https://doi.org/10.48048/tis.2024.8245

Combination of Green Tea, Green Coffee, and Turmeric Extract Improve the THOC5 and AIF1, but not ACTA2 and CNN1 Gene Expression in the Aortic Tissue of Metabolic Syndrome Model

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Received: 28 March 2024, Revised: 8 July 2024, Accepted: 15 July 2024, Published: 15 October 2024

Abstract

Metabolic syndrome (MetS) is a group of risk factors in the form of central obesity, insulin resistance, dyslipidemia, and hypertension, increasing oxidative stress. This pathological event leads to the development of cardiovascular disease, for instance, atherosclerosis. Besides modifiable risk factors, non-modifiable risk factors such as genetic factors also play a role in the formation of atherosclerosis in MetS conditions such as THOC5, AIF1, CNN1, and ACTA2. Recently, natural compound derivatives, such as epigallocatechin-3-gallate (EGCG), chlorogenic acid (CGA), and turmeric, have shown beneficial effects in MetS improvement. This study aimed to investigate the effect of green tea, green coffee, and turmeric extract on the expression of THOC5, AIF1, CNN1, and ACTA2 genes that contributed to atherosclerotic vasculopathy development in the MetS rat model. Twenty-five MetS rat models were grouped into 4 groups (n = 5): Standard control (SC), MetS (MetS), a combination of green tea, green coffee, and turmeric extract with treatment doses: 300/100/150 mg/BW(C1) and 400/200/250 mg/BW(C2) group. The THOC5, AIF1, CNN1, and ACTA2 expression were measured at the end of treatment periods. This study found that administering green tea, green coffee, and turmeric extract can lower the expression of THOC5, AIF1, CNN1, and ACTA2. The correlation test showed that there is a strong correlation between THOC5 and AIF1 gene expression, with positive value. In summary, the combined effects of green tea, green coffee, and curcumin extract show significant promise as a potential anti-atherosclerosis treatment by improve the THOC5 and AIF1, but not ACTA2 and CNN1 gene expression in the aortic tissue of metabolic syndrome model.

Keywords: Green coffee, Green tea, Curcumin, THOC5, ACTA2, CNN1, AIF1, Metabolic syndrome

Introduction

MetS, which affects 20 - 25 % of the world's adult population, is a group of risk factors in the form of central obesity, insulin resistance, dyslipidemia, and hypertension (National Cholesterol Education Program (NCEP ATP III, 2002) [1]. Individuals with MetS have an increased risk of developing atherosclerotic cardiovascular disease [2]. The presence of metabolic disorders in MetS causes an increase in reactive oxygen species (ROS) and a decrease in antioxidants, increasing oxidative stress. This condition causes endothelial dysfunction that leads to the development of atherosclerosis through increased adhesion molecules, macrophage differentiation, and the release of pro-inflammatory cytokines such as interleukin-1 beta (IL-1β), tumor necrosis factor-α (TNF-α), interferon-γ (INF-γ), and the formation of oxidized Low-Density Lipoprotein (ox-LDL) [3]. Apart from intimal injury, this pathological event also affects vascular smooth muscle cells (VSMC), critical participants in early and late-stage atherosclerosis development [4]. Upon inflammation and increased ROS production in MetS, VSMC undergoes phenotype switching towards a synthetic phenotype with proliferation and migration capabilities [4,5].

Besides modifiable risk factors such as dyslipidemia and hyperglycemia, non-modifiable risk factors such as genetic factors also play a role in the formation of atherosclerosis in MetS conditions. THO Complex 5 (THOC5) is one of the genes found in atherosclerosis. THOC5 regulates the expression of a subset of genes post-transcriptionally, thereby playing an essential role in disease progression [3]. In atherosclerosis, THOC5 plays a role in the proliferation and migration process of vascular smooth muscle cells (VSMC) [6]. THOC5 regulates the expression of VSMC marker genes (ACTA1 and CNN1). Downregulation of ACTA2 and CNN1 will increase VSMC proliferation [7]. In addition, the VSMC migration process is also mediated by the Allograft Inflammatory Factor 1 (AIF1) gene, which encodes the AIF1 protein [8]. AIF1 is an actin and calcium-binding protein expressed in medial aortic smooth muscle cells induced by pro-inflammatory cytokines (IL-1β, IL-16, INF-γ). Through colocalization with Rac Family Small GTPase 1 (RAC1), AIF1 enables chemotaxis and migration of VSMC [9]. It is known that the AIF1 gene is one of the genes that are dependent on the THOC5 transport gene at the post-transcriptional level [10].

