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Dietary Steamed Tomato Inhibits Hyper-production of Inflammatory Markers and Enhance miR-29b-3p Expression in Atherosclerosis Rats

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Abstract. Atherosclerosis is triggered by cholesterol accumulation in endothelial layers that induces the production of inflammatory cytokines, including TNF-α, IL-6, and IL-10. This condition disrupts microRNA homeostasis like miR-29b-3p, generally maintaining artery health. Steamed tomatoes contain higher antioxidant properties than raw, which might be better against atherosclerosis. However, its influence on inflammation and miR-29b-3p balance remains unclear. This study aims to elucidate the effect of steamed tomatoes on the TNF-α, IL-6, and IL-10 levels and miR-29b-3p expression under atherosclerosis conditions. Sprague Dawley male rats were equally divided into K1 group of healthy rats given a placebo; K2 group was atherosclerosis rats induced with 2 m of cholesterin/ 200 g/KgBW per day, then K3 and K4 groups were atherosclerosis rats supplemented with 20 mg/ 200 g/KgBW per day of atorvastatin and 16 mg/ 200 g/KgBW per day of steamed-tomato extract for 60 days. Steamed tomato decreases cytokine level in the K4 group and significantly differs from all groups ($p < 0.050$). This study showed that increased proinflammatory cytokine, including TNF-α and IL-6, is hypothetically involved in plaque formation and lamina layer destruction in endothelial. Steamed tomato supplementation also significantly increases miR-29b-3p expression to 0.98 ± 0.33 $\log^{10}$ fold change higher than K2 and K3 ($p = 0.000$). This study demonstrated the potential of steamed tomatoes to improve dyslipidemia, atherosclerosis-related inflammation, and miRNA homeostasis. This research provides additional knowledge regarding determining the expression of miR-29b-3p, which has the potential to be developed as a diagnostic or therapeutic target.

Keyword: atherosclerosis; IL-6; IL-10; miR-29b-3p; steamed tomato; TNF-α

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INTRODUCTION

High accumulation of low-density lipoprotein-cholesterol (LDL-C) in blood vessel walls causes atherosclerosis, clogged arteries that increase heart disease risk, characterized by oxidized LDL-C (ox-LDL), which triggers the inflammation (Libby, 2021; Malekmohammad et al., 2021; Park et al., 2018). These condition surges high cytokines production (Abdolmaleki et al., 2019; Fatkhullina et al., 2016; Taleb, 2016), such as interleukin 1β (IL-1β) (Libby, 2017; Ridker et al., 2017), IL-6 (Eltoft et al., 2018; Huang et al., 2017), tumor necrosis factor-alpha (TNF-α) (Sterpetti et al., 2017; Tay et al., 2016), and IL-10 (Kamaly et al., 2016; Puz & Lasek–Bal, 2017). The inflammation damages blood vessels, causing lesions, leading to smooth muscle cell (SMC) migration, and the formation of plaques that can block blood flow (Hartmann et al., 2016).

Previous research on experimental animals has revealed the potential of tomatoes in immune therapy (Iswari et al., 2016), and atherosclerosis (Iswari et al., 2020), without toxicity indication for daily consumption (Iswari et al., 2020). Giving tomatoes as paste extract improves lipid metabolism in healthy people on a high-lipid diet (Deplanque et al., 2016). In addition, tomatoes

reduce triglycerides and LDL-cholesterol and increase HDL-cholesterol levels (Mozos et al., 2018). In addition, experimental studies in animals and humans with cardiovascular disorders who are supplemented with tomato extract or purified lycopene and carotenoid have same improvement effects that are not significantly different (Cheng et al., 2019; Rattanavipanon et al., 2021). The process of tomato consumption is reported to contribute to changes in nutritional content, especially carotenoids, where the steaming process for 15 minutes produces the best nutritional content (Iswari & Susanti, 2016). Thermal processing increases the concentration of trans-lycopene, which has better bioavailability and bioactivity than cis-lycopene in raw tomatoes (Jayathunge et al., 2019; Shahidi & Pan, 2022; Yang et al., 2021). The high content of carotenoids such as β-carotene, lycopene, and vitamin A in tomatoes is involved in activating the nuclear retinoid A receptor (RAR) and retinoid X receptor (RXR), leading to improved lipid metabolism and reduced inflammation (Mounien et al., 2019).

