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Phytochemical and Hypoglycemia Effect of Tomato Lycopene Extract (TLE)

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ABSTRACT

Background: High blood glucose level is one of the Diabetes Mellitus (DM) symptoms. DM type 2 occurs when the body's cells experience insulin resistance. Insulin resistance caused by an increase of lipids in the body, so insulin can not be used properly.
Objective: To determine the effect of giving tomato lycopene extract (TLE) to reduce levels of rat blood glucose induced by hyperglycemia.
Method: Tomato skin extracted with four kinds of solvents, namely acetone, ethanol, *n*-hexane, and a combination of *n*-hexane: acetone: ethanol 96% (1: 2: 1). The identification of lycopene compounds was carried out by the TLC derivatization method. Hyperglycemia rats were induced by a high-fat diet (HFD) for 30 days and dexamethasone 1 mg/kg BW (i.p). Blood glucose levels were measured before and after the administration of the extract. TLE 5, 15, and 50 mg/kg BW given for 14 days.
Results: The combination of *n*-hexane: acetone: ethanol 96% (1: 2: 1) produces the most lycopene content. This extract contains the fewest phenol and flavonoid compounds, so it is called a tomato lycopene extract (TLE). LTE 5, 15, and 50 mg/kg BW can reduce blood glucose levels. On the 7th and 14th day, the rat blood glucose level of the treatment group was not statistically different from the normal group.
Conclusion: The hyperglycemic rat pancreas did not show any significant difference to the pancreas of normal rats. TLE 5, 15, and 50 mg/kg BW can reduce blood glucose levels.
Keywords: tomato, TLE, blood glucose level, hypoglycemia
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INTRODUCTION

Indonesia, the incidence rate of diabetes mellitus (DM) was 8.4 million people in 2000 and expected will increase by 21.3 million in 2030 [(1)]. DM is a metabolic disorder that can be caused by insulin damage or insulin resistance, which is characterized by hyperglycemia. The prevalence of pre-diabetes in Indonesia is quite high, at 10%. DM type 2 could be caused by the lifestyle shift, namely: lack of exercise, consuming a high-fat diet, etc. A high-fat diet (HFD) can increase glucose blood levels due to the decreased expression of protein GLUT4 [(2)]. Moreover, dexamethasone sort of corticosteroid drug is also capable of increasing blood glucose levels [(3)].

Lycopene is a remarkable compound contained in tomatoes (*Solanum lycopersicum*). Lycopene is very beneficial for health, which functions as a natural antioxidant, preventing prostate cancer, preventing breast cancer, and suppressing the occurrence of osteoporosis [(4)]. Lycopene can be used as an alternative choice to overcome hyperglycemia. Lycopene can be extracted using the right solvent. In this study, the extraction of tomatoes was carried out using a combination of solvents in order to obtain more lycopene compounds. Lycopene, phenol, and flavonoid compounds from each extract were identified. The extract with the highest lycopene content was further tested for the hypoglycemia studies in rats.

MATERIAL AND METHOD

Material

Ethanol 96%, acetone, *n*-hexane, TLC silica gel GF_{254} plate, toluene (pa), ethyl acetate (pa), formic acid (pa), acetic acid (pa), diethylamine, ammonia (pa), chloroform (pa) pa), methanol (pa), Follin chiochalteu, $AlCl_3$ (pa), Na acetate (pa), $NaCO_3$ (pa), anisaldehyde, sulfuric acid, vanillin, $FeCl_3$, GOD-PAP (Dyasis) were purchased from PT. Kurniajaya Sentosa, male Wistar rats

a. Tomato Extraction

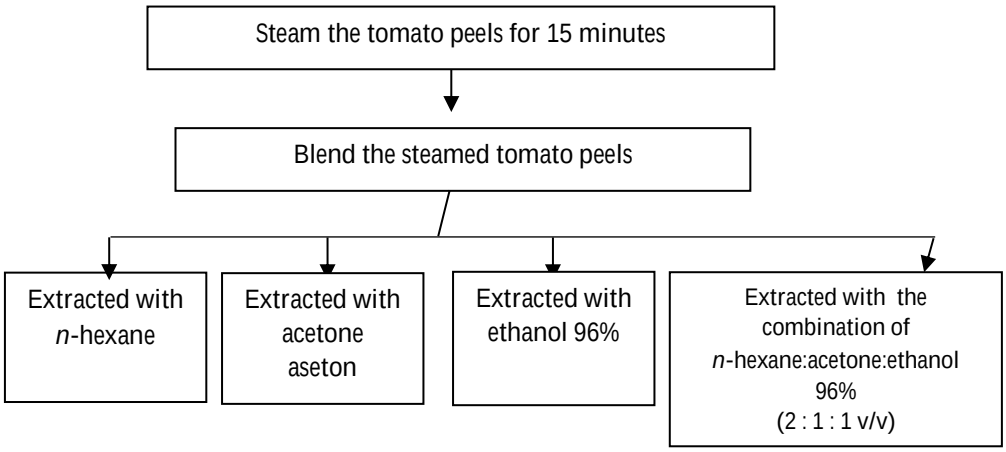

Figure 1: Chart of the extraction of the tomato peels [(5)]