Recently, various natural compounds derived from plant extracts showed a beneficial effect in managing MetS. Anti-inflammatories and antioxidants such as polyphenols can be adjuvant therapy for MetS, mainly to prevent atherosclerosis. Some ingredients with high polyphenol content are green coffee and green tea. The most abundant polyphenolic compound in green coffee beans is chlorogenic acid (CGA), and chlorogenic acid epigallocatechin gallate (EGCG) in green tea leaves [11,12]. Previously, studies had been carried out on the effects of giving a combination of green tea and green coffee extracts to rats with MetS models where the combination of the 2 could improve hyperglycemia conditions by reducing transforming growth factor (TGF-β1) and maintaining the balance of lipid influx and efflux through increasing peroxisome proliferator-activated receptor-γ (PPAR-γ) [13-16]. In addition, EGCG is known to have a 50 times more potent effect than metformin in AMP-activated protein kinase (AMPK) activation and impacts reducing LDL and preventing atherosclerosis. However, besides its role in reducing hyperglycemia and hyperlipidemia, EGCG has low bioavailability [17].

Curcumin is an active compound from Curcuma longa, which is known to have anti-inflammatory effects by reducing nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation and reducing the sensitivity of LDL to beta-oxidation [18]. Curcumin can increase membrane permeability and transporter-mediated intestinal efflux to increase the bioavailability of EGCG and maximize its therapeutic effect 39. Apart from that, curcumin is known to have benefits for reducing the impact of Statin-Associated Muscle Symptoms and has an excellent hepatoprotective effect [19].

The effect of giving green coffee extract and decaffeinated green tea on MetS model rats has been studied [13-16]. However, the impact of 3 combinations of green coffee, green tea, and curcumin on VSMC migration in preventing atherosclerosis in MetS conditions has not been studied. This result is the basis for researching the effects of a combination of green coffee extract, green tea and curcumin on MetS rats, especially regarding the expression of the THOC5, ACTA2, CNN1, and AIF1 genes, which play a role in VSMC migration. However, there was a lack of studies investigating the effect of 3 combinations in the inhibition of signalling transduction responsible for the protective effect of the aorta to the rat model of MetS, especially in proliferative and migration of vascular smooth muscle cells.

Materials and methods

Preparation of coffee-green tea-turmeric extract

Green coffee extract is derived from Robusta coffee beans (*Coffea canephora*) through a series of processes, including sorting, roasting, grinding, and decaffeination. Similarly, the extraction of green tea extract entails a methodical process involving *Camellia sinensis* leaves, which undergo sorting, drying, grinding, and blanching. Moreover, the extraction of turmeric from *Curcuma longa* was done by sorting, drying, grinding, boiling, and filtration. This procedure is consistent with the methodologies established in our previous research by Lukitasari *et al*. [13] and Na *et al*. [22].

Animal and experimental design

Twenty male Sprague-Dawley rats aged 9 weeks weighed 230 - 340 g were obtained from the Indonesian National Agency of Drug and Food Control. Rats were housed individually and acclimatized for 7 days in an ecologically controlled standard polycarbonate cage sized 50×30×15 cm^3 at a temperature of 25 °C and 40 - 70 % relative humidity. The rats were maintained on AIN-93M food and drank ad libitum in a 12:12-hour light-dark cycle environment. Food was provided and replaced daily.