Pathological changes in atherosclerosis influence cell regulation in transcriptomic levels by altering the up- and down-regulated microRNA (miRNA), which is less than 25 base pair RNA involved in gene silencing (Bhat et al., 2016; Kim et al., 2017). From the current studies, miR-29b-3p is a critical miRNA in atherosclerosis, exhibiting paradoxical roles in both pro- and anti-inflammatory processes. On its proinflammatory role, miR-29b-3p promotes specific proinflammatory cytokines production, contributes to plaque formation, contributes to scar tissue formation, and facilitates the buildup of fatty plaques in blood vessels (Wang et al., 2020). Conversely, miR-29b-3p is essential in mitigating inflammation-related oxidative damage and inhibiting macrophage migration to endothelial cells in rats (Qin et al., 2023) and mice (Liang et al., 2019). Furthermore, miR-29b-3p contributes to normal endothelial function (Widlansky et al., 2018) and safeguards it from thrombus formation and migration of SMC to the endothelium (Liu et al., 2021; You et al., 2020).

The study of miR-29b-3p in atherosclerosis appears to be a delicate balance between its pro- and anti-inflammatory functions. Understanding the specific pathways and factors influencing its behavior is crucial for developing diagnosis or targeted therapies to combat this complex disease. This study aims to elucidate the effect of steamed tomatoes on the TNF-α, IL-6, and IL-10 levels and miR-29b-3p expression under atherosclerosis conditions. Overall, the results of this study are intended to explain how the tomato diet diminishes atherosclerosis and affects miRNA expression, which is proposed for a precision diagnostic or therapeutic candidate.

METHODS

All procedure of this animal experiment was approved by the Health Research Ethics Commission, Faculty of Medicine, Universitas Gadjah Mada, registered number KE/FK/1003/EC/2019. A total of 24 healthy 3-month-old Sprague Dawley male rats, with body weights of about 150-200 g, were randomly chosen and placed in a cage for seven days of adaptation. The environmental condition was set at 20-24 °C, 60% humidity, 12-14 hours of light exposure, and continuous ventilation to keep dry. The rats were then divided into four groups, including standard control (K1), healthy rats (no intervention), and high cholesterol diet following Otunola et al. (2010) for K2-K4. Therefore, the control and hypercholesterolemia conditions were induced in the treatment groups, including K2, K3, and K4 were presented in Table 1.

Table 1. Administration of cholesterin and supplementation doses of atorvastatin and steamed tomato extract to the animal model groups.

Groups	Hypercholesterolemia conditioning*	Treatment (Supplementation Doses)
K1	None	Placebo (aqua dest)
K2	2 mL of cholesterin/ 200 g/KgBW per day	Placebo (aqua desr)
K3	2 mL of cholesterin/ 200 g/KgBW per day	20 mg/ 200 g/KgBW of atorvastatin (following human dose conversion)
K4	2 mL of cholesterin/ 200 g/KgBW per day	16 mg/ 200 g/KgBW per day of tomato extract

Note: Cholesterin administration was conducted carefully and precisely every morning (around 07.00-08.00 am) followed by supplementation doses to the treatment groups for 51 days through oral gavage (Tokaç et al., 2015). Furthermore, the tomato extract dose was determined by following the previous study (Iswari et al., 2019).

Tomato extraction

This study was conducted using fresh ripe red tomatoes *Lycopersicum esculentum* var-Lentana F1 collected from Bandungan Sub-district, Central Java, Indonesia. As many as 50 kg of tomatoes were cut into thin slices, then steamed at 120 °C for 30 minutes and grounded. Tomato paste was spread thinly on the baking sheet and then dried in the oven at 40 °C for three days. Dried tomato paste was blended and sieved using a sieve number 100 mesh. The obtained fine tomato powder was weighed as much as 50 g and then prepared for the Soxhlet extraction process using petroleum ether as the solvent. The dregs were dissolved in 500 ml of methanol solvent and mixed with the Soxhlet result. The Soxhlet extraction process was stopped after a clear filtered solution was obtained. The obtained extracts were combined homogenously and poured on the Petri dishes, then re-dried at 50 °C for two days. The extract was suspended in aquadest for administration to the rats (Tokac et al., 2015).

Sample collection and histological specimen processing

The study was terminated on day 60th, and the rats were sacrificed to collect blood vessel organs from the aortic arch to the par's abdominal aorta. Before extraction, the rats were anesthetized by inhaling isoflurane in the anesthetics cage (Parasuraman et al., 2010). The blood was collected using a microhematocrit through the sinus orbitalis, placed into a 2 ml sterile anticoagulated tube, reserved at room temperature for 10 minutes, and subsequently centrifuged for 5 min at 8000 rpm. About 500 µl of blood plasma was separated into a different tube, then 200 µl was used for miR-29b-3p expression analysis, while the remaining blood plasma was used for cytokines analysis.

