Trop J Nat Prod Res, May 2021; 5(5):889-894 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

The Potential of Carotene Compound (Beta-carotene and Lycopene) in Steamed Tomatoes Extract as Atherosclerosis Preventive Nutraceuticals

Ni K. Warditiani[1], Pande M.N.A. Sari[1], Yan Ramona[2], Made I.A.G. Wirasuta[1]*

[1]*Department of Pharmacy, Mathematics and Natural Science Faculty, Udayana University, Indonesia*
[2]*Biology Department, Faculty of Mathematic and Natural Science, Udayana University, Indonesia*

ARTICLE INFO

Article history:
Received 11 March 2021
Revised 08 April 2021
Accepted 11 May 2021
Published online 03 June 2021

ABSTRACT

Tomatoes contain some carotene compounds such as beta-carotene and lycopene, beneficial for human health. Heat pre-treatment prior to tomato processing significantly increased the lycopene but decreased beta-carotene level in the extract. Lycopene have been known to lower blood lipid levels better than beta-carotene. This research aimed to explore the anti-atherosclerosis effect of tomato extract containing increased lycopene content following heat pre-treatment. Steamed and fresh tomatoes were extracted in a mixture of solvents containing 96% ethanol, acetone, and hexane with a ratio of 2:1:1 (v/v), respectively, before measurement of their lycopene and beta-carotene contents. These tomato extracts were then administered for 30 days at the rate of 5 and 15 mg/kg BW in a rat model of atherosclerosis (rats previously fed with a fat-rich diet and Vitamin D3 20,000 IU for 60 consecutive days). The blood lipid levels and histopathology of the aorta were measured or assessed on day 60 and 90. The results showed that the heat-pretreated tomato extracts (L2) contained higher level of lycopene than those extracted from fresh tomatoes (L1). The beta-carotene of the heat-pretreated tomato extracts (L2) however was found to decrease. Administration of L2 was found to decrease the AIP value, and this was positively correlated with a decrease in atherosclerosis incidence in treated rats.

***Keywords*:** Atherosclerosis, AIP, Extract, Lycopene, Steamed, Tomato

Introduction

Tomatoes contain several health-promoting chemical compounds, including lycopene, α-carotene, beta-carotene, γ-carotene, phytoene, phytofluene, lutein and Vitamin C.[1,2] According to Evoli *et al*.[3] heating process of tomatoes can increase the content of lycopene content in the extract and improve antioxidant activity of the extract.[3,4] Conversely, the content of beta-carotene, flavonoids and Vitamin C was found to decrease, and this indicated that the antioxidant activity of the extract must be due to the lycopene compounds.[2]

Lycopene in tomatoes has been used as a nutraceutical to regulate blood lipid levels; hence, it has potential to prevent atherosclerosis. The importance of such compound for this purpose has been reported by many researchers. Tomatoes that contain lycopene, lutein and beta-carotene can reduce triglyceride levels, total cholesterol, and increase blood HDL.[5] Similar report was also given by Nishimura *et al*.[6] who reported that tomato (lycopene 22.0-27.8 mg/day) administered for 3 months to healthy Japanese subjects could reduce LDL blood level.[6] Both lycopene and beta-carotene possess anti-hyperlipidemic properties, but beta-carotene activity is lower than lycopene.[7] When compared to other carotene compounds, lycopene in tomatoes shows the highest antioxidant activity.[7]

Such compound has both antioxidant and anti-hyperlipidemia activity; therefore, it has potential to prevent lipid peroxidation and atherosclerosis. This study was aimed to investigate the effect of steam pre-treatment to tomatoes on the amount of lycopene extracted during processing and to determine the AIP (atherogenic index plasma) value and anti-atherosclerosis capacity of the tomato extract.

Materials and Methods

Materials

Analytical grades of acetone (Bratacho, Bali), ethanol (Bratacho, Bali), hexane (Bratacho, Bali), 2,2-DiPhenyl-1-PicrylHydrazyl (Sigma-Aldrich), Beta-carotene (Sigma-Aldrich), Lycopene (Sigma-Aldrich), THF (Merck, Germany), sodium Carboxy-methyl Cellulose (Na-CMC) (Bratacho, Bali), vitamin D3 1.0 MIU/g (DSM Nutritional Ltd.), chloroform (Merck, Germany), methanol (Merck, Germany), toluene (Merck, Germany), n-Hexane (Merck, Germany), calcium (Bratacho, Indonesia), cholesterol FS (Diasys), triglyceride-FS (Diasys), low-density lipoprotein (LDL) precipitant (Diasys), high-density lipoprotein (HDL) precipitant (Diasys), silica gel TLC plate GF 254 nm (Merck) were purchased from PT Kurnia Jaya Sentosa, Surabaya, Indonesia. The equipment used consisted of a Rotary evaporator (Heidolph) for removing the solvent. UV-Visible (Shimadzu) spectrophotometer for measuring and identifying beta-carotene content and measuring lipid blood content, the light microscope (Olympus BX63 with a DP72 camera, Olympus Corporation, Tokyo, Japan). Antioxidant activity was observed by TLC method. Samples were applied using Automatic TLC sampler 4 (Camag) on TLC plate.

