

Anti-atherogenic properties of Deglet Noor Date seeds (*Phoenix dactylifera*) Methanol extract on Diet-Induced Hypercholesterolemic Rats.

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Abstract. This is the first study to investigate the completely anti-atherogenic effect of Deglet Noor Date seeds methanol extract administration on diet-induced hypercholesterolemic rats. About 24 male Wistar rats were divided into 6 groups. The normal control (NC) group, Hypercholesterolemic Control (HC) group was given high cholesterol diet, and Simvastatin Control (SC) group was given 0.18 mg/200g simvastatin after high cholesterol diet induction. The treatment groups of T0.25, T0.5 and T1 were given supplementation of 0.25, 0.5 and 1 g/kg of dates seed extract after high cholesterol diet induction, respectively for 21 days. Blood was collected from orbitals plexus vein for plasma lipid profile analysis. The levels of Total Cholesterol (TC), Low-Density Lipoprotein (LDL) and Atherogenic Index (AI) values were significantly decreased ($p < 0.05$) on diet-induced hypercholesterolemic rats after supplemented with date seeds extract (T0.25, T0.5 and T1) but not in Triglycerides (TG). Along with that, High Density Lipoprotein (HDL) level was significantly increased ($p < 0.05$). However, the T1 group was the best anti-atherogenic activity in compared to other groups. Results showed that plasma lipid profile was significant to get better after supplemented with date seeds extract.

1. Introduction

Death rate as a result of coronary heart disease is considered high in the world. In the previous epidemiology research, it was found that hypercholesterolemia is the main factor of this disease. High level of cholesterol will result in atherosclerosis that atherogenic index will increase leading to sluggish blood circulation and it a death of heart muscle [1]. The rise of total cholesterol level and low density of lipoprotein (LDL) will increase the risk of coronary heart disease. It is due to high consumption of a saturated fatty meal, low intensity of physical activities and unhealthy lifestyle. Scientific evidence show that oxidative stress is a cause of fat oxidation, functional change of endothelial that leading to atherosclerosis[2].

High consumption of fruits and vegetables is beneficial for health by preventing chronic diseases such as atherosclerosis, cancer, cardiovascular, diabetes, and neurodegenerative diseases. The antioxidant may decrease the risk of chronic diseases and protect health[3]. Most antioxidant capacities are from fruits which contain flavonols and polyphenol. Date seeds contain a lot of



phytochemical compounds such as flavonols, isoflavones, flavones, anthocyanins, catechins and isocatechins which equal vitamin C and E or β -carotene, yet they have not been widely harnessed[4,5].

Many researches about the benefits of date extract have been conducted; however, the use of date seeds as the agent of anti-atherogenic by observing the whole indicators has not been proved. This research aims to investigate the effects of Deglet Noor date seeds as an anti-atherogenic for diet-induced hypercholesterolemic rats.

2. Material and methods

2.1. The preparation of sample and date seed extract

Deglet Noor date seeds were separated from the fruit. They were then washed with flowing water, laid out to dry for one day under the sun. About 2.5 kg of dried date seeds were then pounded and blended resulting in powder. This powder will be macerated for 72 hours with methanol, concentrated under the pressure of rota evaporator to reach the desired volume. Date seed powder is collected in glass objects, added with methanol as high as 2.5 cm above the surface of the date powder, stirring until homogeneous and covered with aluminum foil for 24 hours. The filtrate is transferred to erlemeyer and methanol is replaced back as the procedure until 3x24 hours. Filtrate the first, second and third-mingled, then evaporated with a rotary evaporator. The results of the date seed extract weighed and dissolved with 200 ml aquabidest. Extraction result forms a concentrated solution.

2.2. Experimental design

This research applied true experimental with a complete random design. The total of 24 Wistar male rats weighted around 150-200 g with 2-3 months of age were housed in a plastic cage covered with wire plaited cap. Rats were divided into six groups and acclimated for a week. Standard diet and aqua distillation were given through ad libitum. Air exchange and lighting were provided based on natural condition. The normal control group was given standard diet and the other groups were induced hypercholesterolemic with high fat diet of duck egg yolk for three weeks. After induction, group three, four and five were given date seeds extract of 0.25, 0.5, and 1 g/kg consecutively. A control group of simvastatin (SC) was given 0.18mg/200mg treatment for three weeks consecutively. Later, their blood was taken a day after the treatment.