b. Identify the content of terpenoid compounds from each extract

Determination of chemical compounds of tomato peel extract by the thin layer chromatography (TLC) method. The mobile phase is a combination of chloroform: methanol (10:1). Identification substances are anisaldehyde-sulfuric acid and vanillin 1% sulfuric acid [6]. Identification with anisaldehyde-sulfuric acid, by heating 100°C for 5-10 minutes, visualized at UV 366 nm will appear red or purple spot. Identification with vanillin 1% sulfuric acid, by heating 110°C for 5-10 minutes, visualized at visible light or UV 366 nm will appear purple spot.

c. Identification of total phenols and flavonoids

Identification of this compound using TLC Scanner 4 and TLC-Visualizer (7)

- Total phenol: the extract was dissolved in distilled water and added the Follin chiochalteau reagent (incubated for 3 minutes). Added $NaCO_3$, then incubated for 60 minutes, measured the absorbance at a wavelength of 550 nm.
- Total flavonoid: the extract was dissolved in ethanol 96% then added $AlCl_3$, NaAsetat, and aqueous (incubated for 30 minutes). Absorbance was measured at a wavelength of 434 nm.

d. Hypoglycemia study of extract in rats

Male Wistar rats (4-6 weeks) acclimated for seven days to adjust the environmental conditions of the rats. Rats have induced hyperglycemia with a fat-high diet/FHD (15% pork fat and 5% egg yolk) for 30 days and dexamethasone (Dexa) injection (i.p) 1 mg/kg BW for five days. 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:

Table 1: The rats grouping

No	The rats grouping	Intervention
1	Normal group	Standard diet + water
2	Metformin group	HFD + dexa + metformin 0,45 mg/kg BW
3	Hyperglicemia group	HFD + dexa
4	TLE 5 group	HFD + dexa + TLE 5 mg/kg BW
5	TLE 15 group	HFD + dexa + TLE 15 mg/kg BW
6	TLE 50 group	HFD + dexa + TLE 50 mg/kg BW

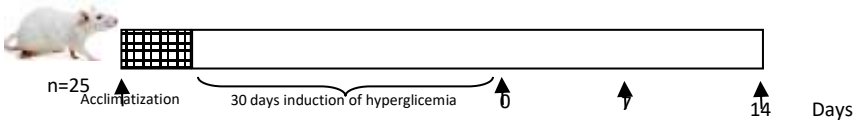

Figure 2: Anti-hyperglicemia activity test model

The blood of the rats were taken on days 0, 7, and 14 after administration of TLE. Obtained blood glucose levels, were further analyzed.

e. Observation of Pancreatic Beta Cell Histopathology

On the last day of treatment, all of the pancreatic organs of the rats were taken. The condition of the pancreatic beta cells of the previous organs were observed within all of the groups. Pancreatic tissue is fixated in Bouin's solution, sliced and stained with Hematoxylin-eosin. The preparations are observed with a light microscope to see the level of damage and repair of pancreatic cells. Pancreatic beta-cell damage analysis was performed.

f. Analysis Method

The data of the blood glucose levels were performed by statistical tests. Obtained datas from each testing group (normal control group, hyperglycemia control, metformin control, and TLE were compared to each other using the parametric paired t-test with a 95% confidencial level. The statistical program used in the recent research was SPSS software (for Windows).

RESULT AND DISCUSSION

Tomatoes have been extracted with a single solvent (acetone, ethanol 96%, and n-hexane) and mixed solvents (combination of acetone, ethanol 96%, and n-hexane). The identification of the terpenoid compounds (TLC derived by vanillin 1% in sulfuric acid, and anisaldehyde in sulfuric acid) showed the presence of the terpenoid which was indicated by the red to purple bands. The Identification of the phenolic compounds (TLC derived by Follin chiocalteu and FeCl3 2%) showed the presence of the phenolic compound which was indicated by the blackish to blackish blue bands. The combination of acetone: ethanol 96%: n-hexane given the highest content of terpenes in the extract (Figures 3.D). The number of terpenoid compounds of each extract could be observed by the chromatograms. Solvents combination extracted tomatoes gave the highest terpenoid compounds (Figure 4.D). The combination of n-hexane: acetone: ethanol 96% (2: 1: 1 v/v) produced non-polar extracted compounds. This non polar extracted compounds are lycopene. Total phenolic and total flavonoid contents of each extract are shown in Figure 5. Total phenolic and flavonoid contents of the combinated solvent (acetone: n-hexane: ethanol = 1: 2: 1) are fewer than the single solvent. The extract that contain highest lycopene we called giving tomato lycopene extract (TLE).