Plant collection

Tomatoes were collected from Kintamani village, Bali, Indonesia in March 2016. The tomato plant was identified at the Indonesian Institute of Sciences (LIPI) number 241/IPH.7/AP/III/2016.

*Corresponding author. E mail: gelgel.wirasuta@unud.ac.id
Tel: +6281337742733

Citation: Warditiani NK, Sari PMNA, Ramona Y, Wirasuta MIAG. The Potential of Carotene Compound (Beta-carotene and Lycopene) in Steamed Tomatoes Extract as Atherosclerosis Preventive Nutraceuticals. Trop J Nat Prod Res. 2021; 5(5):889-894. doi.org/10.26538/tjnpr/v5i5.16

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Test animals
Male Wistar rats with aged of 8-10 weeks (bodyweight 150-200 grams) were acclimated for one week at ambient temperature 27-30^{o}C. These rats were placed in cages and fed with standard B511 chow and water *ad libitum*. During the study period, the rats were placed in a conditioned temperature and appropriate air flow. The ethical clearance for the animal handling was provided by the Faculty of Veterinary Udayana University (Clearance No.: 212/KE-PH-Lit-1/III/2016).

Extraction
Tomato fruits (*Solanum lycopersicum* L.) were sorted according to freshness. The selected tomatoes were steamed for 15 minutes. Fresh and steamed tomatoes were blended separately with a solvent mixture consisting of 96% ethanol:acetone:hexane (2:1:1, v/v). Among 3 layers formed during the extraction, only the middle layer was collected, refluxed for 60 minutes at 60^{o}C, and evaporated with a rotary evaporator (60^{o}C) to remove the solvent.

Identification of lycopene and beta-carotene content
The presence of lycopene and beta-carotene compounds in fresh (L1) and steamed tomato extracts (L2) was confirmed by applying a TLC-spectrophotodensitometry method.[8] L1 and L2 was dissolved in 96% ethanol:acetone:hexane (2:1:1, v/v). Lycopene standard was dissolved in THF. Beta carotene standard was dissolved in hexane. L1, L2, lycopene and beta carotene standard were spotted on the TLC plate, elucidated using a solvent mixture of hexane: toluene (19:1). Both extracts (L1 and L2) were identified for their lycopene and beta-carotene content using TLC-Spectrophoto densitometry method.

Antioxidant activity in vitro test using DPPH
A quantity of 10 mg sample of steamed tomato extract was dissolved in 2 mL methanol and sonicated for 15 minutes. Samples of 100, 300, 500, 700 and 900 µL were pipetted into capped amber vials and added with 1 mL methanol. A volume of 500 µL of each sample was then transferred to new vials, added with 500 µL DPPH solution (0.025 mg/mL), incubated for 60 minutes, spot inoculated (20 µL each) on GF_{254} silica gel TLC plates with an Automatic TLC sampler 4 (Camag), and scanned at wavelength of 530 nm for DPPH[9].

Research design
The study design is shown in Figure 1. The rats were divided into five treatment groups (5 rats per group) and the treatments included: normal (no treatment); atherogenic (given palm oil and Na CMC p.o and fed a fat-rich diet); athorvastatin (given athorvastatin at 7.2 mg/kg body weight + Na CMC p.o and fed a fat-rich diet); E1 (given L1 at 5 mg/kg body weight p.o + palm oil and fed a fat-rich diet); and E2, (given L1 at 15 mg/kg body weight p.o + palm oil and fed a fat-rich diet). The normal rats were fed with B511 at the rate of 25 g/day and they had *ad libitum* access to water. The other four groups of rats were given a fat-rich diet (B511 containing 15% pork fat, 5% duck egg yolk; and 0.1% calcium) at 250g/day per group. All rats received vitamin D3 (20,000 IU/rat/week p.o) for 90 consecutive days.

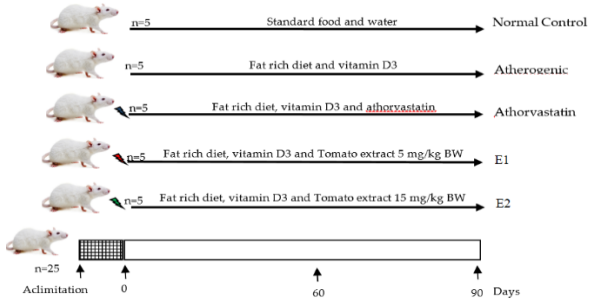


Figure 1: Design of the experiment

Measurement of lipid levels
The fasting blood lipid profiles were determined twice, on day 60 (60 days after induction with fat-rich food) and on day 91 (30 days after receiving the beta-carotene treatment). The levels of total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL-C) and high-density lipoprotein (HDL-C) of rat's serum were measured using enzymatic reactions.[10]

Atherogenic Index of Plasma (AIP)
The AIP is a logarithmic ratio of TG and HDL (TG/HDL). High TG value or low HDL-C value will produce high AIP value.

