'-CCC AAC TTC CAA TGC TCT CCT AAT-3' and reverse: 5'-GCA CAC TAG GTT TGC CGA GTA GA-3'. The PCR cycling conditions were as follows: 5 min at 95 °C; 35 cycles of 30 s at

95 °C, 30 s of annealing at 52.7°C, 52.7°C, 54.7°C, and 55 °C for NF- κ B, TNF- α , IL-6 respectively, followed by extension for 30 s at 72 °C; and a final extension for 10 min at 72°C. The mRNA levels were quantified using spectrophotometer at 260 nm and 280 nm. of the target genes normalized to the expression level of β -actin.

ELISA

For assessing TNF- α and CRP secretion, the blood of the rats collected and serum separated by centrifugation. Serum TNF- α and CRP concentrations were measured using an enzyme-linked immunosorbent assay according to the manufacturer's instructions (Elabscience E-EL-R0019 and E-EL-R0022 respectively). The optical density (OD) value was read by ELISA reader machine (Biotek ELx808) and the standard curve was generated to get the formula for TNF- α and CRP quantification. Measurements performed were in two replicates. The results presented are as ng/ml.

Statistical analysis

The experimental unit was a group of animals and the data obtained was presented in the form of the mean value and standard error of mean (SEM) calculated with GraphPad Prism 8.3.1 software. An independent t-test was used to analyze two of the tested group. Statistically significant differences were defined as $p < 0.05$.

Results

Compounds of green tea and decaffeinated light roasted green coffee

According to HPLC analysis, the concentration of epigallocatechin-3-gallate in our green tea extract was 74,126 μ g/g. While the concentration of chlorogenic acid and caffeine, and polyphenols in green coffee, was 27,134 and 43,473 μ g/g green coffee extract, respectively²⁹.

Animal characteristics

Assessment of body weight, systolic blood pressure, fasting blood glucose level, triglyceride and HDL cholesterol plasma level of combination HFHS diet and a low dose of STZ injection revealed the presence of metabolic syndrome symptoms as classified by the NCEP-ATP III criteria. We observed higher body weight, systolic blood pressure, fasting blood glucose level, triglyceride plasma levels and lower plasma levels of HDL cholesterol in rats that received HFHS diet and STZ injection compared to control rats (Figure 1a and b), as expected.

Then, during the nine following weeks, the rats that met NCEP-ATP III criteria were subjected to HFHS alone or HFHS with 300 mg/BW of green tea extract (GT), 200 mg/BW of decaffeinated light roasted green coffee extract (GC), and combination of those extracts (CM) (Figure 1a).

Combination of green tea and decaffeinated light roasted green coffee does not improve weight loss and food efficiency ratio

At the end of the intervention, all HFHS-fed- and STZ-injected rats had significantly lower body weight compared to normal

chow-fed rats (Figure 1c). Additionally, all metabolic syndrome rats consumed more food (Figure 1d). These results indicate that the food efficiency ratio (weight gain/food intake) of all STZ-treated animals was significantly lower ($***p < 0.001$, $****p < 0.0001$) compared to that of normal chow-fed rats (Figure 1d).

Green tea extract reduces systolic blood pressure

All supplemented metabolic syndrome rats presented reduced systolic blood pressure (Figure 2a). Green tea and combination of green tea and green coffee could significantly reduce the ($*p < 0.05$) blood pressure after nine weeks of administration; however, the blood pressure of decaffeinated light roasted green coffee only-treated rats was not significantly different compared to their blood pressure before the intervention. Interestingly, compared to the metabolic syndrome rats (MS) group, the green tea extract (GT) group had the lowest systolic blood pressure. Moreover, the green tea treated rats' blood pressure was similar to those in normal chow-fed rats. These results suggest that green tea alone could significantly improve the systolic blood pressure of metabolic syndrome rats.

Combination of green tea and decaffeinated light roasted green coffee improves fasting blood glucose

All treatments successfully lowered the fasting blood glucose (FBG) levels in rats (Figure 2b). Compared to the blood glucose level before the intervention, only green tea-treated and combination-treated rats demonstrated a significant reduction in FBG. Although the FBG of every treated rat was significantly higher compared to its on normal chow group, combining green tea and green coffee could dramatically lower the fasting blood glucose among the treatment group. In general, green tea and decaffeinated light roasted green coffee combination could improve fasting blood glucose.