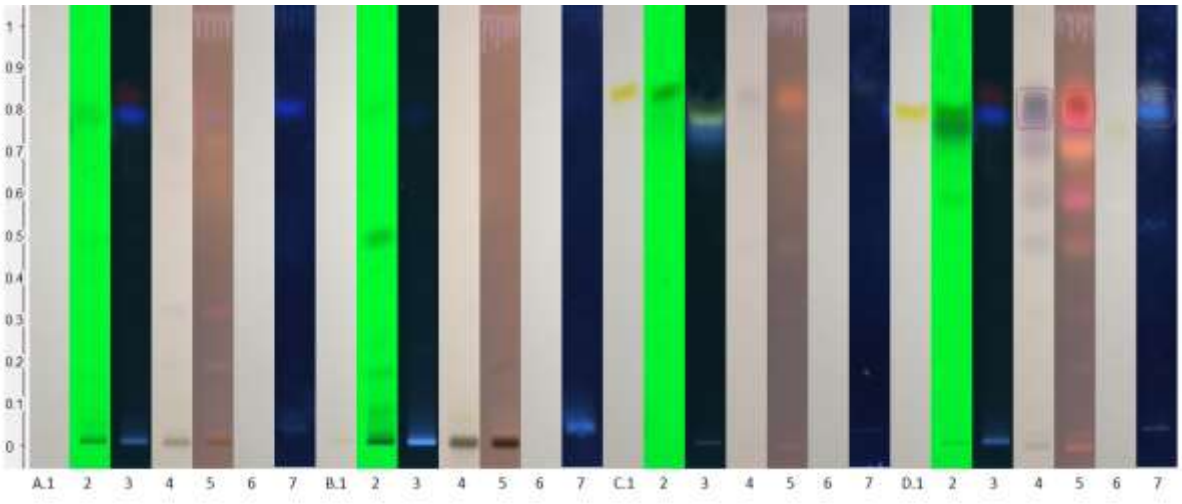

Figure 3: TLC of extract (mobile phase = chloroform:methanol = 9:1)

A = acetone extract of tomato
B = ethanol extract of tomato
C = *n*-hexane extract of tamato
D = extract tomato with solvent combination (*n*-hexane : acetone : ethanol)

1 = before derivatization under visible light
2 = before derivatization under 254nm UV light
3 = before derivatization under 366nm UV light
4 = after derivatization with vanillin 1% sulfuric acid was observed under visible light
5 = after derivatization with vanillin 1% sulfuric acid was observed under 366 nm UV light
6 = after derivatization with sulfuric acid anisaldehyde was observed under visible light
7 = after derivatization with sulfuric acid anisaldehyde was observed under 366nm UV light

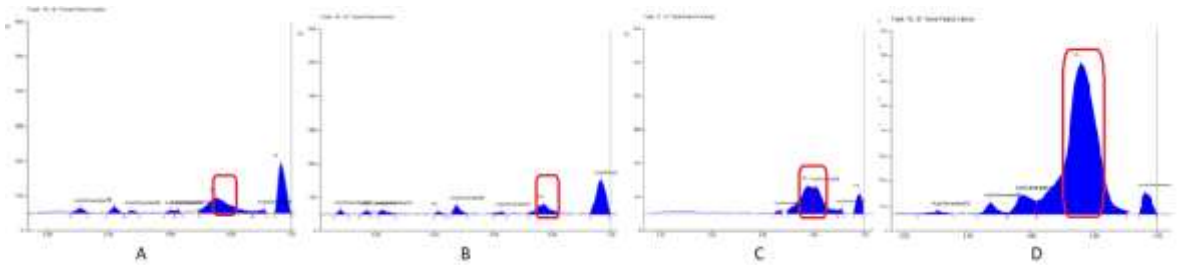

Figure 4: The Chromatogram of the extract (mobile phase = chloroform: methanol = 9: 1)
A = tomato acetone extract; B = tomato ethanol extract; C = tomato *n*-hexane extract; D = tomato solvent combination extract (*n*-hexane: acetone: ethanol)