The collected blood vessel organ was divided into the upper, middle, and lower parts. The tissues were then preserved for 12 hours in 10% formalin, with the volume of formalin approximately ten times the volume of material. The cardiac and aorta organs were prepared for histological specimens preparation using the embedding method (paraffin block) with hematoxylin-eosin (HE) staining by following Hristu et al. (2021). The histopathological specimens were observed under a light microscope examination by an expert pathologist to diagnose atherosclerosis condition.

Lipid and Cytokine Marker Analysis

The lipid biomarkers, such as triglycerides, were analyzed using Triglyceride FS 10, Cat. No 1-1300-99-10-026 (DiaSys, GmbH: Germany); total Cholesterol, LDL-C, and HDL-C were measured using Multi-purpose Kits: Cholesterol FS 10, Cat. No 1-1300-99-10-026 (DiaSys, GmbH: Germany) follows the manufacturer's standard procedures. Triglyceride and HDL-C data were used as a basis for calculating the atherogenic index (AI) with the formula (Dobiášová, 2004):

$$\mathrm{AI} = {}^{10}\mathrm{Log}\left(\frac{\text{Titer of Triglycerides}}{\text{Titer of HDL} - \text{C}}\right)$$

Cytokine analysis was performed using IL10 ELISA Kit for Rat Cat No. BMS629, IL6 ELISA Kit for Rat Cat No. BMS625, TNF-α ELISA Kit for Rat Cat No. KRC3011 (Invitrogen: Vienna, Austria). The analysis step was conducted by following the manufacturer's procedures.

Plasma RNA Isolation and qRT-PCR

The plasma sample was centrifuged at 3000 g for 5 minutes, and then 200 µL of the supernatant was taken for further processing using the miRNeasy Serum/Plasma Kit Cat. No. 217184 (Qiagen: California, USA) according to the manufacturer's standard procedures. This process was initiated by applying miRNeasy Serum/Plasma Spike-In Control alongside *C. elegans* ce-miR-39-1 mimic miRNA as an internal control and following the manufacturer's procedures.

The RNA was converted into cDNA using the miRCURY® LNA® RT Kit (reverse transcriptase-PCR) Cat No: 339340 (Qiagen: California, USA) following the manufacturer's standard procedures. For the next step, the 5 µL cDNA from each group was diluted in 395 µL RNAse-free water. The mixture was then homogenized with a vortex spin down and stored in a tube container lined with an ice pack. This step was followed by quantity real-time PCR (qPCR) method analysis using MiRCURY LNA SYRR Green master mix kit Cat, No: 339345 (Qiagen: California, USA), and primary set as rno-miR-29b-3p, as stipulated in the kit is 5′-GAGGTAGCACCATTTGAAATCAGTGT-3′ (Yang et al., 2020). The rno-miR-29b-3p expression was performed using pooling cDNA plasma samples for each group featuring UniSp6 RNA as a spike in internal control for normalization.

The qPCR program on the Applied Biosystems™ 7500 Real-Time PCR Systems (Thermo Fisher Scientific; Massachusetts, USA) was set up as follows: denaturation 95 °C for 10 minutes, amplification 40 cycles, 95°C for 10 seconds, 60 °C for 1 minute ramp-rate 1.6 °C/s optical read and analyze the melting curve. MiRNA expression was calculated based on the quantification cycle (Cq) read by the qPCR machine connected to Biorad CFX Manager™ Software (Bio-Rad; California, USA) using the Livak & Schmittgen (2001) formula.

Data Analysis

The TNF-α, IL-6, IL-10 concentrations, and miR-29b-3p were analyzed using the Saphiro-Wilk normality test, followed by one-way ANOVA and least significant difference (LSD) with a 95% confidence interval. The statistical analysis was conducted using Statistical Product and Service Solution (SPSS) 24 for Mac. Furthermore, the histopathological condition of the blood vessel was analyzed descriptively by an expert anatomic pathology specialist from the Faculty of Medicine, Universitas Diponegoro, Indonesia

RESULTS AND DISCUSSION

A seven-week cholesterol induction increases the concentration of lipid biomarkers in the blood, such as triglyceride, total cholesterol, and LDL-C, lowering HDL-C levels. It can be used as an indicator of the atherosclerosis condition. The authors also found that high lipid biomarkers are related to the massive production of the inflammation cytokine. The initial average body mass of the rats among groups was homogenously distributed. Then, significant differences were observed in the cholesterol and lipid biomarkers at the termination of the study. Following the induction of cholesterol, the specimen in the K2 group demonstrated the highest triglyceride, total cholesterol, and LDL-C levels and the lowest HDL-C (Table 1). In addition, the atherogenic index calculated by comparing the triglyceride and HDL-C levels revealed high vulnerability and an increased propensity for atherosclerosis.