Combination of green tea and decaffeinated light roasted green coffee improves lipid parameters

Compared to levels before the intervention, all given extracts could significantly reduce triglyceride (TG) levels ($*p < 0.05$) and improve HDL cholesterol levels (HDL-C) ($*p < 0.05$, $**p < 0.01$) (Figure 3a and 3b). After nine weeks of administration, only CM group rats had significantly reduced TG level compared to MS group rats, also, those rats had the lowest TG levels. In contrast, all treated rats had significantly higher HDL-C levels compared to metabolic syndrome rats. These data illustrated that the combined extract group had an improved lipid profile.

Combination of green tea and decaffeinated light roasted green coffee reduces inflammatory markers on plasma and liver

The intervention rats showed a reduction of inflammatory markers in plasma and liver tissue compared to MS rats (Figure 4a and 4b). The combined extract-treated rats had the lowest plasma level of TNF α . Moreover, those rats also had the lowest relative mRNA expression of NF- κ B, TNF α , and IL-6 in liver tissue, suggesting green tea and decaffeinated light roasted green coffee synergistically reduced inflammatory markers in both plasma and liver tissue

Figure 1. Anthropometric characteristics of rats. (a) Schematic diagram of study's experimental design. (b) Body weight, systolic blood pressure, fasting blood glucose, triglyceride level and HDL cholesterol level at 8 weeks. (c) Body weight before extract administration (above) and after 9 weeks administration (above, below). (d) Food intake and food efficiency ratio after 9 weeks intervention. Data are expressed as mean $\pm$ SEM (N=4-5). * P <0.05, ** P <0.01, *** P <0.001, **** P <0.0001 compared with NC.

Figure 2. Measure of systolic blood pressure and fasting blood glucose. Systolic blood pressure (a) and fasting blood glucose (b) before extract administration (left) and after 9 weeks extract administration (left, right). Data are expressed as mean $\pm$ SEM (N=4-5). * P <0.05, ** P <0.01, *** P <0.001, **** P <0.0001 compared with NC. # P <0.05, ## P <0.01, ### P <0.001, #### P <0.0001 compared with MS. † P <0.01 compared with GT. \$\$\$ P <0.01 compared with GC.

Figure 3. Measure of lipid profile. Triglyceride (a) and HDL cholesterol level (b) before extract administration (left) and after 9 weeks extract administration (left, right). Data are expressed as mean $\pm$ SEM (N=4-5). * P <0.05, ** P <0.01, *** P <0.001, **** P <0.0001 compared with NC. # P <0.05, ## P <0.01, ### P <0.001, #### P <0.0001 compared with MS. † P <0.01 compared with GT. \$\$\$ P <0.05 compared with GC.

Discussion

In agreement with the previous literature^{30,31}, we showed that the administration of green tea extract was sufficient to reduce blood pressure by 19%, which was similar to normal chow group. In contrast to green tea treatment, we did not find

any differences in measured blood pressure before and after green coffee extract supplementation alone. Coffee contains many pharmacologically active components that inhibit the anti-hypertensive effect of antioxidative polyphenol, chlorogenic acid, such as hydroxy hydroquinone (HHQ)³². We did not

Figure 4. Measure of inflammatory markers. (a) The plasma level of C-reactive protein (CRP) (above) and TNFα level (below). (b) Relative mRNA expression of NF-κB, TNFα, and IL-6 of liver tissue. Data are expressed as mean ± SEM (N=4-5). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared with NC. #P<0.05, ##P<0.01, ###P<0.001, ####P<0.0001 compared with MS. †P<0.05, ††P<0.01 compared with GT. \$P<0.05, \$\$P<0.01 compared with GC.

fractionate the green coffee extract to eliminate HHQ, thus, this leads us to hypothesize that HHQ might inhibit the hypotensive effect of green coffee extract. Interestingly, the addition of green tea extract to light roasted green coffee extract was shown to help lower blood pressure compared to that of animals treated with decaffeinated light roasted green coffee extract only, suggesting that green tea extract exerts a more favourable influence on blood pressure.