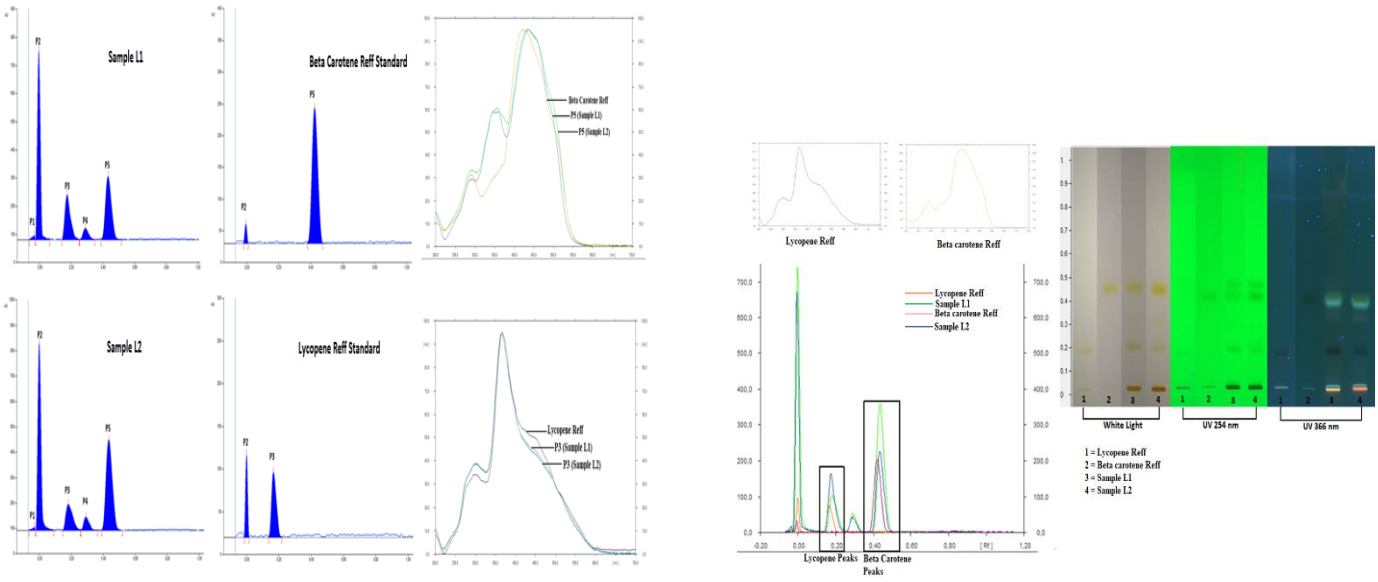
Histopathology of aortic rat
At the end of the experiment, all rats were sacrificed and their aortas were removed for histological evaluations. These aortas were fixed in 10% neutral buffered formalin solution. Fixed aorta tissues were embedded in paraffin wax, cut into 4 µm thick sections, stained with hematoxylin and eosin (H&E), and observed under a light microscope to assess the condition of the aortas. The slides were analyzed under the light microscope (Olympus BX63 with a DP72 camera, Olympus Corporation, Tokyo, Japan) at 400×. Sections were observed for vascular congestion and inflammation and scored. The aortas were scored as follows: 0 for normal aorta; 1 for widening of the constituent cells of the aorta; 2 for fragmentation of elastic fibres and foam cells; 3 for smooth muscle cell proliferation; and 4 for ulceration or calcification of lipid plaques.[10,11,12]

Statistical analysis
All TC, TG, LDL-C, HDL-C, and AIP data and aorta scores were analysed using SPSS software for windows to determine whether there were significant differences that occurred before and after administration of tomato extracts. The normality of the distribution of each variable was measured through means of the Kolmogorov–Smirnov test. The data were expressed as mean ± standard deviation (SD) and examined for their statistical significance of difference with ANOVA and LSD t-test. P-values of less than 0.05 ($p < 0.05$) were considered to be statistically significant.

Results and Discussion

Identification of lycopene and beta-carotene
Figure 2 shows the identification of lycopene and beta-carotene contents found on fresh tomatoes extract (L1) and steamed tomatoes extract (L2). The identification was based on the similarity of the Rf values, colours and the overlay of the spectra among the peaks found on the samples and reference standards tracks. Both samples (L1 and L2) produced 5 peaks, of which peak 3 (P3) showed a similarity to the lycopene reference standard, while peak 5 (P5) showed a similarity to the beta-carotene reference standard. Therefore, both lycopene and beta-carotene compound were identified on both extracts (L1 and L2). Densitograms presented in Figure 2 also shows the effect of steaming on the lycopene and beta-carotene levels of the tomato extracts. Tomatoes steamed before extraction was found to increase the level of beta-carotene in the extract, and this value was significantly higher than that of non-steamed tomatoes (Figure 2. a and b).
The extract with the highest beta-carotene level was further tested for antioxidant activity and was administered to the rats. Tomato fruit extracts contain terpenes and flavonoids. Beta-carotene was the most abundant terpene compounds contained in the extracts in the present study. The extraction method determined the amount of lycopene in the extracts. The combination of hexane, ethanol and acetone in the extraction provided substantial beta-carotene content in the extract. Dewanto *et al*.[3] also reported that heat pre-treatment increased the beta-carotene content to 2.81 ± 0.136 mg in 100 mg extract.[3]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)



a b

Figure 2a: Densitogram of lycopene in extracts from fresh tomatoes (L1) or from steamed tomatoes (L2); **2b:** Spectra of the L1 and L2