Table 1. Initial body mass of rats and lipid biomarkers after treatment.

Parameter	K1	K2	K3	K4	p-value
Initial Body Mass (g)	172.00±3.46	175.17±3,76	174.17±7.41	171.83±6.11	0.211
Triglyceride (mg/dL)	93.10±2.21 [a]	171.01±3.51 [b]	115.20±2.73 [c]	108.05±4.49 [d]	0.000
Total Cholesterol (mg/dL)	109.24±2.83 [a]	213.66±6.60 [b]	121.29±2.99 [c]	121.02±2.42 [c]	0.000
LDL-C (mg/dL)	39.29±1.69 [a]	84.19±2.09 [b]	46.84±2.55 [c]	44.04±2.64 [c]	0.000
HDL-C (mg/dL)	69.49±1.82 [a]	27.09±2.59 [b]	63.02±2.55 [c]	65.02±2.68 [c]	0.000
Atherogenic index	0.13±0.02 [a]	0.80±0.05 [b]	0.26±0.02 [c]	0.22±0.03 [d]	0.000

Note: Different letters of the alphabet (a-d) show significant variation between treatment groups ($p<0.05$). Also, an atherogenic index [Log (triglyceride/ HDL-C) value of between −0.3 to 0.10 indicates low cardiovascular risk; 0.10 to 0.24 for moderate and above 0.24 is high risk (Cai et al., 2017; Shen et al., 2018).

The inflammation condition is initiated by activating immune cells, such as polymorphonuclear neutrophils (PMN). During their activity, the immune cells produce radical oxygen species (ROS), which promotes endothelial dysfunction by oxidizing tyrosine phosphatase cellular signaling proteins (Qi et al., 2017). Therefore, inflammation as an effect of highly induced cholesterol is depicted by the significant production of TNF-α, IL-6, and IL-10 concentrations ($p <0.05$) in blood plasma. This phenomenon occurred in all groups, including the healthy rats (K1), while a higher amount of proinflammatory cytokines were produced in the K2 group (Figure 1). In addition, K3 and K4 demonstrated a significantly lower TNF-α, IL-6, and IL-10 titer than K2 ($p \leq 0.050$). Furthermore, decreased proinflammatory cytokines in K3 and K4 may be related to low cholesterol levels due to atorvastatin or tomato extract supplementation. Atorvastatin inhibits an enzyme crucial for cholesterol production, effectively lowering blood cholesterol levels. This reduces the inflammatory response triggered by excess cholesterol deposits in the blood vessel walls (Bruder-Nascimento et al., 2019).

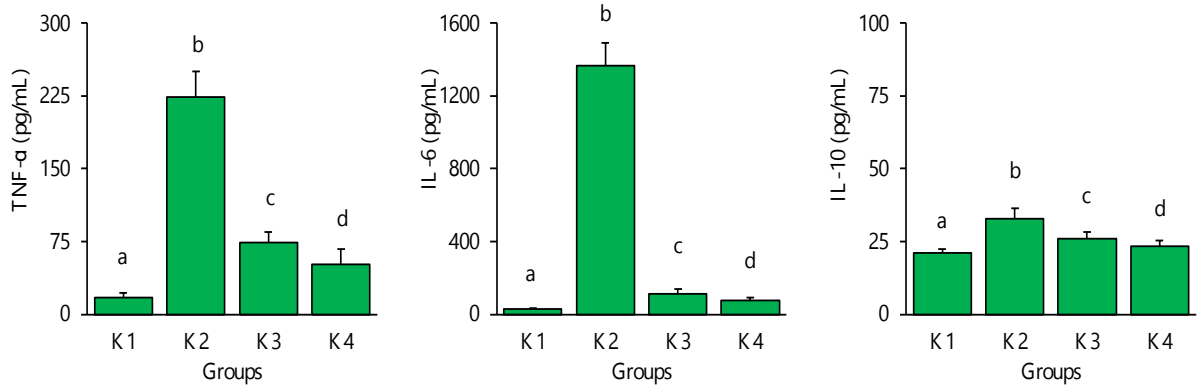

Figure 1. Inflammatory cytokine concentration in atherosclerosis rats supplemented with atorvastatin and steamed tomato extract. The alphabetic (a-d) letters showing significant differences were analyzed using an LSD test with confidence level = 95%.

