Original article

Comparison between Effectiveness of Pomegranate Juice (*Punica granatum*) and Simvastatin for Lowering Blood LDL Level in Hypercholesterolemic Male Rats (*Rattus novergicus*)

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Abstract:

Background: Coronary heart disease (CHD) is one of the leading causes of death in developed and developing countries. In Indonesia 26% of death is caused by CHD. Hypercholesterolemia is one of the risk factors for CHD. Simvastatin is a hypolipidemic drug which has a significant number of side effects such as myopathy. While, Pomegranate juice (*Punica granatum*) contains anthocyanin, cathechins, tanin, vitamin C and vitamine E which have beneficial effects to decrease blood LDL level. ***Objective***: The aim of this study was to compare the effectiveness of pomegranate juice (*Punica granatum*) and simvastatin in lowering blood LDL level of hypercholesterolemic male rats (*Rattus novergicus*). ***Methodology***: This experimental study was executed following by pre and post control group design. This study used 15 male Wistar rats that were divided into three groups as 2% DMSO, simvastatin and pomegranate juice. Blood LDL level was examined at day-0 and day-15 of trial period. Result: Dependent T-test reveals that both pomegranate juice and simvastatin group showed significant difference in lowering blood LDL level before and after treatment. The result of Kruskal Wallis test showed that both pomegranate juice and simvastatin group have more significant efficacy than 2% DMSO. On the other hand, there was no significant difference between effectiveness of pomegranate juice and simvastatin group. ***Conclusion***: Both Pomegranate juice and simvastatin are effective in lowering blood LDL level of hypercholesterolemia male rats and there is no significant difference between effectiveness of pomegranate juice and simvastatin.

Keywords: hypercholesterolemia; simvastatin; pomegranate juice; blood LDL level

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Introduction:

Presently coronary Heart Disease (CHD) is one of the key causes of death in developed and developing countries. Based on the information of World Health Organization (WHO) in 2002, 4.4 million deaths are due to CHD caused by hypercholesterolemia and 7.9% of deaths occurred at young age.[1]

Hypercholesterolemia is a disorder of lipoprotein levels in the blood, which is characterized by elevated levels of blood LDL.[2]

The main principle for the treatment of hypercholesterolemia is to adjust the diet by maintaining normal body weight. Besides, there is pharmacological treatment by medicine such as Simvastatin.[3] Statin group of drugs are the most effective to lower cholesterol by inhibiting the enzyme HMG-CoA (3-hydroxy-3-metiglutaril coenzyme A) reductase. The key side effect of simvastatin is myopathy.[3,4]

Pomegranate *(Punica granatum*) is known to have many health benefits. The peel of Pomegranate contains tannins, anthocyanins, vitamin C and E and catechins that can lower total cholesterol, triglycerides, LDL and blood glucose.[5] Previously conducted in vivo studies showed catechins are able to reduce the oxidation of lipids in plasma.[6]

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The purpose of this study was to compare the effectiveness of pomegranate juice (*Punica granatum*) with simvastatin to decrease LDL levels in the blood of hypercholesterolemia male rats (*Rattus novergicus*).

Research Methodology:

This research was an experimental study that used white male rats (*Rattus novergicus*) of wistar strain. The study was conducted by 60 days following 45-days as induction period and 15 days of treatment. The research was executed in the Laboratory of Research and Testing Integrated-4 (LPPT-4) at the University of Gajah Mada (UGM). Previous research has been approved by the Research Ethics Committee of the Medical Faculty of Medicine and Health, University of Muhammadiyah Yogyakarta with number 017 / EP-FKIK-UMY / I / 2015. 3 months old male rats of Wistar strain having 200-250 grams body weight collected from LPPT -4 UGM used as sample in this study. Usually based on the minimization of feeder formula, each research group is consisted of 6 rats. However, in this study 5 rats having hypercholesterolemia were used. Researchers in this research studied on 3 kinds of groups. Each group consisted of 5 mice tails. Group I was DMSO2% provided control solvent, group II was Simvastatin treated group and the third group was pomegranate (*Punica granatum*) juice group. Pomegranate juice was obtained from the fleshy part of pomegranate that had been separated from its skin and pulverized using a blender without the addition of water. Induction period of this study lasted for 45 days. 15 rats were induced with glucose monohydrate solution that administered orally once a day and a high-fat feed with a ratio of 9: 1 were provided in ad libitum so that the rats would have induced by hypercholesterolemia. In the next 15 days the mice received treatment on the basis of predetermined dose. The first group was given a 2% DMSO solution as 2ml, group II was given simvastatin at a dose of 0.18mg / kg / day previously dissolved using 2% DMSO and group III was given pomegranate juice (*Punica granatum*) with a dose 259.2mg / 200gr BW / day. Treatment was executed continuously 15 full days, once a day at 8 o'clock every day morning.

