

Anti-Dyslipidemia Activity of Tomato Extract in Wistar Male Albino Rats Induced Fat-Rich-Diet and Dexamethasone

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Abstract

Lycopene is a compound that contained in tomatoes which has pharmacological activities such as antioxidants, anti-inflammatory, anticancer and anti-hyperlipidemia. Lycopene belongs to a terpenoid compound that has a long carbon chain. Lycopene is more easily attracted to non-polar solvents. The aim of this study was investigating anti-dyslipidemia activity of tomatoes extract (TE) in Wistar male rats. The parameters measured were total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels on the 7th and 14th days of TE administration. Result: TE was made using a combination of ethanol: n-hexane: acetone resulted in only lycopene compounds was attracted. Wistar male rats were fed rich-fat-diet (RFD) for 30 days and dexamethasone 1 mg/kg BW for five days (i.p), could cause an increase in blood lipid levels. TE 15 and 50 mg/kg BB after giving 14 days can reduce levels of TC, TG, LDL, and increase HDL in the blood.

Keywords: tomatoes, lycopene, dyslipidemia, tomato extract, fat-rich-diet (RFD)

INTRODUCTION

Tomatoes are fruits mostly used as food and beverage ingredients. Tomatoes can be served in a fresh form (such as tomato juice) and cooked (such as chili or tomato sauce) (Alda et al., 2009). Lycopene is a compound contained in tomatoes. With the heating process of tomatoes, resulting in increased absorption of lycopene in the blood when compared with fresh tomatoes. This process also causes an increase in antioxidant activity from heated tomatoes (Dewanto, Xianzhong, Adom, & Liu, 2002).

Dyslipidemia is a condition of increasing levels of total cholesterol (TC), triglyceride (TG), and low-density lipoprotein (LDL) and decreasing high-density lipoprotein (HDL) levels in the blood. Obesity-induced test animals have increased the production of ROS, which may cause vascular inflammation and increase adipogenesis (Youn et al., 2014). In mice induced by dyslipidemia, administration of 0.06% BW tomatoes was able to reduce blood levels of TC and TG (Chattopadhyay et al., 2015). This study examined the effect of extracted steamed tomatoes on the anti-dyslipidemia effect. The benefits of this study will provide information on the efficacy of steamed tomato extract against blood lipids and their potential in overcoming atherosclerosis.

MATERIALS AND METHODS

a. Material

Tomatoes, 96% ethanol technically, technical acetone, technical n-hexane, KLT silica gel GF 254 plate, toluene pa, ethyl acetate pa, diethylamine pa, ammonia pa, chloroform pa, methanol pa, male Wistar strain white rat, cholesterol reagent (Dyasis), triglycerides (Dyasis), HDL precipitant (Dyasis), LDL precipitant (Dyasis).

b. Tomato extract

Tomatoes washed, then steamed for 15 minutes. Tomatoes were blended, then extracted using a mixture of solvents

(n-hexane: acetone: ethanol 96%, 2: 1: 1 v / v) with a ratio of 1: 1 (extract: solvent) (Barba, et al., 2006). The organic phase separated from the globul phase and the water phase. A rotary evaporator dissolves the organic phase.

c. Phytochemical screening

The phytochemical test was carried out to identify the presence of compounds in tomato lycopene extract. Identification of compounds includes an examination of polyphenols, alkaloids, terpenoids, flavonoids, saponins, and tannins. The phytochemical test method used is TLC (Harbone, 1984; Reich and Blatter, 2004).

d. Determination of Lycopene Levels

ET dissolved in n-hexane. Lycopene absorbance measured in the wavelength of 503 nm. The formula calculates lycopene levels:

$$A = a \times b \times c$$

A is the absorbance of the sample at a wavelength of 503 nm, a is the expansion coefficient ($1.585 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$ at a maximum wavelength of 503 nm), b is the thickness of the cuvette used, and c is the concentration of the sample (Lavecchia and Zuurro, 2008; Merck & Co 1989).