During hypercholesterolemia conditions, exceeded LDL-C discharges cholesterol, deposits it in the tunica layers, undergoes oxidation, and produces ROS that initiates inflammation (Ince et al., 2016). The ROS then diffuses into endothelial cells, resulting in the synthesis of intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM1), which subsequently triggers the migration of neutrophils and macrophages to the inflammation site (Taleb, 2016). High lipid deposition and ROS concentration damage the tunica and increase the production of inflammatory cytokines, including TNF-α, IL-6, and IL-10 (primarily found in the K2 group). This outcome is consistent with the elevated levels of TNF-α, IL-1β, IL-6, and IL-10 as a response to excess ROS (Fatkhullina et al., 2016; Taleb, 2016).

The ROS activity on the cell membrane is perpetrated through electron release and the subsequent destruction of ester bonds in the phospholipid molecules (Ghosh et al., 2018). This high electronegative property of ROS also impacts the release of H^+ ions from the hydrogen and peptide bonds (Hameister et al., 2020). In addition, excess cholesterol will be engulfed by macrophages and SMCs. Then proteases discharged after the lysis of macrophages or SMCs, result from the effect of excess cholesterol intake (Wang et al., 2020) and release matrix metalloproteinases as a fibrotic factor (Rosshirt et al., 2018).

Furthermore, the ROS also induces phosphorylation of intermediate proteins signaling in lymphocytes and macrophages, including tyrosine phosphorylase and IκBα, to activate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway (Shi et al., 2016). TNF-α, which automatically acts as an autocrine stimulator, increases IL-6 and interferon-γ (IFN-γ) (Reiss et al., 2017; Stefanutti et al., 2017). These pathway outcomes have been affiliated with the continuous formation of adaptive gene-phenotype expressions. Hence, all related immune cells may change phenotypes to respond to the inflammation, which is subsequently inherited after cell proliferation (Abdolmaleki et al., 2019). These transformations result in hypersensitivity despite the removal of stimulus. Furthermore, the inflammatory process involves various tissues and applies systemically chain-reaction processes, leading to a long recovery time for impaired tissues (Sezgin et al., 2023).

In addition, atorvastatin administered to specimens in K3 was made systemically available as an inhibitor of 3-hydroxy 3-methylglutaryl coenzyme-A (HMG-CoA), which directly limits the biosynthesis of hepatic cholesterol (Shamsuddin et al., 2016; Watts et al., 2017). The mechanism of action also involves reducing the synthesis of de novo cholesterol, followed by an increase in the expression of HDL receptors in hepatocytes, increased LDL absorption, and prohibited its accumulation in tissues, then prevents ROS production (Watts et al., 2017).

In the tomato supplementation group, the massive reduction in proinflammatory cytokine production may be caused by various antioxidants from tomato extract, including carotenoid groups and vitamins. In this research, the authors propose two mechanisms contributing to inflammation suppression. The first mechanism, the antioxidant, especially carotenoid, e.g., lycopene, plays an essential role as a ROS scavenger, which prevents the phosphorylation of epidermal growth factor

receptor (EGFR), cell surface receptors; and other second messengers in the inflammation process (Zeboudj et al., 2018). It then constrains the expression of TNF-α through a suppression in the NF-κB signaling pathway and prevents macrophage migration, mediated by ICAM-1 and VCAM1 (Ba et al., 2023). Declined ROS content hinders the phosphorylation of IκBα that is required to release p50 (NF) and p65 (κB) proteins, the protein complex bond that is needed in NF-κB promoters gene activation (Brazão et al., 2023; Feng et al., 2019). It then reduces the production of proinflammatory cytokines, especially TNF-α and IL-8 (Fatimatuzzahro et al., 2022). Meanwhile, the increased IL-6, IFN-γ, and ROS act as paracrine communicators that trigger the secretion of IL-10 by lymphocytes T_{reg} cells to suppress inflammation (Munjal & Khandia, 2020).

The second mechanism, the lycopene, and other carotenoids are metabolized in the cell and converted into a 9-cis retinoid (9cRA) (Karkeni et al., 2017; Krężel et al., 2021). The 9cRA activates the retinoid acid receptor (RAR) and retinoid X receptor (RXR) in the macrophage's nucleus receptor cell complex; it reduces lymphoproliferative capacity and IFN-γ secretion (Jetten & Cook, 2020). This contributes to the dwindling interaction between macrophages and lymphocytes, which prevents the production of antibodies responsible for increasing opsonization and macrophage aggressiveness toward cholesterol (Cui et al., 2021). Furthermore, The RAR/RXR activation contributes to controlling cholesterol synthesis through the liver X receptor (LXR) and peroxisome proliferator-activated gamma receptor (PPPRγ) pathway (Brtko & Dvorak, 2020; Königshofer et al., 2021). Tomato diet may also affect Ras homolog family member A (RhoA), which is associated with the cellular activity of hepatocytes (Frambach et al., 2020). It respectively controls the multilevel pathway in cellular cholesterol by increasing the efflux of cholesterol and phospholipids from macrophages to HDL and depositing lipids from the liver to adipose tissue via LDL (Ikhlef et al., 2016).