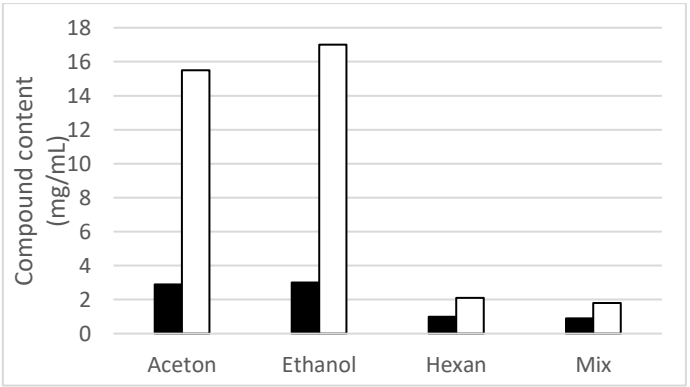

Figure 5: Quantification of total phenolic content and total flavonoid content from each extract.
□= total flavonoid contenst ; ■ = total phenolic content

The rats were induced hyperglycemia using HFD for 30 days and given Dexa 1 mg/kg BW for five days. Rat blood has taken on the 30th day of induction. HFD and Dexa (i.p) can increase rat blood glucose levels. Blood glucose levels in the normal and rat induced by HFD and Dexus injection (figure 3, day 0) were significantly different ($p < 0.05$). It means that induction has been able to cause hyperglycemia.

TLE 5, 15, and 50 g/kg BW and metformin 0.45 mg/kg BW given on the 31st day for 14 days. Glucose levels in the blood measured on days 0, 7, and 14 (Figure 6). Data shows that the administration of LTE can reduce blood glucose levels. On the 7th and 14th day, the rat blood glucose level of the treatment group was not statistically different from the normal group.

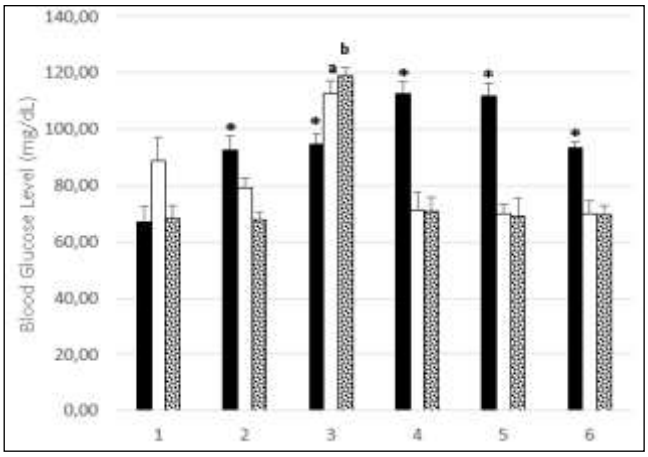

Figure 6: Blood glucose profile on days 0, 7 and 14; 1 is a group of normal rats; 2 is a group of hyperglycemic rats given metformin; 3 is a group of hyperglycemic rats without treatment; 4 is a group of hyperglycemic rats given 5 mg/kg BW of lycopene extract; 5 is a group of hyperglycemic rats given a lycopene extract dose of 15 mg/kg BW; 6 is a group of hyperglycemic rats given 50 mg/kg BW of lycopene extract.

Note: * = 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.

HFD, and Dexa (i.p) does not cause pancreatic damage (Figure 7). The purple cells are pancreatic beta cells. Hyperglycemic-induced rats show a smaller number of pancreatic beta cells than other groups, but there are still several pancreatic beta cells (Figure 7.c). It indicated that rat pancreas beta cells induced by street vendors and Dexa injection are still able to produce insulin. As for the other groups, the number of pancreatic beta cells does not appear to be very different (Figure 7.a, b, d, e, f). The number of pancreatic beta cells in normal rats appeared to be quite large (Figure 7.a). The pancreatic beta cells of rats given lycopene extract treatment (Figure 7.d-f) appear to be quite numerous, as well as the rats were given metformin (Figure 7.b).