Antioxidant activity

The tomato extracts showed free radical scavenging ability when tested with a free radical DPPH, with an IC_{50} value of 0.496 mg/mL. The IC_{50} was obtained from the linear equation generated from a curve of extract concentration VS DPPH per cent inhibition (Table 1).

The tomato extract had radical scavenging ability against DPPH, with an IC_{50} value of 0.496 mg/mL. This was due to the presence of beta-carotene and other flavonoid compounds in the extract. The ability to capture free radicals can represent a synergism of beta-carotene and these flavonoid compounds.[3]

Table 1: Free radical scavenging activity of tomato extract

No	Concentration (mg/mL)	% Inhibitor
1	0.121	29.996
2	0.242	41.788
3	0.483	51.843
4	0.725	59.339

Blood Lipid levels

The effects of tomato extracts to improve lipid profiles (decreased TC, TG, and LDL-C level of rat's blood; and increased HDL-C level of rat's blood) following administration of tomato extract at the rate of 5 and 15 mg/kg bodyweight for 30 days to rats previously fed with a fat-rich diet and Vitamin D 20,000 IU are shown in Figure 3.

Dyslipidemia is a risk factor for atherosclerosis to occur. Lycopene is a compound with capability to prevent dyslipidemia condition. Homeostatic of cholesterol in our body can be a solution to overcome dyslipidemia. Cholesterol homeostatic can be overcome by regulation uptake pathways, storage, synthesis, and efflux of cholesterol. The synthesis of cholesterol is affected by *β*-Hydroxy *β*-methylglutaryl-CoA (HMG-CoA) enzyme.[13,14,15] Inhibition of such enzyme will interfere of even inhibit mevalonate, and therefore, formation of cholesterol will not happen. Lycopene is a terpene compound synthesized from mevalonate through reductase HMG-CoA pathway.[16] Lycopene consumption can reduce lipid levels in the blood, and this was claimed to be due to its ability to inhibit the HMG Co-A reductase enzyme.[5] Rats supplied with tomato juice (containing lycopene at 1 mg/kg bodyweight) for four weeks showed reduced levels of TC, TG and LDL and an increase in HDL blood levels.[5]

Provision of tomatoes containing 100-800 ppm lycopene for ten weeks reduced the levels of TC and LDL in rat blood.[17] Lycopene and β-carotene play essential roles in the cholesterol metabolism as an inhibitor in the cholesterol formation.[18] Zhou *et al*.[19] provides evidence that the conversion of beta-carotene to vitamin A regulates hepatic lipoprotein secretion and atherosclerosis development in mice.[19] Combination of lycopene and beta-carotene could increase the potency to reduce lipid levels in the blood.

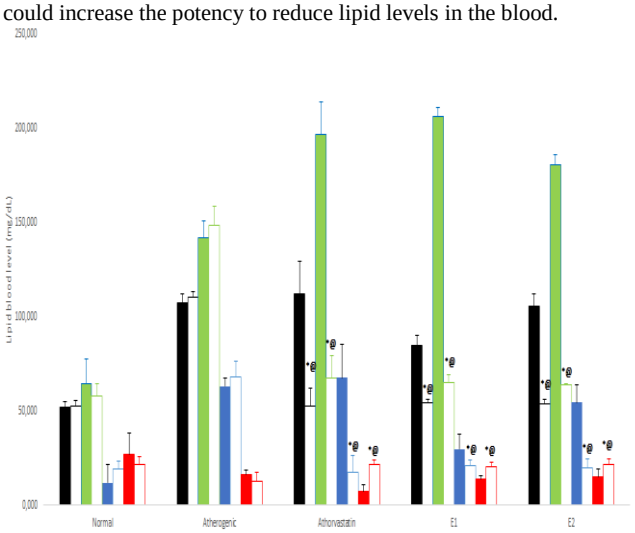


Figure 3: Lipid profiles before and after feeding tomato extract.

E1 = atherogenic rats feeding tomato extract 5 mg/kg body weight; E2 = atherogenic rats feeding tomato extract 15 mg/kg body weight; black solid square = TC levels before treatment; black open square = TC levels after treatment; green solid square = TG levels before treatment; green open square = TG levels after treatment; blue solid suare = LDL levels before treatment; blue open square = LDL levels after treatment; red solid square = HDL levels before treatment; red open square = LDL levels after treatment; * = significantly different from the group before treatment ($p < 0.05$); @ = significantly different from the atherogenic group ($p < 0.05$).