The aorta histological examination observes inflammation-related endothelial destruction and atherosclerosis formation (Figure 2). Each preparation was analyzed for the presence or absence of plaque, endothelial damage, lamina damage, myocyte fattening, and the presence or absence of lesions. The necrotic core is identified as part of the atherosclerotic plaque, which appears white, and the black core at the edge represents acellular cholesterol crystals (hematoxylin negative) (Wihastuti et al., 2015). Furthermore, plaque area can be spotted by a lesion characterized by the destructed area of SMC beneath endothelial to fibrous tissue and protrusion of the thrombus from the tunica towards the lumen (Andrés-Manzano et al., 2015; Conti et al., 2020; Hu et al., 2016).

The health condition of tunica media and tunica adventitia was depicted on the aorta specimens in K1, K3, and K4, shown by the solid and non-fragmented layer in endothelial tissue. Furthermore, the SMC nuclei were noticeable from the dark coloration recognized between the lamina elastica layers. This histological appearance confirms the absence of inflammatory conditions and damage in the rats' aorta. The lack of tissue rupture in groups K1 and K3 indicates no cholesterol deposition inside the tunica layers. However, some fattened SMC was found in the K4 group, which may show cholesterol deposition during a high lipid diet. There are clear patches around the nucleus (black arrow) in several parts of the tunica media in the K4 rat's aorta specimen (Figure 2H). Furthermore, a loose bound within SMC is also found in the K4 rat's aorta specimen; however, it is not followed by the endothelial and lamina elastica rupture (Figure 2H).

Based on the observation, a destructed intima is observed in the K2 group, showing a formation of plaque in the ascendance aorta, featuring 1) the protrusion of tunica, where SMC is fattened and migrates from the tunica media to the aortic lumen; 2) Lamina layer damage, indicated by unclearly visible line, fragmentation and disconnection of each lamina layer and the basement membrane; and 3) the lysed fattened SMC, deformed with nucleus (black spots) and pushed to the edge (Figure 2E & F). However, the authors assumed that fattened macrophage and SMC, converted to foam cells, cannot be clearly distinguished.

Increased ROS production and inflammation during plaque development in atherosclerosis influence several complex genes, including the miR-29b-3p gene. This study shows that the miR-29b-3p expression decreases along with the increased production of proinflammatory cytokine, the deformation of tunica layers, and the development of endothelial plaque. The expression of miR-29b-3p in normal mice was used as a calibrator for calculating miRNA, so it had a value of 0.