After 1 week of the acclimatization process, the rats were randomized into 2 groups based on the diet administered: A normal diet (n = 5) and a high-fat high-sucrose (HFHS) diet (n = 15). The diet regimen was maintained throughout the 17-week protocol. In the 2nd week of the protocol, the HFHS diet group received an intraperitoneal injection of a low dose of streptozotocin (STZ) (30 mg/kg BW). In contrast, the normal group was injected with a citrate buffer as an STZ mimic. On the 8th week of protocol, the rats were considered to have MetS based on NCEP-ATP III if it meets at least 3 of the following criteria: 1) Fasting blood glucose levels over 126 mg/dL, 2) Triglyceride level over 150 mg/dL, 3) Systolic blood pressure over 140 mmHg and 4) High-Density Lipoprotein (HDL) cholesterol lower than 40 mg/dL. The rats were then divided into 4 experimental groups: Normal control group (NC, n = 5), metabolic syndrome positive control group (MetS, n = 5), HFHS + CGA/EGCG/CUR 100/300/150 (C1, n = 5) and CGA/EGCG/CUR 200/400/250 mg/kg BW group (C2) (n = 5). The extracts were given in milliliters based on weekly measured body weight and administered daily via oral gavage for 9 weeks. At the end of the protocol, the rats were sacrificed by cervical decapitation with the methodologies established in our previous research [13,20].

Gene expression quantification

The aorta was obtained after 17 weeks of treatment and preserved in an RNA Buffer solution. The extraction of total RNA was done using the Total RNA Extraction Kit easy-BLUE (Intron Biotechnology, South Korea) reagent. The total RNA was stored at –80 °C until gene expression analysis. The reverse transcription reaction was converted by ReverTra Ace (Ref FSK-101, Toyobo, Japan). The RNA expression levels were measured using the PCR LightCycler 96 system (PCR, Takara, Japan). Each tube of the PCR

mixture contained the specific primers, cDNA, and GoTaq Master Mix (Ref M7122, Promega, Madison, USA) (Integrated DNA Technologies, Singapore). Primer sequences created using NCBI PrimerBLAST were as follows:

THOC5	Forward 5'-GATGCTGAAGAGGAGCAGAC-3'
	Reverse 5'-ATACAAGCAGCTCAAGACCG-3'
CNN1	Forward 5'-AACTTGTCTGGGTCATCATCTCG-3'
	Reverse 5'-TTCGCAAAGAATGATCCCGT-3'
AIF1	Forward 5'-GAGCTATGAGCCAGAGCAAG-3'
	Reverse 5'-TGGCTTCTGGTGTTCTTTGT-3'
ACTA2	Forward 5'-GAGCTATGAGCCAGAGCAAG-3'
	Reverse 5'-TGGCTTCTGGTGTTCTTTGT-3'
β-ACTIN	Forward 5'-CGAGTACAACCTTCTTGCAG-3'
	Reveres 5'-CATTGTAGAAAGTGTGGTGC-3'

The PCR amplification protocol included an initial pre-denaturation step at 95 °C for 5 min, followed by 10 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. It was followed by an additional 30 cycles of denaturation at 95 °C for 30 s, annealing at 5 °C below the melting temperature for 30 s, and extension at 72 °C for 30 s. The reaction was maintained at 4 °C. The PCR products were separated by electrophoresis using the Mupid-exU Submarine Electrophoresis System (Advance, Japan) and visualized with the ImageQuant LAS 500 Chemiluminescence CCD Camera (Guangdong Denley Technology, China). Band intensities were semi-quantified using ImageJ software to determine the relative expression levels of each gene, normalized to the housekeeping gene (β-actin).

Data and statistical analysis

Statistical analysis was performed using SPSS 26. Data were presented as mean ± standard deviation (SD) and analyzed by a one-way ANOVA test followed by an LSD test. Significant statistical differences in all tests were considered when p-values < 0.05.

Ethical clearance

This experimental design and protocols have been approved by the Health Research Ethics Committee of the Faculty of Medicine, Brawijaya University, Malang, Indonesia, by registered number 152/EC/KEPK-S2/06/2022. The animal care in all experimental procedures was under the rigorous supervision of the ethical commission. This study used the minimum number of rats, and appropriate measures were taken to minimize pain or discomfort.

Results and discussion

The combination of coffee, green tea, and turmeric extract attenuated the metabolic characteristics of the MetS rat model

Examination of food intake, systolic blood pressure, fasting blood glucose, HDL, LDL, total cholesterol level, and body weight already described by Rohman *et al*. [16]. In this study, the rats were treated with 2 concentrations of CGA/EGCG/CUR (100/300/150 and 200/400/250 mg/kg BW). The results of the descriptive analysis can be seen in **Table 1**.