LDL level of blood was tested on day 0 (pre-test) and day 15 (post-test) during the treatment period. Before blood sampling, firstly rats were anaesthetized using ketamine. In later, blood samples were taken into Eppendorf the blood cholesterol level. Blood LDL level was calculated using precipitation method. The blood cholesterol level in rat over 54mg / dl was considered as hypercholesterolemia.[9]

Analysis of data:

The data were presented in this research in the form of diagrams and tables. IBM SPSS Statistics software 21 was used for analysis. Data were analyzed by one way ANOVA and dependent t-test.

Result of Research:

The purposes of test of blood LDL levels on day 0 and day 15 were to observe the reduction of blood LDL levels before and after treatment at each group and comparison of inter-group effectiveness. The result of the LDL level is presented in the form of diagram 1 in below

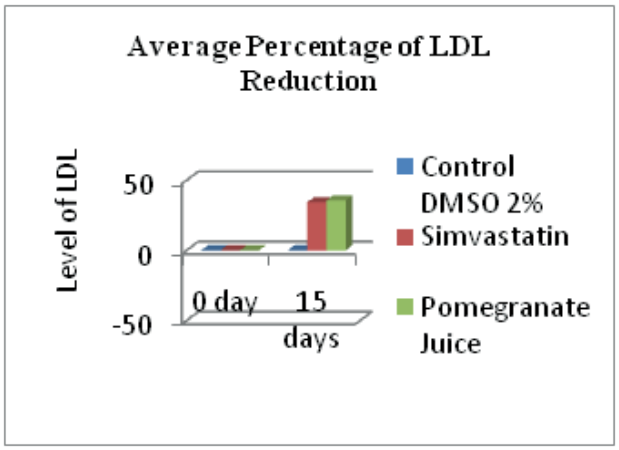

Figure 1 : Average percentage of reduction in LDL levels of rat blood on day -0 and day 15

Data showing percentage reduction of LDL levels were analyzed statistically using One Way Anova. One Way Anova is used where the data are normally distributed and the variance of data is homogeneous with $P > 0.05$. Test of data distribution was done by Shapiro-Wilk variance and Levene test. The distributed and variance data are presented in tabular form in below.

Based on tables 1 and 2, the difference of average reduction of LDL levels does not fulfill the condition for One way ANOVA test because variance of data is not homogeneous ($p = 0.047$).The calculation of the data was followed by transformation test to normalize the data and did not succeed. Therefore, blood LDL level was analyzed with the alternative test, i.e Kruskal Wallis test followed by Mann Whitney test.

The result of Kruskal Wallis test in table 3 shows that there is significant difference in lowering blood LDL level between the groups. The purpose

Table 1: Results of Shapiro-Wilk test

Variable		P Value	Distribution
Reduction of LDL 0-15 days (%)	Control	0.552	Normal
	Simvastatin	0.162	Normal
	Pomegranate Juice	0.097	Normal

Table 2 : Result of Levene test

Variable	P-value	Variance of data
Reduction of blood LDL 0-15 days (%)	0.047	Not homogenous

Table 3: Result of comparative hypothesis testing and numerical variables Kruskal-Wallis test on blood LDL levels.

Variable	P-value	Status
Reduction of blood LDL 0-15 days (%)	0.009	Significant

Tabel 4. Result of Mann Whitney Test

Group	P-value	Status
Control	0.941	insignificant
Simvastatin	0.08	significant
Pomegranate juice	0.011	significant

Tabel 5. Result of T-test on day 0 and day 15

Group	Comparative group	P-value	Status
Control	Simvastatin	0.009	significant
Control	Pomegranate juice	0.009	significant
Simvastatin	Pomegranate juice	0.917	Not significant

of Mann Whitney test was to determine the most distinctive group for lowering blood LDL levels effectively, which is presented rats in table 4.