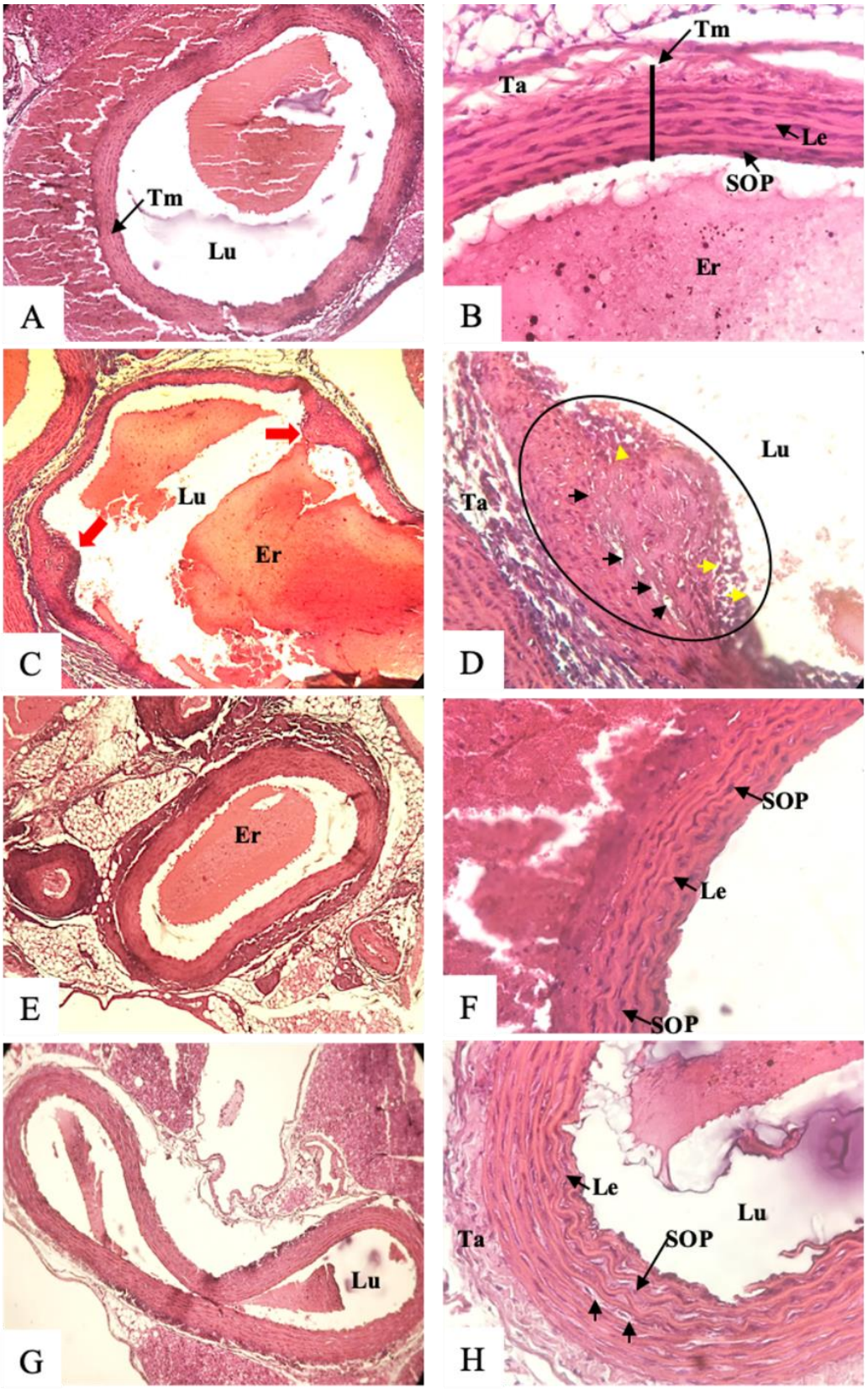

Figure 2. Cross-section specimen of rats' aortic in normal group K1, and after 52 days of treatments in the K2 (C-D), K3 (E-F), and K4 (G-H) groups. Intima damage in the form of fragmentation and deformation (D: black circle); plaque (C: red arrows); SMC fattening (D, H: black arrowhead); and lesions (D: yellow arrows). Tm: tunica media; Ta: tunica adventitia; Le: lamina elastica; Er: erythrocytes; Lu: lumen vessel. HE staining, microscopic observation of 40x magnification (A, C, E, G) and 400x, bar scale 200 μm (B, D, F, H).

Meanwhile, in the K2 group, the expression of miR-29b-3p decreased significantly compared to K3 and K4 ($p \leq 0.050$). Apart from that, the analysis results also showed that miR-29b-3p in K3 also experienced a decrease in expression, while K4 experienced a significant increase in expression ($p = 0.000$) up to 0.98 ± 0.33 $\log^{10}$ fold change (Figure 3).

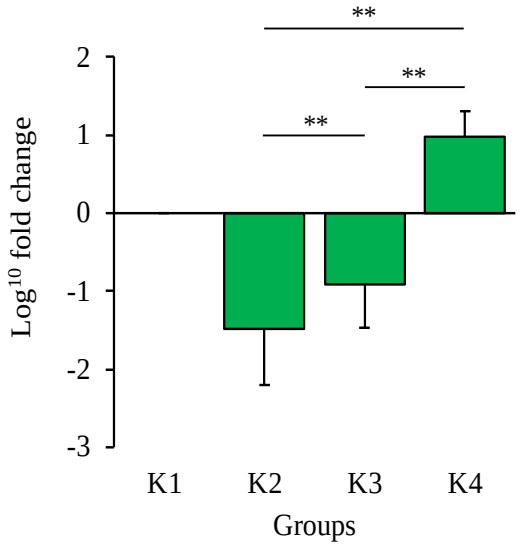

Figure 3. The differences in miR-29b-3p expression in atherosclerosis rats supplemented with atorvastatin and steamed-tomato extract. The stars (**) showing significant differences were analyzed using the LSD test with confidence level = 95%.