Table 1 Result of the measurement and analysis on THOC5, AIF1, ACTA2, and CNN1.

Variable	Group (n = 5)	Min	Max	Mean Square^	F	*p*-value
THOC5	NC	0.733	1.077	0.226	5.771	0.007
	METS	1.010	1.759			
	C1	0.859	1.251			
	C2	0.609	0.995			
AIF1	NC	0.012	0.774	0.689	4.833	0.014
	METS	0.509	1.309			
	C1	0.557	1.766			
	C2	0.176	0.901			
ACTA2	NC	0.477	1.446	0.010	0.093	0.003
	METS	0.239	1.269			
	C1	0.649	1.210			
	C2	0.716	1.155			
CNN1	NC	0.636	1.185	0.571	7.293	0.963
	METS	0.552	1.049			
	C1	1.089	2.195			
	C2	0.793	1.261			

Note: ^Means square in between group. *Significant different (*p*-value < 0.05).

The EGCG, CGA, and curcumin concentration in coffee, green tea, and turmeric extract combination

The result of the EGCG, CGA, and curcumin extraction process was analyzed by LC-HRMS as described in the previous study. The peak chromatogram of coffee, green tea, and curcumin identified the possibility of EGCG, CGA, and Curcumin as the main secondary active compounds

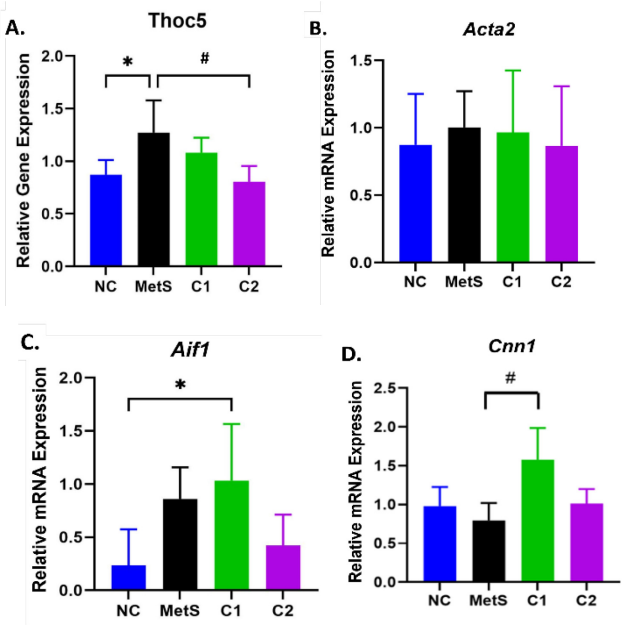

Figure 1 Effect of coffee, green tea, and turmeric extract combination on vascular smooth muscle gene (A) THOC5, (B) ACTA2, (C) CNN1, and (D) AIF1 expression in rat's aortic MetS model. Data were expressed in Mean ± SD (n = 5). *$p < 0.05$ compared to NC group, #$p < 0.05$ compared to MetS group, NC: Normal control, MetS: Metabolic syndrome, C1: Combination dose of CGA/EGCG/CUR 100/300/150 mg/kg BW and C2: Combination dose of CGA/EGCG/CUR 200/400/250 mg/kg BW.

The effect of coffee, green tea, and turmeric extract combination lowering the THOC5 gene expression in aortic rat MetS model

The effects of green tea, green coffee, and curcumin extract combination on the THOC5 gene are shown in **Figure 1**. MetS induction using an HSHF diet and low-dose STZ injection showed an increase in THOC5 gene expression compared to those in the healthy control group (NC). The expression of THOC5 gene in the MetS group has a significant value (p-value < 0.05) compared to the NC group. This result shows that feeding an HFHS diet, accompanied by a low dose of STZ injection, can induce THOC5 gene expression. Furthermore, administration of lower doses of coffee, green tea, and curcumin extract combination (C1) resulted in a decrease in THOC5 gene expression. The gene expression after C2 administration was statistically significant compared to the MetS group (p-value < 0.001). These results showed that increasing the dose of a combination of CGA/EGCG/CUR 200/400/250 mg/kg BW can reduce the expression of the THOC5 gene.