On the basis of Mann Whitney test results in Table 4 it can be seen that there is significant difference between the control group with simvastatin and the control group with pomegranate juice for the effectiveness of lowering blood LDL levels on day 0 to day 15 as $P < 0.05$. While there is no significant difference in effectiveness between Simvastatin and pomegranate juice group as obtained $P > 0.05$.

The data were analyzed using test T-test to determine the effect of control DMSO 2%, simvastatin and pomegranate juice on the reduction of blood LDL levels in hypercholesterolemia mice before and after treatment.

T- test results on blood LDL levels between day 0 and day 15 showed a significant difference ($p < 0.05$) of blood LDL level in the simvastatin and pomegranate juice group before and after treatment , Whereas in the control group ($p > 0.05$) demonstrated no difference significant of decrease in LDL levels before and after treatment.

Discussion:

In this study, pomegranate juice was made using blender for 2 days treatment. Storage of fresh fruit juices should be stored in cold temperatures. It aimed to provide fresh fruit juice during treatment. Rats were given a dose of pomegranate juice 259.2mg / 200gr body weight or equal to 3.6ml / 200gr body weight.[8]

In this study, t-test result showed that there is significant difference pomegranate juice and simvastatin effectiveness for lowering blood LDL levels before and after treatment. The results of Kruskal Wallis demonstrated that pomegranate juice and simvastatin have distinct effect on lowering blood LDL levels in comparison with 2% DMSO control. While there is no considerable difference between the effectiveness of pomegranate juice and simvastatin.

Antioxidant compounds such as tannins, flovanoid, catechins, vitamin C and vitamin E containing in pomegranate juice might be responsible for lowering blood LDL level. These substances work synergistically. It is known that tannins and anthocyanine contained in pomegranate juice can lower LDL levels by increasing the activity and stability of the enzyme paraoxonase-1 (PON1) in type 2 diabetes mellitus patients.[5] Other studies have shown that catechins in pomegranate juice may inhibit LDL oxidation and decrease lipid peroxidation in plasma.[6] Flavonoids may decrease the risk of atherosclerosis by preventing the oxidation in blood.[8] Regular consumption of vitamin E can lower blood LDL level by disrupting a free radical reactions network..[5,8]

Simvastatin is the standard drug for hypercholesterolemia. Statin is provided to reduce blood LDL levels by inhibiting HMG Co-A reductase. The inhibition of HMG Co-A reductase means HMG Co-A cannot convert into mevalonate. As a result synthesis of cholesterol is inhibited, which leads to a decrease in concentration of

cholesterol in the liver cells and increases the LDL receptor.[3] Simvastatin can lower blood LDL levels effectively, which is supported by previous studies.[11,12] Simvastatin as a positive control were capable of lowering LDL cholesterol levels in hypercholesterolemia induced mice. However, in another study, administration of simvastatin for 21 days was not able to lower the blood LDL levels in hypercholesterolemia mice.[8] The effect of simvastatin therapy has not been tested completely during examination. As it takes at least 4 weeks as hypolipidemic therapy in cases of mild hypercholesterolemia with gradual increased dose.[3] On the basis of above discussion it can be reported that pomegranate juice and simvastatin have the same effectiveness of lowering blood LDL level in hypercholesterolemia white male rats (*Rattus novergicus*) in comparison with 2% DMSO.

However, this study still has shortcoming such as variance of dose given on pomegranate juice was limited to one dose, so it is need to execute more research related to the optimization of dose that can be given to human.

Conclusion and Suggestion

Conclusion:

From this study it can be concluded that pomegranate juice (*Punica granatum*) and simvastatin have the same effectiveness in lowering blood LDL hypercholesterolemia white male rats (*Rattus novergicus*). Besides, based on the inter-group comparison the efficacy of pomegranate juice and simvastatin found no significant difference.

Suggestion:

In future juice or extract can be made from various active compound contained part of pomegranate tree such as fruit, seeds, leaves, stems, flowers, leaves or roots, which can be given to hypercholesterolemia white male rats at various doses for lowering blood LDL level.

Conflict of interest: None declared.

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