Several studies explain that miR-29b-3p expression decreases in hypercholesterolemia and cardiovascular decrease, even though other research states the opposite. MiR-29b-3p plays a complex and fascinating role in atherosclerosis and inflammation, with both pro- and anti-inflammatory effects that change periodically and are affected by various factors. In a previous study, miR-29-3p had anti-fibrotic activity and contributed to normal endothelial function (Widlansky et al., 2018). The high production of ROS in arterial inflammation also causes endothelial cells to express miR-29b-3p, which is required to inhibit fibrosis (Chu et al., 2017). In this study, the miR-29b-3p noticeably decreased in atherosclerosis and inflammation, then increased during tomato supplementation, which improved the dyslipidemia and oxidative stress condition.

MiR-29b-3p also possesses anti-inflammatory properties that might offer protection against atherosclerosis by targeting sterol regulatory element-binding protein 2 (*SREBP2*) and adenosine triphosphate binding cassette subfamily A member 1 (*ABCA1*) genes (Khan et al., 2022). Furthermore, miR-29b-3p overexpression led to decreased matrix metalloproteinase-2 (*MMP2*) gene expression that was involved in vascular calcification in rat's vascular SMC (Jiang et al., 2017). Besides, silencing family members of miR-29b increases *MMP2* expression, calcification, aggravated aortic fibrosis, and stiffness (Wang et al., 2023). MiR-29b-3p also confirmed attenuates atherosclerosis by inhibiting the aorta's sprout receptor tyrosine kinase (RTK) signaling antagonist 1 (*SPRY1*)/ mitogen-activated protein kinase 1 (*MAPK*) signaling pathway and inflammation (Lu et al., 2018). Furthermore, miR-29b-3p protects the vascular lining (endothelium) from inflammatory damage by targeting genes like *VCAM1* and *ICAM1*, reducing the adhesion of inflammatory cells (Maucher et al., 2020).

However, a recent study also explains that miR-29b-3p acts as a proinflammatory switch, facilitating plaque formation through several mechanisms, including targeting cyclin-dependent kinase (*CDK6*) and wildtype activating factor-1/cyclin-dependent kinase inhibitory protein-1 (*WAF1/CIP1*) or *p21* genes (Andrikopoulou et al., 2021). This unchecked proliferation leads to an overabundance of SMC within the artery walls, the main building blocks of plaques (Lee & Kang, 2019). MiR-29b-3p also increases the production of proinflammatory cytokines including IL-1β, IL-6, and IL-8 by inhibiting the activation of the AMPK signaling pathway (J. Wang et al., 2020). In advanced stages, miR-29b-3p can promote scar tissue formation within the arteries by targeting connective tissue growth factor (*CTGF*) and transforming growth factor beta 1 (*TGFB1*) genes. It activates pathways leading to excessive collagen production, further restricting blood flow (Jiang et al., 2017; Liang et al., 2019).

Factors like the specific gene targets, the overall inflammatory state, and the disease stage likely play a crucial role in determining its ultimate impact. Understanding the complex interplay between miR-29b-3p, its target genes, and other factors in atherosclerosis and inflammation is vital for developing effective therapeutic strategies. Based on the extrapolation of the findings above, both the increase in pro- and anti-inflammatory cytokines and the expression of

miR-29b-3p are natural mechanisms that external and internal factors may influence. Consequently, anti-inflammatory cytokines such as IL-10 may increase inflammation as a negative feedback on proinflammatory cytokines (Clark et al., 2019). Likewise, miR-29b-3p has a paradoxical function, so in some studies, it shows an increase in cardiovascular disease and inflammation, but on the other hand, the opposite is true. By deciphering its two-faced nature, we may unlock new avenues for preventing and treating this life-threatening disease. Through this research, the role of miR-29b-3p in atherosclerosis has the potential to be clarified and decisive in developing a disease diagnosis.

CONCLUSION

Dietary 16 mg/ 200 g/KgBW per day of steamed tomato in high-cholesterol diet rats (K4) inhibits atherosclerosis formation, reflected by decreased atherosclerosis index and total lipid level in blood plasma. Even though there are several patches found within lamina layers that show low cholesterol deposition, the plaque structures and thrombi are not formed. The tomato supplementation also produces a better reduction in lipid profile proinflammatory cytokines, including TNF-α, IL-6, and IL-10, compared to the administration of statins (K3). Furthermore, this study found that steamed-tomato supplementation also increases the expression of miR-29b-3p up to 0.98 ± 0.33 $\log^{10}$ fold change, while it decreases in atherosclerosis conditions. We suggest that steamed tomatoes' bioactive compounds are more effective in the inflammatory responses involved in cholesterol biosynthesis reduction. Moreover, the miR-29b-3p status has the potential to be developed as a new approach to the diagnosis and therapeutic of inflammatory atherosclerosis. A further study should be conducted to elucidate the mechanism pathway involved in miR-29b-3p expression-related atherosclerosis, inflammation, or a high-cholesterol diet.

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