Atherogenic Index of Plasma (AIP)

Lycopene's ability to reduce blood lipid levels in rats is comparable to the decrease in AIP values. AIP is a new index lipid used as a

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

predictor of risk factors for heart problems. A high AIP value indicates a greater chance of heart problems. The rat's AIP values were calculated before and after the administration of the tomato extracts. A decrease in the AIP value was observed, following administration of tomato extract for 30 consecutive days (Figure 4). This data gives an insight that tomato extract given consecutively for 30 days may have potential to reduce risk factors from obtaining cardiovascular disorders. The AIP value is positively correlated with the risk of cardiovascular disorders (i.e. a high AIP value is associated with a higher risk of cardiovascular disorders). A high AIP value indicates high TG levels and low HDL levels in the blood. A high AIP is a risk factor for atherosclerosis and can be used to monitor the CVD events index on a daily, especially in patients with risk factors for heart problems.[20] This test was carried out on human subjects (500 men and 500 women) from 2008 to 2010 and an increase in the AIP value was positively correlated with an increase in TC, TG and LDL and a decrease in HDL.[21] Similarly, three hundred patients aged 30-60 years with diabetes mellitus-associated dyslipidemia had a high AIP values of 99.3% (out of the total number of patients). A positive correlation exists between the increase in TC and TG and the decrease in HDL level in patients with an increase in AIP value.[21]

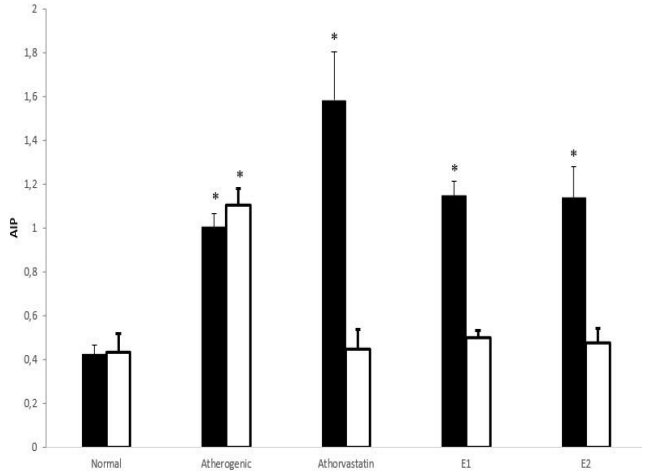


Figure 4: AIP values before and after administering tomato extract.
E1 = atherogenic rats feeding tomato extract 5 mg/kg body weight; E2 = atherogenic rats feeding tomato extract 15 mg/kg body weight; Black solid square = AIP before treatment; black open square = AIP after treatment; Significant differences compared to the normal control group are designated as * $p < 0.05$

Anti-atherosclerosis activity

The ability of tomato extract to reduce lipid levels and to scavenge DPPH free radicals provides us with information about its potential as an anti-atherosclerosis compound. The average aortic histopathological scores of the rats previously treated with tomato extract for 30 days was found to be comparable (statistically non-significant at $p<0.05$) when compared to those of normal rats (Figures 5 and 6). Normal rats had normal aortas with neat and tight cell constituents (Figure 6 A). The rats fed with fat-rich diet and Vitamin D 20,000 IU for 60 days (Figure 6 B1) or 90 days (Figure 6 B2) showed changes in the aortic cells, as smooth muscle cells (SMC) seemed to be undergoing proliferation in the aorta. The rats fed a fat-rich diet and Vitamin D 20,000 IU for 60 days before treatment also showed morphological changes in their aortic cells (Figure 6 C1, D1, E1). On day 61, all treated rats were administered with atorvastatin (Figure 6 C2) or tomato extract with doses of 5 or 15 mg/kg bodyweight (Figure 6 D2, E2) for 30 days. On the last day of the treatment, all rats were sacrificed and their aortic conditions were analysed. Significant changes were again observed in the rat aortas (Figure 6 C2, D2, E2). The aorta appeared to be normal and there was no SMC proliferation.

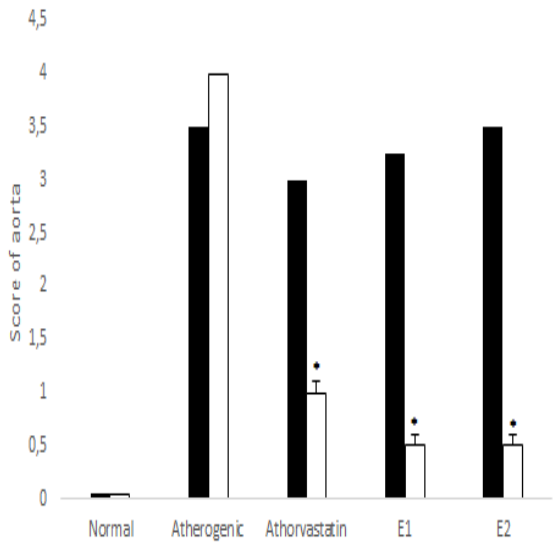


Figure 5: The average of histopathological score of rats aortas (n = 5); E1 = atherogenic rats feeding tomato extract 5 mg/kg body weight; E2 = atherogenic rats feeding tomato extract 15 mg/kg body weight; black solid square = pre-treatment; black open square = post treatment; Significant differences compared to the atherogenic group are designated as *$p < 0.05$.