The THO complex (Thoc) is a subunit of the Transcription-EXport (TREX) ribonucleoprotein complex that binds transcription to nascent RNA splicing, elongation, and export. Notably, Thoc6 acts as a Tho scaffold, and THOC5 acts as an adaptor for spliced mRNA release from the nucleus. THOC5, which links transcription and mRNA processing to mRNA export, is an essential molecule for stem cell maintenance, organ development, and proliferation [10]. In this study, we found that THOC5 increased in rat aorta with MetS, suggesting an early stage of vascular dysfunction. Several mechanism Genome-wide association studies from about 100,000 people have revealed that CXCL12 becomes a new atherosclerosis locus both in mice and humans. THOC5 is a vital gene product in stem cell biology and is post-translationally regulated by stem cell ligands (CXCL12) [23]. External stimuli, such as growth factors, cytokines, chemokines, and DNA damage, can cause phosphorylation of THOC5 in numerous locations [10]. However, little is known about the signalling pathway involved in this inhibitory effect. Several reports have shown that CXCL12 induced phosphorylation of THOC5 [10-25]. The stimulation of CXCR4 triggers G protein to activate Phosphoinositide 3-kinases (PI3K), NF-κB, Wingless-related integration site (Wnt), Janus kinase (JAK), and Mitogen-activated protein kinase (MEK) signalling. These data imply that THOC5 may have a role in macrophage differentiation [10,23]. The study conducted by Liu *et al.* [6], found that carriers of THOC5 p.V525I (rs737976) allele C have a high risk in ISR and have high mRNA expression, which may lead to vascular smooth muscle cell migration and proliferation and ISR. The effect of administering a combination of green coffee extract, green tea and curcumin directly on THOC5 gene expression has not been observed in previous studies. However, several studies explain that the active compound in green tea, EGCG, has the potential to inhibit THOC5 expression through its activity of inhibiting extracellular stimuli, such as cytokines, chemokines (CXCL12) and growth factors (M-CSF) [10]. Furthermore, in the study by Wang *et al.* [26], EGCG effectively inhibited the expression of CXCL12/CXCR4, thereby preventing macrophage recruitment and differentiation. In other studies, EGCG and CGA each have an anti-inflammatory role by inhibiting M-CSF [27]. Similar to EGCG, curcumin compounds have the ability to suppress the expression of CXCL12/CXCR4 and the NF-κB signaling pathway preventing cell proliferation [28].

The effect of coffee, green tea, and turmeric extract combination lowering the migration gene AIF1 expression in aortic rat MetS model

In the NC group, AIF1 gene expression was low. The expression of the AIF1 gene in the MetS group increased significantly compared with the NC group. This result shows that the HFHS diet and low-dose STZ injection in rats increased the expression of the AIF1 gene in the aorta of the Rat MetS model. In the intervention group, results showed a significant reduction in AIF1 gene expression in overall MetS. There

was no statistically significant difference regarding AIF1 gene expression in the NC and MetS groups ($p > 0.05$). However, the mean can be seen between the NC and MetS groups, where the results are higher in the MetS group compared to the NC. This result shows that the HFHS diet, accompanied by a low dose of STZ injection in rats, decreased AIF1 gene expression in the aorta, although it was not statistically significant. The administration of the C1 combination intervention showed a decrease in the average expression of the AIF1 gene compared with the MetS group. This result was also proven by a statistically significant difference ($p < 0.05$). However, there was no significant difference between the MetS and C2 treatment groups ($p > 0.05$), even though the mean showed a substantial decrease in AIF1 gene expression. A trend of AIF1 gene expression continued to decrease with increasing doses of green tea, green coffee and turmeric extract. This result shows that administration of a combination of green tea, green coffee, and turmeric extracts can reduce the expression of the AIF1 gene in the aorta of Rat MetS model with CGA at a dose of 100, EGCG 300, CUR 250 mg/kg BW as the effective dose.