Compared to metabolic syndrome rats, our study showed either green tea or decaffeinated light roasted green coffee supplementation after nine weeks was sufficient to decrease fasting blood glucose and plasma triglyceride (TG) levels, and these results are supported by previous studies^{33,34}. Unlike the plasma TG levels, findings on the potential effects of tea on HDL-C are mixed. Some studies reported null effects^{35,36}, whereas some showed a significant increase⁵. However, few investigate the effect of green coffee on plasma HDL-C level. Here, our study presented green tea or green coffee extract alone after nine weeks of administration significantly increased plasma HDL-C level which was similar to normal chow-fed mice. Moreover, we showed that a synergistic effect was not found for the plasma HDL-C levels.

Many studies reported that metabolic syndrome may induce and may be caused by low-grade chronic inflammation that can be detected systematically and within affected tissues, such as adipose tissue, liver, and vasculature³⁷⁻³⁹. As shown in the present study, both levels of systemic markers of inflammation, including C-reactive protein (CRP) and TNFα, and relative

mRNA expression of NF-κB, TNFα, and IL-6 of liver tissue were increased in metabolic syndrome model rats. The liver plays a pivotal role in glucose and lipid homeostasis. Hepatic steatosis and its inflammatory state (steatohepatitis) are closely related to metabolic syndrome⁴⁰. A recent review has established the critical role of NF-κB on the liver for the development of insulin resistance⁴¹. Chronic inflammation activation by NF-κB in the liver activates IKK-β in hepatocytes that exhibit hepatic and systemic insulin resistance, as expected, hepatic overexpression of IκBα, a repressor of NF-κB signalling, reverses the phenotype⁴².

Recent reviews showed that targeting inflammation in chronic disease may be beneficial in metabolic disorders^{43,44}. While only focusing on inflammation may be harmful to metabolic conditions due to potential immunosuppression, a review suggested anti-inflammatory nutritional intake as a new alternative in modulating metabolism and inflammation⁴⁵. Several studies have revealed that green tea and green coffee reduced inflammatory markers in animal models^{12,46-48}. Similar to those studies, we demonstrated that green tea or green coffee alone decreased plasma CRP and TNFα levels. Moreover, decreased mRNA expression level of inflammatory genes in the liver was found in rats treated with green tea or green coffee supplementation. Combining those compounds exacerbated the reduction of plasma TNFα and transcription of NF-κB, TNFα, and IL-6. Previous studies have shown that targeting hepatic inflammation by specific deletion of downstream NF-κB signalling, IKK-β and p65, shows an improvement in glucose homeostasis^{49,50}. However, both studies showed that the

improvement in insulin sensitivity is limited to the liver. Mice with IKK- β deletion in hepatocytes exhibited liver insulin sensitivity, but insulin resistance in muscle and fat in response to a high-fat diet⁴⁹. Together these studies conclude that hepatic inflammation contributes to the development of the metabolic disorder, however, further detailed studies are needed to elucidate the beneficial effect of targeting inflammation in the liver.

There are some limitations to this study. First, we did not have data regarding inflammation profiles on other affected tissues, such as adipose tissue, which is directly associated with metabolic syndrome. Second, we could not establish a molecular mechanism for the beneficial effects of combined green tea and green coffee.

Conclusion

In our data highlight that, in metabolic syndrome rats, the combination of green tea and decaffeinated light roasted green coffee ameliorate hepatic inflammatory through suppression of NF- κ B, TNF α , and IL-6 gene expression have synergistic metabolic and anti-inflammatory effects.

Data availability

Figshare: Data for Combination Green Tea and Green Coffee Extract on Inflammatory in Metabolic Syndrome Rat Model. <https://doi.org/10.6084/m9.figshare.13249163.v3>²⁹.

This project contains the following underlying data:

- Data of Metabolic Syndrome Rat Model.xlsx (This file contains the analysed 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](#) (CC-BY 4.0).