2.3. Measurement of lipid profile

Blood samples were taken from rats after being starved for 8 hours on the last treatment day. The blood was taken as much as 3 cc from orbitalisplexus vein and then contained in EDTA vacutainer tube to check their Total cholesterol (TC), Triglycerides (TG), Low-Density Lipoprotein (LDL) and High-Density Lipoprotein (HDL). All profile lipid parameters applied Enzymatic Colorimetric Assay method. Atherogenic Index (AI) was measured with $\log_{10}(\text{TG}/\text{HDLc})$.

2.4. Statistical Analysis

Analysis of Variance and LSD Post Hoc were carried out to compare group mean. The significance is accepted if $p < 0.05$. Ethical approval for this research was obtained from the ethical committee of health research, medical faculty of Padjajaran University Bandung.

3. Research Result

Concentrations of TC, LDL, TG and AI after treatment for treatment and SC groups were lower compared with NC and HC groups significantly ($p < 0.05$) (Table 1). Hypercholesterolemic diet could increase TG, TC, AI and LDL levels before treatment. Date seeds extract supplementation could decrease, TC, AI and LDL significantly than TG level.

Table 1. Lipid profile as atherogenic indicator after 21 days experimental

| Group | TG | HDL | TC | LDL | AI |
|-------------------------------------|--------|---------|---------|---------|---------|
| Negative control (NC) | 98.3 | 38.0 | 83 | 21.9 | 0.49 |
| High fat diet (HC) | 117.3 | 30.8 | 86.3 | 28.1 | 0.35 |
| Date seed extract 0.25 g/Kg (T0.25) | 103.5 | 45.3 | 72 | 9.8 | 0.31 |
| Date seed extract 0.5 g/Kg (T0.5) | 76.8 | 60.5 | 72.8 | 5.3 | 0.12 |
| Date seed extract 1 g/Kg (T1) | 73.8 | 57.3 | 70 | 4.2 | 0.09 |
| Simvastatin 0.18 mg/200g (SC) | 69.0 | 60.3 | 70.3 | 16.3 | 0.17 |
| Anova test | p=0.07 | p=0.006 | p=0.004 | p=0.002 | p=0.002 |

The analysis result of one-way ANOVA shows a significant difference of HDL level ($p < 0.05$) in HDL level, total cholesterol, LDL, and atherogenic index; yet the difference was not significant for triglyceride level. Post hoc analysis of LSD reveals that 1 g/kg date seed extract was more significantly effective than other doses ($p < 0.05$) in restoring rats' lipid profile after diet-induced hypercholesterolemia.

4. Discussion

It is the first research to use complete indicators to figure out the use of date seeds to restore lipid profile after diet-induced hypercholesterolemia rat. High saturated fat and cholesterol diets may increase triglyceride and total cholesterol blood levels, resulting in the increase of LDL synthesis by lipoprotein lipase (LPL) enzyme and decrease of HDL synthesis. High level of cholesterol may precipitate in vascular and result in cholesterol oxidation in the arterial wall leading to atherogenic level increase (atherogenic index increase). Atherogenic index is a predictor of cardiovascular diseases [6]. Cholesterol will be fixated to triglyceride and apoprotein to generate lipoprotein. Therefore, high cholesterol diet can increase the level of LDL in specimens.

Triglyceride increase will result in the enhanced activities of *Cholesteryl Ester Transfer Protein* (CETP) which participates as a distribution mediator of triglyceride to HDL and ester cholesterol to LDL. Distribution of triglyceride to HDL generates HDL mature leading to the increase of LDL and decrease of HDL level. Antioxidant may also inhibit CETP activities. CETP mediates CE (cholesterol ester) transfer from HDL to pro-atherogenic apoB-lipoprotein and transfers ester cholesterol from triglyceride. CETP inhibition is necessary to develop to decrease the risk of coronary heart disease. The reduced CETP activities will be beneficial for the existence of HDL; thus triglyceride will not mostly be exchanged with HDL particle [7].

Date seeds contain various beneficial components for health [8,9]. The level of flavonol, polyphenol, unsaturated fatty acid, vitamin C and vitamin E, and fibers of date seeds are proved to be beneficial for anti-atherogenic [10]. These compounds act as antioxidants. The evidence from previous studies shows that the consumption of date seeds can increase the body's antioxidant (3). Antioxidants prevent the free radicals oxidation thus decreasing LDL-ox. The research result reveals that date seed extract can decrease LDL, TC and AI level as well as increase HDL level.