Administration of a fat-rich diet and Vitamin D 20,000 IU caused an increase in the number of chylomicrons in the rat intestines. These results indicated an increase in free fatty acids entering the liver. According Warditiani *et al.*[11,22] the hepatic fatty acids undergo esterification to TG, and the TG accumulation in hepatocytes produces very low-density lipoprotein, which will be processed into LDL.[11,22] An excessive LDL burden in the blood due to excessive formation and due to a lack of LDL receptors will in turn stimulate LDL oxidation to produce ox-LDL. Excessive levels of ox-LDL in blood vessels triggers macrophages to prey on ox-LDL, and this lead to the formation of foam cells.[23] The foam cells induce the proliferation of SMC in the aortic intima tunica.[24,25] Therefore, consumption of a fat-rich diet and Vitamin D 20,000 IU for 60 days induced atherosclerosis in the rat aortas (Figure 6). A combination of phenolic compound and lycopene protect LDL from oxidation so that formation of ox-LDL will not occur.[26]

An excessive LDL burden in the blood due to excessive formation and due to a lack of LDL receptors will in turn stimulate LDL oxidation to produce ox-LDL. Excessive levels of ox-LDL in blood vessels triggers macrophages to prey on ox-LDL, and this lead to the formation of foam cells.[23] The foam cells induce the proliferation of SMC in the aortic intima tunica.[24,25] Therefore, consumption of a fat-rich diet and Vitamin D 20,000 IU for 60 days induced atherosclerosis in the rat aortas (Figure 6). A combination of phenolic compound and lycopene protect LDL from oxidation so that formation of ox-LDL will not occur.[26]

Lycopene reduced cholesterol levels in the blood, probably by suppressing cholesterol uptake rather than by cholesterol excretion from the blood. Lycopene can reduce cholesterol levels in human blood by reducing the expression of HMG-CoA reductase expression, which is required for the biosynthesis of cholesterol.[27] Lycopene can reduce the biosynthesis of cholesterol by inhibiting HMG-CoA reductase and acyl-CoA and cholesterol O-acyltransferase (ACAT) activity and can reduce the absorption of cholesterol from food, thereby increasing the excretion of cholesterol in faeces in test rabbits.[16,18] The anti-atherosclerotic activity of tomato extract may be due to an increased LDL receptor activity caused by lycopene; therefore, lycopene protects LDL against oxidation.[16] This causes the formation of foam cells so that the next process does not occur.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Lycopene can inhibit pro-inflammation so that it decreases the production of ROS and activities of MAPK and NF-kB.[28] Other reports showed that lycopene prevented the proliferation of SMC to occur. Lycopene prevents SMC's proliferation by activating protein kinase C that plays an important role in the transduction signal pathways.[29] Its ability to inhibit SMC proliferation may be due to its antioxidant effect. Lycopene is a carotene compound with a strong capability to inhibit synthesis of SMC in human aortas.[30,31] Another mechanism that may occur is its ability to interfere with growth signal factors.[32] Principally, the antioxidant effect of lycopene is to inhibit proliferation, a result of peroxidation of lipid and enzymatic hydrolysis of lysophosphatidylcholine causing mitogenic for SMC. [32]

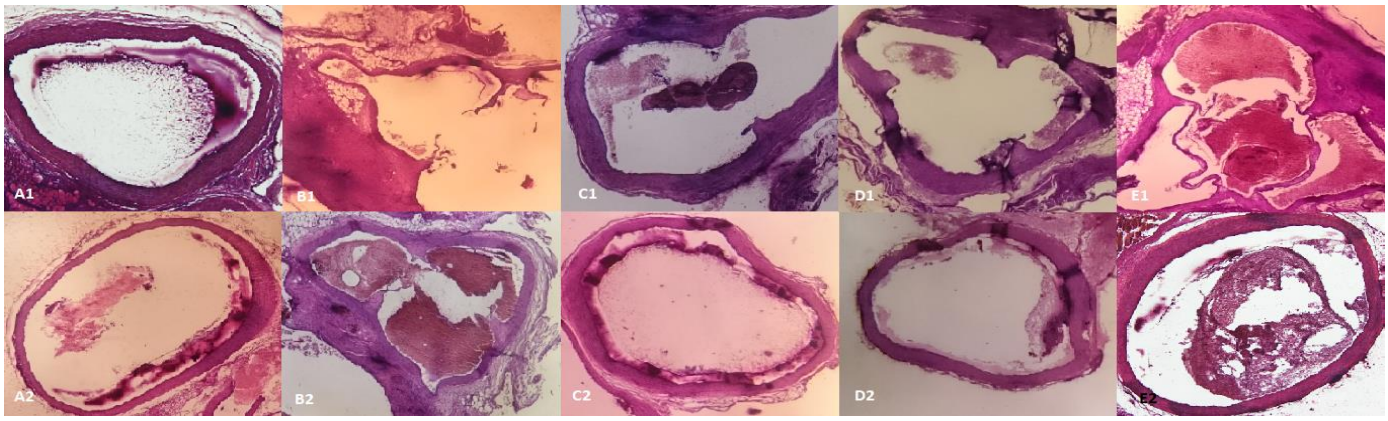


Figure 6: Morphology of rat aortas in hematoxylin eosin stained tissues in rats fed atherogenic foods and after treatment with tomato extracts (magnification by 100x).