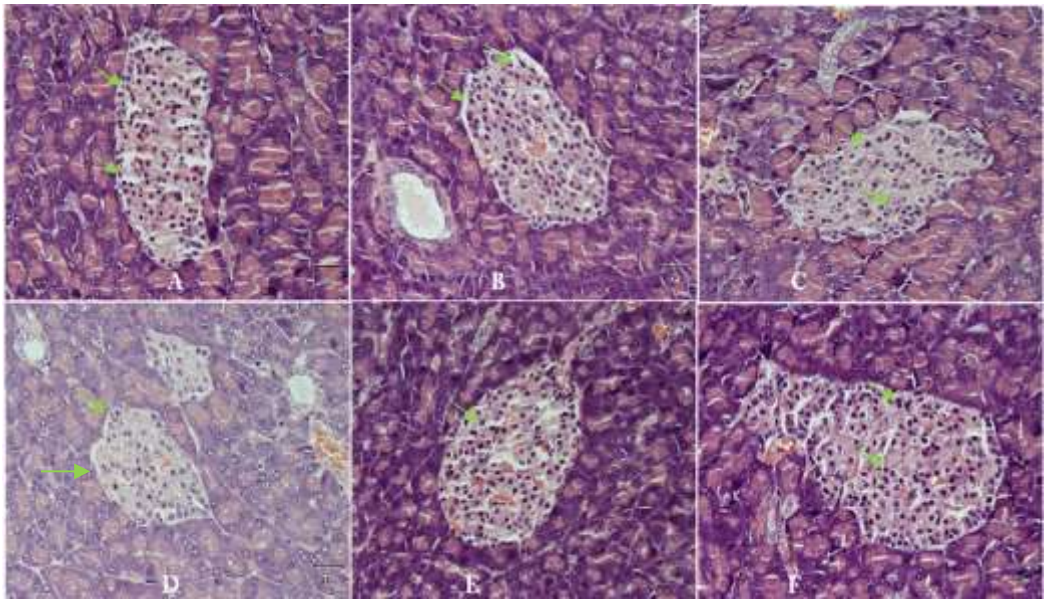

Figure 7 : Histopathology of pancreas stained with colorant HE (magnification 100x), A = pancreatic beta cells of normal rats; B = pancreatic beta cells of DM rats given metformin; C = pancreatic beta cells of hyperglycemic rats; D = pancreatic

beta cells DM rats were given TLE dose of 5 mg/kg BW; E = DM pancreatic beta cells were given TLE dose of 15 mg/kg BW; F = pancreatic beta cell DM rats were given TLE dose of 50 mg/kg BW, green arrows indicate pancreatic beta cells

Hyperglycemia is the result of uncontrolled conditions in glucose regulation. Hyperglycemia generally caused due to excessive production of free radicals or the decreased ability of antioxidants in the body. It will contribute to the onset and progression of hyperglycemia [8]. Free radicals through glucose oxidation, glycation of both non-enzymatic and enzymatic proteins, will cause hyperglycemia. Excessive amounts of free radicals can cause cellular oxidation of organelles and enzymes, thereby causing lipid oxidation and causing insulin resistance. It will trigger the occurrence of hyperglycemia [9].

Giving Dexa 1 mg/kg BW for five days (i.p) can cause a decrease in insulin secretion so that it can cause hyperglycemia and insulin resistance [10]. Glucocorticoid compounds can damage pancreatic beta cells, thereby disrupting glucose metabolism [11]. Dexamethasone induction of 10 mg/kg BW for ten days could use to make a model of diabetes test animals with insulin resistance [12]. FHD can induce metabolic disorders such as DM, hypertension, and hypercholesterolemia [13].

Lycopene 10, 20, and 40 mg/kg BW provide hypoglycemic effects in STZ induced rats 60 mg/kg BW [14]. Lycopene 5 mg/kg BW can reduce glucose levels in the blood of rats experiencing hyperglycemia induced by alloxan [15]. Foods rich in carotene compounds can overcome the condition of hyperglycemia in patients with type 2 DM [16].

The choice of solvent affects the quantity of lycopene that can withdraw. The use of single and combination solvents causes differences in the content of lycopene compounds in the extract. The content of lycopene in the extract (mg/100g) extract extracted in *n*-hexane: acetone: ethanol: combination (*n*-hexane: ethanol: acetone) is 0.54; 3.79; 1.15; 5.59. It shows that the combination of solvents produces the highest lycopene content [17].

Regular consumption of tomatoes will be able to reduce the risk of degenerative diseases. This positive effect is due to the presence of antioxidant molecules, namely lycopene, ascorbic acid, vitamin E, and phenol compounds [18]. Tomato peels contain lycopene, vitamin C, and phenol compounds [19]. The antioxidant compounds contained in the extract will be able to overcome the increase in free radicals so that the condition of hyperglycemia is resolving. This antioxidant ability will also be able to protect pancreatic tissue against oxidative damage.

CONCLUSION

LTE, which gives 50 mg/kg BW, can reduce blood glucose levels. It caused by the ability of lycopene in counteracting free radicals (antioxidants) and protecting pancreatic beta cells against HFD induction and Dexa injection.

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