e. Anti dyslipidemia activity

Wistar male albino rats (4-6 weeks) conditioned for seven days in laboratory animals. Rats induced dyslipidemia by the administration of FRD (standard diet: lard: yolk = 85: 10: 5) and injection of dexamethasone 1 mg/kg BB. The Faculty of Veterinary provided ethical Clearance of the animal's handling, Udayana University no: 392/KE-PH-Lit-3/IV/2018. Animals were grouped as follows:

1. Normal group: standard diet + water.
2. Dyslipidemia group: FRD for 30 days and dexamethasone injection (i.p) 1 mg/kg for five days.
3. Dyslipidemia + extract 5 mg/kg BW: FRD for 30 days and dexamethasone injection (i.p) 1 mg/kg for five days. On the 31st day, TE 5 mg/kg BW given for 14 days

4. Dyslipidemia + extract 15 mg/kg BW: FRD for 30 days and dexamethasone injection (i.p) 1 mg/kg for five days. On the 31st day, TE 15 mg/kg BW given for 14 days
5. Dyslipidemia + extract 50 mg/kg BW: FRD for 30 days and dexamethasone injection (i.p) 1 mg/kg five days. On the 31st day, TE 50 mg/kg BW given for 14 days
- Blood lipids measured were TC, TG, LDL, and HDL. Blood lipid levels measured on days 0, 7, and 14 after administration of extracts. Serum blood of the rat reacted

with reagent TC, TG, precipitated HDL, and LDL precipitates to obtain lipid levels in rat blood.

f. Data Analysis

Blood lipid levels (TC, TG, LDL, and HDL) displayed in the mean \pm SD. Data obtained in the normal control group, dyslipidemia control, and treatment, were compared with each other using the parametric t paired test with a confidence level of 95%. The statistical program used in SPSS software.

Table 1. Determination chemical compounds of TE by the TLC method

No	Group	Mobile phase	Substance	Identification
1	Polyphenol	Toluene: Ethyl Acetate (93:7)	Folin	Blackish blue spots (on visible light)
			FeCl ₃ 2%	Blackish spots (on visible light)
2	Alkaloid	Toluene: Ethyl Acetate: Diethylamine (70:20:10)	Dragendrorrf	Orange-brown spots with a yellow background, yellow on UV 254 nm and light green at UV 366 nm
			Wagner	Light brown to yellow spots
3	Flavonoid	Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:26)	Ammonia vapor	Greenish spots on visible light
4	Saponins	Chloroform: Methanol: Aquades (70:30:4)	Lieberman Burcard	Spots are green to blue by heating 90°C for 10 minutes
			Vanillin sulfuric acid	The spots are gray by heating 120°C for 10-20 minutes
5	Tannin	Toluen: Ethyl Acetate (93:7)	Ammonia vapor	Purple patches under UV 366 nm
			FeCl ₃	Brownish blue spots at visible light
6	Triterpenoid	Chloroform: Methanol (10:1)	Anisaldehyde-sulfuric acid	Spots are red to purple by heating 100°C for 5-10 minutes at UV 366 nm
			Vanillin 1% sulfuric acid	Purple spots by heating 110°C for 5-10 minutes at visible light or UV 366 nm