A. Normal rat aorta; B. Aorta from negative control rat fed with fat-rich diet; C. Aorta from a positive control rat fed with fat-rich diet and treated with atorvastatin; D. Aorta from a rat fed with fat-rich diet and administered with tomato extract at the rate of 5 mg/kg body weight; E. Aorta of a rat administered with tomato extract at the rate of 15 mg/kg body weight; 1 = after atherosclerosis induction for 60 days; 2 = after 30 consecutive days of tomato extract treatment.

Conclusion

L2 contained higher level of lycopene L1, but lower level of beta-carotene content. Administration of L2 to rats reduced the AIP value, and this was positively correlated with a decrease in atherosclerosis in this animal. A decrease in atherosclerosis was suspected to be related to the ability of tomato extract to reduce blood TC, TG and LDL levels and to increase the HDL levels. Antioxidant properties of lycopene in the tomato extract was found to prevent SMC proliferation in rat's aortas.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Nour V, Panaiteb TD, Ropotab M, Turcub R, Trandafir I, Corbua AR. Nutritional and bioactive compounds in dried tomato processing waste. CyTA. J Food. 2018; 16(1):222-229.
2. Borycka B. Tomato fibre as potential functional food ingredients. Polish J Nat Sci. 2017; 32:121-130.
3. Dewanto V, Wu X, Kafui K, Adom, Liu RH. Thermal Processing Enhances the Nutritional Value of Tomatoes by Increasing Total Antioxidant Activity. J Agric Food Chem. 2002; 50(10):3010-3014.
4. Thanuja S, Sivakanthan S, Vasantharuba S. Effect of different cooking methods on antioxidant properties of Tomato (*Lycopersicon esculentum*). Ceylon J Sci. 2019; 48(1):85-90.
5. Valderas-Martinez P, Chiva-Blanch G, Casas R, Arranz S, Martínez-Huélamo M, Urpi-Sarda M, Torrado X, Corella D, Lamuela-Raventós RM, Estruch R. Tomato Sauce Enriched with Olive Oil Exerts Greater Effects on Cardiovascular Disease Risk Factors than Raw Tomato and Tomato Sauce: A Randomized Trial. Nutrients. 2016; 8(3):170.
6. Nishimura M, Tominaga N, Ishikawa-Takano Y, Maeda-Yamamoto M, Nishihira J. Effect of 12-Week Daily Intake of the High-Lycopene Tomato (*Solanum lycopersicum*), A Variety Named "PR-7", on Lipid Metabolism: A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study. Nutrients. 2019; 11:1177-1190.
7. Salem AS. Effect of Two Carotenoids (Lycopene and β-Carotene) Supplementation on Hyperlipidemia and Lipid Peroxidation in Experimental Albino Rats. JHIPH. 2015; 45(1):1-7.
8. Warditiani NK, Arisanti CIS, Wirasuta IMAG. Anti-Dyslipidemia Activity of Tomato Extract in Wistar Male Albino Rats Induced Fat-Rich-Diet and Dexamethasone. J Pharm Sci Res. 2019; 11(7):2684-2688.
9. Shimaa SE, Abd El-NG, El G, Abdelsamed IE, Mahmoud IN, Hesham RES. Chemical Composition and TLC-DPPH-Radical Scavenging Activity of *Cyperus alternifolius* Rottb. Essential Oils. J Essent Oil-Bear Plants. 2017; 20(4):1125-1130.
10. Warditiani NK and Susanti NMP. Antidyslipidemia activity of ethanol, methanol and ethyl acetate extract of *Zingiber montanum* rhizome. RJPT. 2018; 11(4):1381-1385.
11. Warditiani NK, Sari PMNA, Ramona Y, Wirasuta I MAG. Molecular Pharmacology Study of Andrographolide Extracted from *Andrographis paniculata* on Atherosclerosis Preventive Effect. Sys Rev Pharm. 2020; 11(9):201-206.
12. Warditiani NK, Widjaja INK, Larasanty LPF, Noviyanti NWR, Gitarini NM, Juniari NPM, Siahaan TF, Nugroho AE, Ramona