The AIF1 gene codes for the AIF1 protein, calcium, actin-binding protein in VSMC, also encodes for inflammation-responsive gene and plays a central role in the regulation of VSMC activation, especially regarding the development of vasculopathy [29]. AIF1 is not expressed in normal VSMC but is rapidly expressed in response to injury and pro-inflammatory cytokine stimulation [30]. This study confirmed a significant increase in AIF1 gene expression in the aorta of MetS model rats compared with the healthy group. This condition followed research by Jia *et al.* [31] and Zhou *et al.* [32], where inflammatory stimuli, such as IFN-γ, TNF-α, and IL-1β regulated AIF1 gene expression. Apart from that, another mechanism is endothelial cell dysfunction, which releases growth factors and chemotaxis, which then recruits mononuclear cells that secrete inflammatory cytokines. AIF1, which encodes the AIF1 protein, is expressed in medial aortic smooth muscle cells induced by pro-inflammatory cytokines (IL-1β, IL-16, INF-γ) through colocalization with RAC1 [30,33]. AIF1 allows chemotaxis and migration of VSMC. This localized cytokine production will activate the VSMC response to injury, and contractile changes occur, differentiating into differentiated synthetic cells capable of migration. These findings are in line with research by Sommerville *et al*. [33], which found increased AIF1 expression in atherosclerotic plaque VSMC and AIF1 expression caused increased VSMC chemotaxis to ox-LDL, increased NF-κB activation and lipid uptake.

VSMC migration is one of the earliest cellular events in vascular dysfunction and plaque formation. An increase in AIF1 expression might result from low-grade inflammation under the MetS condition, and it exerts VSMC migration mainly by TNF-α stimulation. Furthermore, it may also activate PPAR-γ through activation of the NF-κB pathway [34]. A previous study by Shankar *et al*. [35] showed that EGCG significantly reduced ERK activity and enhanced p38 and JNK activities in a human pancreatic tumour xenograft model [33]. Lukitasari *et al*. [13] were the 1st to show a higher improvement of metabolic profile in rats in the MetS model treated with a combination of coffee and tea than in single coffee or single green tea extract. The report showed that coffee and tea infusions could lower blood pressure, improve lipid profile, and increase the peroxisome proliferator-activated receptor gamma (PPARγ) and mitogen-activated protein kinase (MAPK) expression [13]. Under this report, the current study showed that EGCG significantly decreased the AIF1 mRNA expression dose-dependently. Previous studies also found that VSMC, which over-express AIF-1, have increased matrix metalloproteinase (MMPs) expression and increased uptake of oxidized LDL, stimulating vasculopathy development. However, little is known about the signalling pathway involved in this inhibitory effect.

The effect of coffee, green tea, and turmeric extract combination lowering the proliferation gene CNN1 expression in aortic rat MetS model

This study showed a significant difference in CNN1 gene expression ($p < 0.00$). There was a decrease in CNN1 gene expression in the MetS group between the NC group, although it was not statistically significant ($p > 0.05$). Administration of the C1 intervention group showed a considerable increase in CNN1 gene expression between MetS groups ($p < 0.00$). Meanwhile, the C2 group also showed increased CNN1 gene expression between Mets, but no significant difference was proven ($p > 0.05$). The increase in the trend of CNN1 gene expression was shown after administering a combination of green coffee, green tea, and curcumin extract. **Figure 1** shows the most effective dose in the group given the combination of 100 mg/kg BW of green coffee extract, 300 mg of green tea, mg/kg BW and 150 mg/kg BW curcumin.

CNN1 plays a role in maintaining vascular tone and contraction, which is highly expressed in healthy adult aorta and VSMC differentiation and contractility [36]. This study shows a decrease in the expression of the CNN1 gene in the aorta of rats from the Mets group compared to the regular group. The reduction of CNN1 gene expression was found to be consistent with the research of Lu *et al*. [37], on the aorta of cardiomyopathic rats and Belo *et al.* [38], 2016 on the aorta of hypertensive rats [39].

CNN1 is part of the cyclic guanosine monophosphate (cGMP) kinase signalling complex, which is widely recognized as a mediator of numerous physiological processes, including smooth muscle relaxation, platelet inhibition, and cell growth and differentiation [40]. The cGMP signaling complex is commonly inhibited due to chronic inflammation, causing vascular dysfunction. Several studies show that chronic exposure to ROS inhibits cGMP-dependent kinase activity due to the suppression of NO production [41]. These findings might be the reason for the CNN1 downregulation in this study.