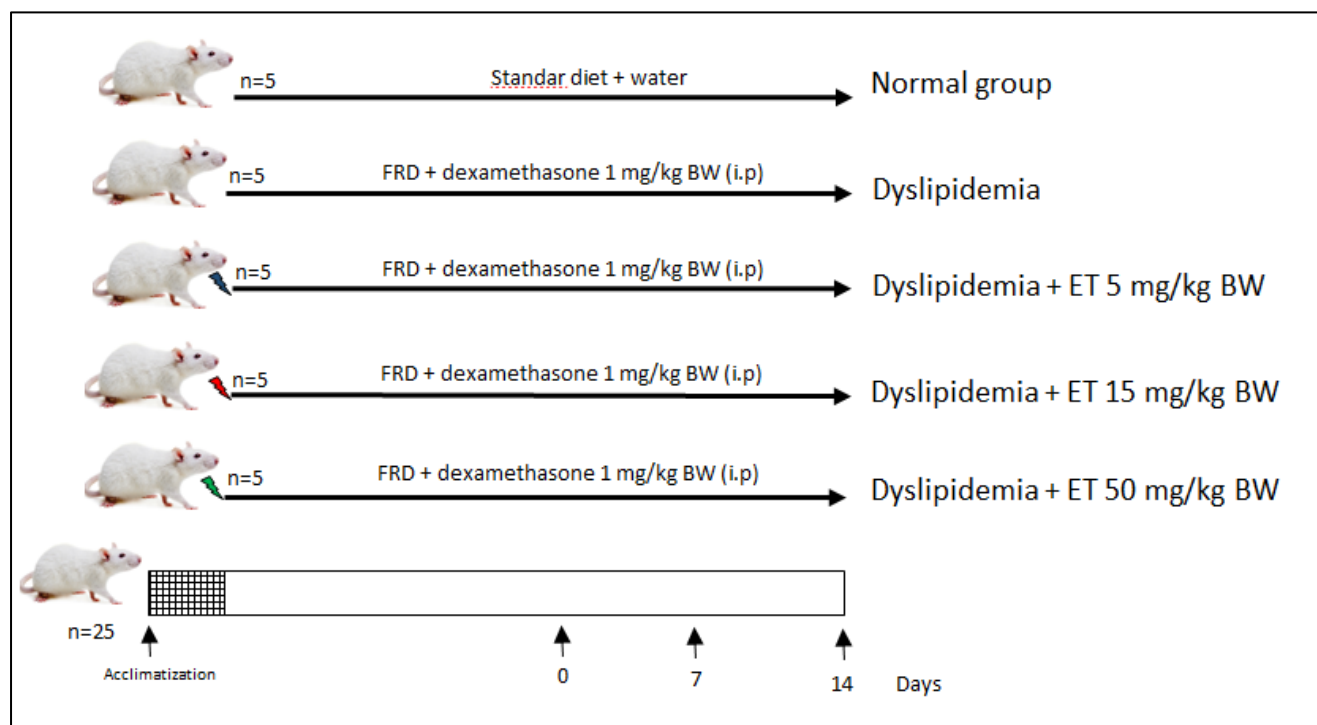


Figure 1. Anti-dyslipidemia activity test model

RESULTS

Tomato is a yellowish red fruit. This color shows the presence of lycopene and carotenoids. The content of lycopene is abundant in the skin of tomatoes. Lycopene is more extracted from tomatoes if it is warmed up first (Xu, Adyatni, & Reuhs, 2018). Lycopene is one of the non-polar terpenoid compounds. Lycopene tends to be non-polar. The n-hexane solvent mixture: acetone: ethanol 96% (2: 1: 1 v / v), lycopene was dissolved in n-hexane part (the top part) (Mohammed and Malami, 2013).

TE contains triterpenoid compounds which lycopene is a terpenoid compound (Table 2, Figure 2). Lycopene content was measured quantitatively by the spectrophotometric method. The content of lycopene in the TE is 2.81 ± 0.136 mg in 100 mg extract (Table 3). The yield of TE on fresh tomatoes is 0.0082% b / b, so in 1 kg of fresh tomato contains 2.304 mg of lycopene. Lycopene content in TE was quite high because tomatoes steamed first then extracted with a solvent. Other studies

that the amount of lycopene contained in tomatoes ranges from 3.8 to 6.6 mg/100 g (Health, 1999). Thompson et al. (2000) stated that in ripe tomatoes contain 7.05 mg/100 grams of tomatoes.

FRD given for 30 days and injection of dexamethasone 1 mg/kg BW for five days could influence blood lipid levels. It has been able to increase TC, TG, and LDL levels and decrease HDL blood levels (Figure 3). It is mean, FRD and dexamethasone able to cause dyslipidemia. Giving fat-rich feed for six weeks could increase the levels of TC, TG, LDL, and reduce HDL blood levels of rats (Warditiani et al., 2016). Giving dexamethasone 1 mg/kg BW for five days (i.p) is also able to increase blood lipid levels. Wistar male white rats injected with dexamethasone 1 mg/kg BW (i.p) for ten days were able to increase blood lipid levels (Dolatabadi and Mahboubi, 2015).