Y, Wirasuta IMAG, Pramono S. Anti-atherosclerosis Effect of Purified *Andrographis paniculata* Extract, IJPCR. 2016; 8(5):457-460.
13. Bao Z, Li L, Geng Y, Yan J, Dai Z, Shao C, Sun Z, Jing L, Pang Q, Zhang L, Wang X, Wang Z. Advanced Glycation End Products Induce Vascular Smooth Muscle Cell-Derived Foam Cell Formation and Transdifferentiate to a Macrophage-Like State. Mediators Inflamm. 2020; 6850187:12.
14. Heery JM, Kozak M, Stafforini DM, Jones DA, Zimmerman GA, McIntyre TM, Prescott SM. Oxidatively modified LDL contains phospholipids with platelet-activating factor-like activity and stimulates the growth of smooth muscle cells. J Clin Invest. 1995; 96(5):2322-2330.
15. Wang HH, Garruti G, Liu M, Portincasa P, Wang DQH. Cholesterol and Lipoprotein Metabolism and Atherosclerosis: Rec Adv Rev Chol Trans. 2017; 16(Suppl. 1):s27-s42.
16. Fuhrman B, Elis A, Aviram M. Hypocholesterolemic effect of lycopene and beta-caro-tene is related to suppression of cholesterol synthesis and augmentation of LDL receptor activity in macrophages. Biochem Biophys Res Commun. 1997; 233(3):658-662.
17. Basuny AM, Gaafar AM, Arafat SM. Tomato lycopene is a natural antioxidant and can alleviate hypercholesterolemia. Afr J Biotechnol. 2009; 8(23):6627-6633.
18. Verghese M, Richardson JE, Boateng J, Shackelford LA, Howard C, Walker LT, Chawan CB. Dietary Lycopene has a protective effect on cardiovascular disease in New Zealand male rabbits. J Biol Sci. 2008; 8(2):268-277.
19. Zhou F, Wu X, Pinos I, Abraham BM, Barrett TJ, von Lintig J, Fisher EA, Amengua J. B-Carotene conversion to vitamin A delays atherosclerosis progression by decreasing hepatic lipid secretion in mice. J Lipid Res. 2020; 61(11):1491-1503.
20. Yang RL, Shi YH, Hao G, Li W, Le GW. Increasing Oxidative Stress with Progressive Hyperlipidemia in Human: Relation between Malondialdehyde and Atherogenic Index. J Clin Biochem Nutr. 2008; 43(3):154-158.
21. Niroumand SH, Khajedaluee M, Khadem-Rezaiyan M, Abrishami M, Juya M, Khodaee GH, Dadgarmoghaddam M. Atherogenic Index of Plasma (AIP): A marker of cardiovascular disease. Med J Islam Repub Iran. 2015; 29(240):1-9.
22. Bezerra MA and Cohen DE. Triglyceride metabolism in the liver. Compr Physiol. 2017; 8(1):1-8.
23. Tomkin GH and Owens D. LDL as a Cause of Atherosclerosis. J Atheroscler Thromb. 2012; 5:13-21.
24. Bennett MR, Sinha S, Owens GK. Vascular smooth muscle cells in atherosclerosis. Circ Res. 2016; 118(4):692-702.
25. Khatana C, Saini NK, Chakrabarti S, Saini V, Sharma A, Saini RV, Saini AK. Mechanistic Insights into the Oxidized Low-Density Lipoprotein-Induced Atherosclerosis. Oxid Med Cell Longev. 2020; 5245308:14.
26. Milde J, Elstner EF, Grassmann J. Synergistic effects of phenolics and carotenoids on human low-density lipoprotein oxidation. Mol Nutr Food Res. 2007; 51(8):956-961.
27. Alvi SS, Iqbal D, Ahmad S, Khan MS. Molecular rationale delineating the role of lycopene as a potent HMG-CoA reductase inhibitor: *in vitro* and *in silico* study. Nat Prod Res. 2016; 30(18): 2111-2114.
28. Palozza P, Simone R, Catalano A, Monego G, Barini A, Mele MC, Parrone N, Trombino S, Picci N, Ranelletti FO. Lycopene prevention of oxysterol-induced proinflammatory cytokine cascade in human macrophages: inhibition of NF-κB nuclear binding and increase in PPARγ expression. J Nutr Biochem. 2011; 22(3):259-268.
29. Keri LH, Carpenter, Simon J, Hardwick, Albarani V, Malcolm J, Mitchinson. Carotenoids inhibit DNA synthesis in human aortic smooth muscle cells. FEBS Lett. 1999; 447(1):17-20.
30. Sharoni Y and Levy J. Natural Antioxidants and Food Quality in Atherosclerosis and Cancer Prevention. In: Kumpulainen JT, Salonen JT (Eds.). UK. Royal Soc Chem Cambridge; 1996. 378-385 p.
31. He Y, Xia P, Jin H, Zhang Y, Chen B, Xu Z. Lycopene Ameliorates Transplant Arteriosclerosis in Vascular Allograft Transplantation by Regulating the NO/cGMP Pathways and Rho-Associated Kinases Expression. Oxid Med Cell Longev. 2016; 3128280:9.
32. Lo HM, Hung CF, Tseng YL, Chen BH, Jian JS, Wu WB. Lycopene binds PDGF-BB and inhibits PDGF-BB-induced intracellular signaling transduction pathway in rat smooth muscle cells. Biochem Pharmacol. 2007; 74(1):54-63.