Studies have shown that EGCG enhances cGMP concentrations by activating the cell surface receptor 67LR, which stimulates the Akt/eNOS pathway and causes vasodilation and improved cardiovascular function [42]. CGA in coffee is a non-selective inhibitor of phosphodiesterases (PDEs), which inhibit the degradation of cGMP, leading to an increase in the level of intracellular cGMP [42]. Like green tea, curcumin stimulates the Akt/eNOS pathway by activating PPARγ, inhibiting Ang II-induced cardiac hypertrophy *in vitro* and pressure overload-induced cardiac hypertrophy *in vivo*. Other studies have shown that curcumin inhibits vascular NADPH oxidase expression brought on by Ang II, resulting in a reduction in superoxide generation and ROS levels, leading to increased eNOS production [43]. The combination of EGCG and curcumin increases cGMP, along with inhibition of PDE by CGA, leading to sustained elevation of intracellular cGMP.

The effect of coffee, green tea, and turmeric extract combination lowering gene ACTA2 expression in aortic rat MetS model

Analysis conducted from aortic tissue suggested higher ACTA2 gene expression in the MetS group compared to the NC group, but it was not significantly different ($p > 0.05$). The administration of the C1 combination intervention showed decreased ACTA2 gene expression compared with the MetS group, but there was no statistically significant difference ($p > 0.05$). However, the C2 intervention group also showed decreased ACTA2 gene expression, which was not significantly different from the MetS group ($p > 0.05$). The trend of ACTA2 gene expression showed a decrease in the administration of the combination of green coffee, green tea and curcumin among the MetS group. However, it was not statistically significant ($p > 0.05$).

This study showed increased of ACTA2 gene expression in atherosclerosis is closely linked to the appearance of mutations in this gene. Variation of p.Arg149C in ACTA2 is a predisposing factor for atherosclerosis [44]. A study by Guo *et al.* [45] found that ACTA2 mutations caused atherosclerosis in

young patients without other risk factors. In an *in vivo* research, on a rat model, it was found that there was a 2.5-fold increase in the incidence of atherosclerotic plaque in Acta2R149C/+Apoe-/- mice compared to Apoe mice -/- with no difference in serum lipids [46]. Based on previous studies, R149C misfolding in Acta2 activates heat shock factor 1 (HSF1), which then increases endogenous cholesterol biosynthesis by increasing HMG-CoA reductase expression. Elevated endogenous cholesterol levels induce ER stress, activate PERK, and increase SMC modulation and atherosclerotic plaque formation [47]. Furthermore, this mutation alters the process of filamin formation and stability in VSMCs, leading to an increase in atherosclerosis risk factors. In this study, we assume that Acta2 expression is not significant because the rat model was not designed undergo mutations. Thus, further research is needed to explain it.

Oxidative stress increases ACTA2 gene expression through SRF activity [48]. Oxidative stress, leading to atherosclerosis pathogenesis, resulted in endothelial dysfunction [49]. It can result from the accumulation of oxidized LDL, which can induce the proliferation of smooth muscle cells in MetS [50]. In the process of atherogenesis, ACTA2 plays a role in the formation of the fibrous cap, producing progeny that migrate into the core and differentiate into distinct mesenchymal phenotypes that promote lesion progression [51,52]. Overexpression of the ACTA2 gene causes excessive fibrosis, which leads to a progressive decrease in tissue compliance, supply of nutrients, and oxygen delivery, as well as increased cardiomyocyte atrophy and cell death [53]. A recent study reported that a combination of EGCG and CGA could potentially reduce the progression of cardiac fibrosis in MetS model rats [54]. According to Islam *et al.* [55], EGCG treatment has an anti-fibrotic effect, significantly reducing ACTA2 in fibroid cells. Anti-fibrotic effects were also found in CGA, which reduced hyperglycemia-induced cardiac fibrosis by substantially reducing the levels of α-smooth muscle actin (α-SMA) and ki67 through the eNOS/NO/sGC/cGMP/PKG signalling pathways [42]. Another study found that curcumin inhibited the production of α-SMA via upregulated PPARγ expression in hepatic cells [56]. This study shows that curcumin can reduce ACTA2 gene expression in the aorta cell, but the mechanism is unclear. Therefore, further research is still needed.