Table 2. Identification of TE Chemical Compounds

No	Group	Substance	Result
1	Polyphenol	Folin	Negatif
		FeCl3 2%	Negatif
2	Alkaloid	Dragendrorff	Negatif
		Wagner	Negatif
3	Flavonoid	Ammonia vapor	Negatif
4	Saponins	Lieberman Burcard	Negatif
		Vanillin sulfuric acid	Negatif
5	Tannin	Ammonia vapor	Negatif
		FeCl3	Negatif
6	Triterpenoid	Anisaldehyde-sulfuric acid	Positif
		Vanillin 1% sulfuric acid	Positif

Table 3. Lycopene levels in TE

Absorbance	1	0.744	Conct (%)	1	2.76
	2	0.785		2	2.96
	3	0.721		3	2.70
Mean					2.81
SD					0.136

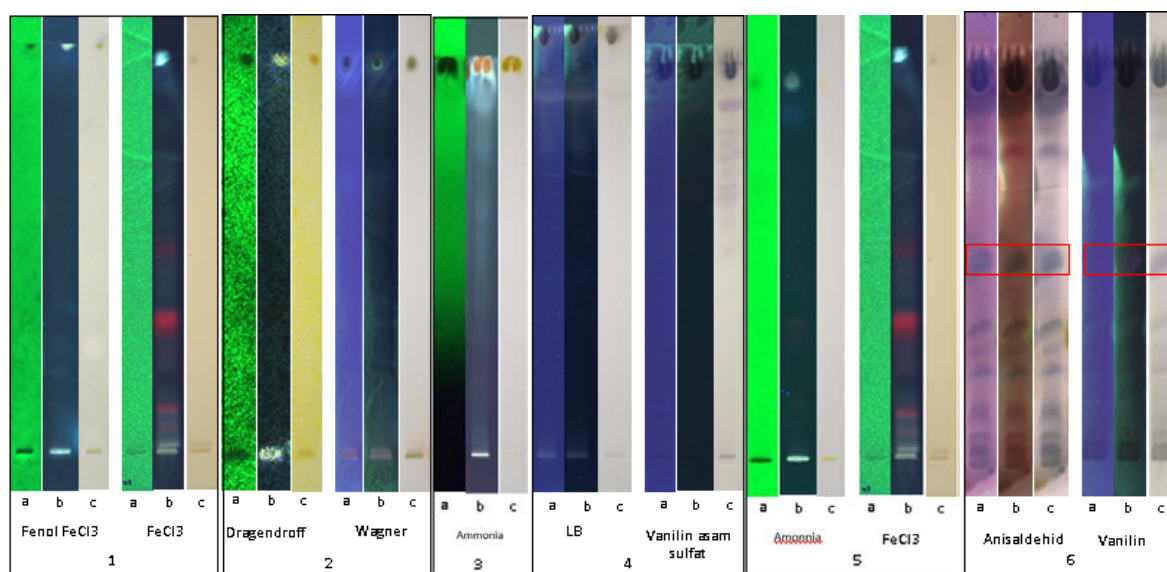


Figure 2. Chemical compounds identification of TE by TLC method

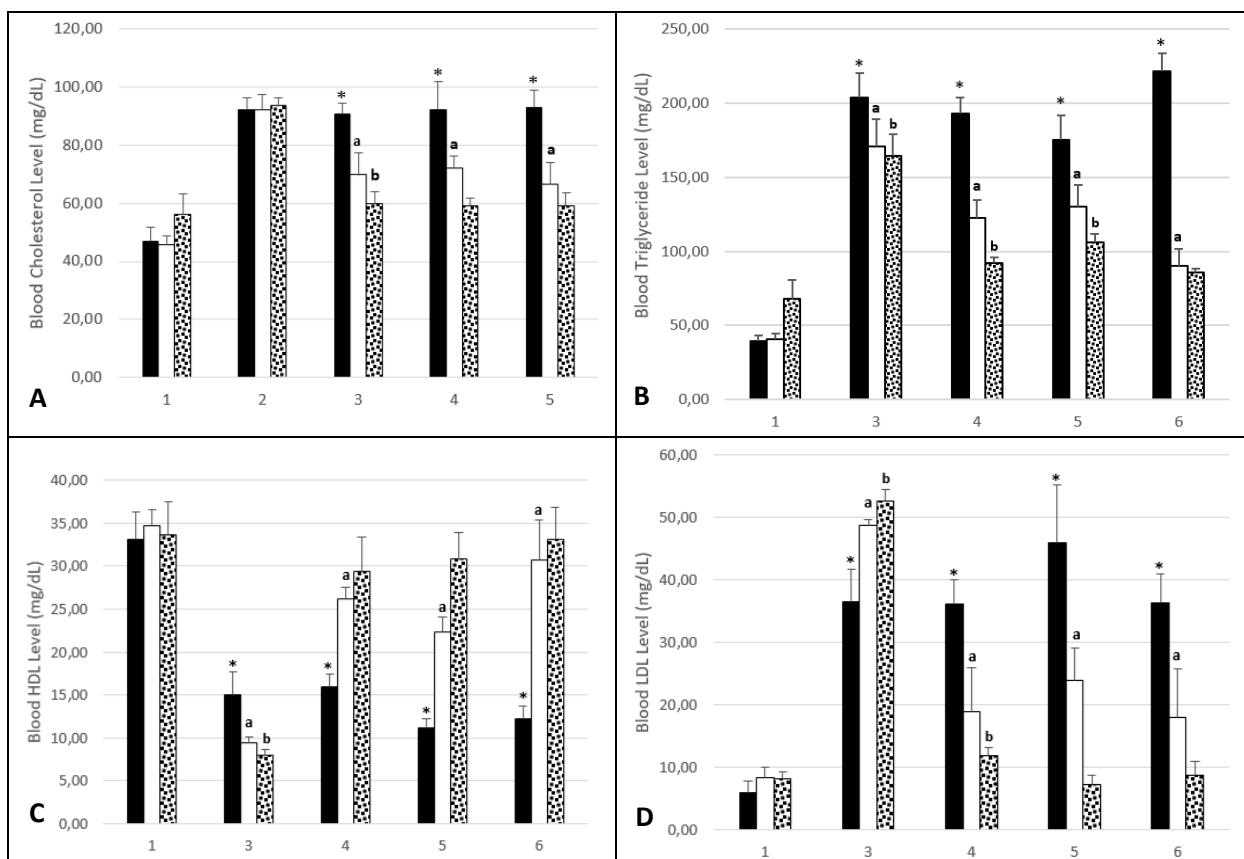


Figure 3. Lipid profile in blood on day 0 (■), 7 (□) and 14 (▨). A is the TC profile; B is a TG profile; C is an HDL profile; D is an LDL profile. 1 is a normal group; 2 is the dyslipidemia group; 3 is a group of dyslipidemia rats given TE 5 mg/kg BB; 4 is a group of dyslipidemia rats given TE dose of 15 mg/kg BB; 5 is a group of dyslipidemia rats given TE dose of 50 mg/kg BW. * = significantly different from the normal group on day 0; a = significantly different from the normal group on day 7; b = significantly different from the normal group on day 14.

TE dose 15 and 50 mg/kg BW could reduce TC levels after 14 days of administration. TC blood level of rats given TE doses of 15 and 50 mg/kg BW did not differ significantly from those in the normal group (Figure 3.A). TE dose 50 mg/kg BW could reduce TG levels after 14 days. TG blood level of rats given TE 50 mg/kg BW were not significantly different from the normal group (Figure 3.B). TE dose 5, 15, and 50 mg/kg BW could increase HDL levels after 14 days of administration. The HDL blood level of rats given TE doses 5, 15, and 50 mg/kg BW did not differ significantly from the normal group (Figure 3.C). TE dose of 15 and 50 mg/kg BW could reduce LDL levels after 14 days of administration. LDL blood level of rats given TE doses of 15 and 50 mg/kg BW were not significantly different from the normal group (Figure 3.D).

DISCUSSION

Lycopene content in TE was quite high because tomatoes steamed first then extracted with a solvent. Other studies that the amount of lycopene contained in tomatoes ranges from 3.8 to 6.6 mg/100 g (Health, 1999). Thompson et al. (2000) stated that in ripe tomatoes contain 7.05 mg/100 grams of tomatoes.