Quercetin in date seeds as a flavonol has equal activities as α -tokoferol; thus protecting LDL from oxidation reaction. Quercetin reacts with free radical by donating its proton and transforms into radical compounds; yet, unpaired electron resulted from delocalized by resonance thus the energy content will be low enough to become reactive radical. Moreover, Quercetin may also inhibit secretion of Apo-B100 to intestine, hence Apo-B number will decrease. It will cause the decrease of LDL generation in the human body.

Vitamin C content (ascorbic acid) in date seeds can also be used in hydroxylation reaction in the process of generating bile acids. The rise of bile acids will increase cholesterol excretion of LDL leading to the decrease of LDL cholesterol level in blood. The antioxidant of ascorbic acid also possesses the effect of slowing down autoxidation process through various mechanism such as metal

ion fixation, oxidation, hyperoxide degradation to non-radical products, UV radiation absorption, and oxygen singlet deactivation[11].

Phenolic content in date seeds will inhibit the generation of HMG Ko-A reductase enzyme which functions in converting Asetil Ko-A to mevalonate in lipid metabolism in the human body. With no production of mevalonate in the body, cholesterol synthesis will be inhibited leading to the decrease of cholesterol concentration, triglyceride, total cholesterol and plasma LDL level and the rise of LDL receptor number. This LDL receptor works in Cholesterol LDL clearance; thus the increase of its level may cause the rise of LDL plasma cholesterol clearance[12]. Phenolic also works as chain reaction breaker, reacting with lipid radical thus transforming it to more stabilized products and as a preventive antioxidant to reduce initiation reaction speed, prevent lipid auto oxidation through fast hydrogen atom input in lipid radical[13]. Inhibition of HMG-CoA reductase activities may increase LCAT (*Lecithin Cholesterol Acyltransferase*) activities. LCAT is believed to be able to maintain cholesterol gradient which is not stratified between peripheral cells and HDL.

Phenolic is a type of polyphenol. According to recent research[14], polyphenol compounds may decrease cholesterol plasma level by inhibiting cholesterol absorption in the intestine and increase reactions in generating bile acid from cholesterol which is excreted through feces later.

Date seeds also contain Mono Unsaturated Fatty Acid (MUFA) and fibers of pectin[15,16]. Food fibers may fixate LDL cholesterol directly, fixate bile acid and inhibit enterohepatic circulation of bile acid. This mechanism will support the excretion of cholesterol LDL through feces. Methanol extract of date seeds will also decrease LDL serum level through phagocytosis process by preventing oxidized LDL stack on the wall of vascular with contained antioxidant[17].

Fatty acid contents (MUFA and PUFA) of date seeds participate in increasing HDL level. Main fatty acid components in date seeds consist of oleic acid (50.10%), linoleic acid (19.23%), lauric acid (10.24%), palmitic acid (9.83%), and stearic acid (7.51%)[5]. High MUFA diet affects the rise of HDL[18]. MUFA consumption of oleic acid in a high concentration will decrease CETP activities significantly[19].

Phenolic and gallic acid in date seed extract can increase activities of paraoxonase enzyme 1 which possesses protective effects towards LDL oxidation. A research conducted by Takaeidi *et al.*, (2014) revealed a significant difference on the increase of PON1 activities in the 1000mg/kg/weight date seed diet given to hypercholesterolemia rats in the control group[20]. PON1 enzyme prevents lipoprotein oxidation resulting in the prevention of oxidized HDL. The rise of PON1 activities has protective effects towards atherosclerosis process. Paraoxonase is an enzyme found in a human body participating in lipid metabolism. HPON1 enzyme may protect vena from arteriosclerosis by hydrolyzing derivate cholesterol or oxidized phospholipid in atherosclerotic lesions and in oxidized LDL. PON1 has two active points involving hydrolysis reaction and protecting LDL from oxidation.

5. Conclusions

All combinations of active compounds found in date seeds are assumed to be able to affect lipid profile by increasing HDL level and decreasing total cholesterol, triglyceride and LDL level, thus atherogenic index will decrease as well. This research reveals that effective dose of date seed extract to decrease LDL, triglyceride, and AI level is T1g/kg. This dose equals that of the groups which was given simvastatin (SC).

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