Results and discussion

Results of correlation analysis of THOC5, AIF1, ACTA2 and CNN1

Based on the results obtained, it is known that there is a strong correlation between THOC5 and AIF1 gene expression, with positive value. Meanwhile, the correlation values calculated for the other 2 gene expressions were not significant. The correlation between THOC5 with ACTA2 and CNN1 shows the opposite relationship, indicated by a negative value. This indicates a strong relationship between THOC5 and AIF1 expression in the regulation of atherosclerosis, while the relationship between THOC5 with ACTA2 and CNN1 indicates the opposite regulation in this process (**Table 2**).

Table 1 Results of correlation of THOC5, AIF1, ACTA2 and CNN1 gene expression.

Variable	THOC5	AIF1	ACTA2	CNN1
THOC5	1	0.570**	–0.191	–0.262
AIF1	0.570**	1	0.234	0.186
ACTA2	–0.191	0.234	1	0.251
CNN1	–0.262	0.186	0.251	1

Note: **Correlation is significant at the 0.01 level (2-tailed).

The strong and positive correlation between THOC5 and AIF1 gene expression indicates that there is a close connection between these 2 genes in the regulation or course of atherosclerosis. THOC5 and AIF1 may play a role in the same biological pathway or influence each other in triggering or moderating the atherosclerosis process. These results suggest that higher expression of 1 gene may be associated with increased expression of the other gene, or vice versa, in the context of regulation or progression of atherosclerosis. On the other hand, the negative correlation between THOC5 gene expression with ACTA2 and CNN1 is interesting to note. ACTA2 and CNN1 are genes involved in the regulation of vascular smooth muscle contraction, which is an important process in atherosclerosis. This negative correlation may indicate that high expression of THOC5 may be associated with decreased expression of ACTA2 and CNN1, or vice versa. This suggests that THOC5 may be involved in mechanisms that inhibit or suppress the activity of these genes, which in turn may influence the regulation of vascular smooth muscle contraction and contribute to the development of atherosclerosis. Therefore, further understanding of the interactions and regulation between these genes may provide valuable insights into the mechanisms underlying atherosclerosis and may pave the way for the development of new therapeutic strategies in its management.

The limitation of this study is that the effects of administering green tea, green coffee, and turmeric extract combination were only measured at the transcriptional level. Therefore, it is recommended for future research to assess the effects of the extract combination on translational level.

Conclusions

This study found that administering green tea, green coffee, and turmeric extract can lower the expression of THOC5, AIF1, CNN1, and ACTA2. The correlation test showed that there is a strong correlation between THOC5 and AIF1 gene expression, with positive value. In summary, the combined effects of green tea, green coffee, and turmeric extract show significant promise as a potential anti-atherosclerosis treatment by improving the THOC5 and AIF1, but not ACTA2 and CNN1 gene expression in the aortic tissue of metabolic syndrome model. Although further research and clinical trials are necessary to fully confirm and understand the extent of these benefits, the initial findings indicate a hopeful direction for combating atherosclerosis in this particular model of MetS.

In summary, the combined effects of green tea, green coffee, and curcumin extract show significant promise as a potential anti-atherosclerosis treatment in a rat model of metabolic syndrome (MetS). This promise stems from their capacity to inhibit THOC5 and AIF1, 2 crucial factors linked to the development of atherosclerosis. By targeting these pathways, this combination could offer a comprehensive approach to slowing down the progression of atherosclerosis, thus holding important therapeutic implications for managing cardiovascular health in individuals with MetS. Although further research and clinical trials are necessary to fully confirm and understand the extent of these benefits, the initial findings indicate a hopeful direction for combating atherosclerosis in this particular model of MetS.

Acknowledgements

We thank the Ministry of Education, Culture, Research, and Technology and the Faculty of Medicine, Brawijaya University for the professorship grant funding provided through contract number 3173.2/3/UN10.F08/PN/2023. We are also grateful to all parties who helped carry out this study.

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