Tomato juice can prevent or treat hyperlipidemia condition in hamster (feed containing 0.2% cholesterol and 10% lard) (Lee et al., 2015). In vitro test, lycopene can inhibit HMG Co-A reductase so that it can reduce TC levels in the blood (Palozza et al., 2011). Lycopene can prevent hypercholesterol, plasma lipid oxidation, and atherosclerosis after being given for 16 weeks in hyperlipidemia rabbits (Frederiksen et al., 2007). Lycopene contained in red tomatoes can overcome the condition of hyperlipidemia. Cooked tomatoes will produce more lycopene and are more ready to be absorbed in the intestine so that the blood level is higher, which makes it more effective in reducing blood lipid levels. The high concentration of lycopene in the blood produces a better effect on reducing lipid levels. Lycopene can help the excretion of feces by reducing the absorption of abdominal fat (Obono and John, 2018).

CONCLUSIONS

TE 15 and 50 mg/kg BW given for 14 days can reduce TC, TG, LDL, and increase HDL blood level. Tomato extraction method is better starting with preheating, to get more lycopene.

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REFERENCES

- [1] Youn, J.Y., Siu, K.L., Lob, H.E., Itani, H., Harrison, D.G., Cai, H., Role of vascular oxidative stress in obesity and metabolic syndrome. *Diabetes*. 2014;63(7):2344-55
- [2] Obono, O.N., and John, E.J., Nutritional and therapeutic importance of lycopene: a strong antioxidant present in red tomatoes for effective reduction of plasma lipid profile (hyperlipidemia) and prevention of cardiovascular diseases in humans, *International Journal of Applied Research* 2018; 4(9): 242-247
- [3] Thompson, K.A., Marshall, M.R., Sims, C. A., C. I. Wei, S. A. Sargent, J. W. Scott. Cultivar, Maturity, and Heat Treatment on Lycopene Content in Tomatoes. *Journal of Food Science* 2000. 65(5), 791-795
- [4] M. I. Mohammed and D. I. Malami, Effect of Heat Treatment on the Lycopene Content of Tomato Puree, *ChemSearch Journal* 4(1): 18 – 21, June, 2013
- [5] Dolatabadi A A, Mahboubi M A, study of the influence of dexamethasone on lipid profile and enzyme lactate dehydrogenase, *Journal of Medicine and Life* Vol. 8, Special Issue 3, 2015, pp.72-76
- [6] Lee LC, Wei L, Huang WC, Hsu YJ, Chen YM, and Huang CC, Hypolipidemic Effect of Tomato Juice in Hamsters in High Cholesterol Diet-Induced Hyperlipidemia, *Nutrients*, 2015,7, 10525–10537
- [7] Warditiani NK, Milawati, Susanti, NMP. Anti dyslipidemic activity of Katuk leaves saponins fraction (*Sauropus androgynus* (L) Merr) in rats induced with fat-rich diet. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2016; 8(5): 418-420
- [8] Palozza P, Simone R, Gatalano A, Parrone N, Monego G, Ranelletti F. Lycopene regulation of cholesterol synthesis and efflux in human macrophages. *J. Nutr. Biochem.*22, 971–978 (2011) .
- [9] Frederiksen H, Rasmussen SE, Schröder M, Bysted A, Jakobsen J,Frandsen H, Ravn-Haren G, Mortensen A, Dietary supplementation with an extract of lycopene-rich tomatoes does not reduce atherosclerosis in Watanabe Heritable Hyperlipidemic rabbits, *British Journal of Nutrition*(2007).97, 6–10
- [10] Xu, Q., Adyatni, I., & Reuhs, B. (2018). Effect of Processing Methods on the Quality of Tomato Products. *Food and Nutrition Sciences*. <https://doi.org/10.4236/fns.2018.92007>
- [11] Merck & Co (1989) *The Merck Index*, 11th edn. Rahway, NewYork, p 884
- [12] Harborne, J.B., *Phytochemical Methods: A Guide to Modern Technique of Plant Analysis*. (2nd edn). Chapman and Hall. London 1984.