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Nelly Mayulu

Nutrition and Food, Faculty of Medicine, Sam Ratulangi University, Manado, Indonesia

This study presents the results of Decaffeinated light-roasted green coffee and green tea extract combination to improve metabolic parameters and modulated inflammatory genes in metabolic syndrome rats. Interesting research, but the writing needs to be improved, as follows:

1. In the abstract please state how many mice were used in total and in each group.
2. In the abstract, state how many doses are used for STZ injection?
3. In the abstract, the results for C-reactive protein (CRP), NF- κ B, TNF α , and IL-6 have not been mentioned, please add them in the abstract.
4. In the Methods: Biochemical analysis and ELISA section, the detailed kit of IL-6 and NF- κ B has not been mentioned.
5. High fat, or high sucrose (HFHS) diet needs to be clarified the percentage content of the feed (e.g. what percentage of fat and what percentage of sucrose, referring to which research HFHS is used?).
6. The analysis of the levels of chlorogenic acid and caffeine, and polyphenols (epigallocatechin gallate) in green coffee using HPLC was carried out how many times it was repeated? Duplo? Triplicate? If there is please provide SD (Standard Deviation).
7. Writing the Latin name of the species, please pay attention again (italics).
8. Pay attention to the writing in the "Isolation of total RNA and RT-PCR" section, please correct the last sentence, (of the target genes normalized to the expression level of -actin).
9. In the discussion, add references to the benefits of epigallocatechin-3-gallate and polyphenols in green coffee which are thought to contribute to having synergistic metabolic and anti-

inflammatory effects.

10. Why don't you test the levels on the combination of the two mixed extracts (Combination of Green Tea and Green Coffee Extract)?

I recommend to be indexed after some improvements.

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?

Partly

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

Partly

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

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Functional Food, Natural Product, Non-communicable Diseases, Food and Nutrition, Antioxidants, Polyphenols,

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.

Reviewer Report 06 September 2021

<https://doi.org/10.5256/f1000research.30875.r92580>

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Alex Boye

Department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health and Allied Sciences, University of Cape Coast, Cape Coast, Ghana

In this manuscript authors investigated how a combination therapy comprising extracts of decaffeinated light-roasted green coffee and green tea influence metabolic parameters and pro-inflammatory factors in a rat model of metabolic syndrome. I find the study to be interesting and relevant in the specific topic area. Nonetheless, the study will benefit from the following:

1. The title needs to capture the key metabolic factors as well as the name of the chemical used to induce metabolic syndrome in the rats.
2. Authors should avoid personalizing the study by limiting or better still avoid use of “we, our, us etc”.
3. Dose of STZ administered at week 2 should be mentioned in the abstract.
4. Language should be improved throughout manuscript.
5. Authors should replace “constructed” with “established” in the abstract, line 6.
6. These factors (systolic blood pressure, plasma triglyceride level, plasma fasting blood glucose level) on their own do not represent phenotypes of MS, rather elevated levels of these factors.
7. What was the dose of “combined green tea and green coffee extract (CM)”?
8. Light roasted coffee should be hyphenated, i.e., light-roasted coffee. Use this throughout manuscript.
9. “Drink” replace with “water”.
10. Improve “...to get experimental an animal model that represented...”
11. “with 4500 rpm “should be “at 4500 rpm”.
12. How was MS confirmed in rats?
13. Improve “STZ injection, and combination extract of green tea and green coffee at 300 mg/kg and 200 mg/kg B.W, respectively”.
14. State the dose of diethyl ether used to induce anesthesia.
15. Italize *Coffea canephora*.
16. Check this “gallat” should be “gallate”.
17. The organization of the “materials and method” section should reflect the order in which experiments were conducted. For example, collection, and preparation of extracts preceded animal studies.
18. Improve the statistical analysis subsection. State how parameters measured were presented in figures and tables, mean $\pm$ s.e.m or SD. State the statistical software used in

the analysis of data and its source (manufacturer, country of origin). State the specific statistic that were measured for comparison among groups (control and treatment groups). Follow convention by stating the P-value at which differences in mean was considered statistically significant.

19. Both language and results reporting under the results section need to be improved.
20. Provide HPLC chromatogram for the two extracts as part of the results.
21. Please, avoid use of "we, our, us etc" throughout manuscript.
22. Improve the discussion, e.g., "Coffee contains many pharmacologically active components that inhibit the anti-hypertensive effect of antioxidative polyphenol, chlorogenic acid, such as hydroxy hydroquinone (HHQ)³²."
23. Rewrite the conclusion by improving language. Also, it should advance future direction of the topic area while reflecting on the key findings of the current study.
24. Consider replacing reference numbers 14 and 18, with a recent one if possible.

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?

Partly

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

Partly

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

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pharmacology, natural product pharmacology, toxicology, cancer pharmacotherapy

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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