Soy and Dragon Fruit Functional Drink: The Potential to Reduce Cholesterol Total Levels in Hypercholesterolemic Sprague Dawley Rats

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Abstract: Hypercholesterolemia is a lipid metabolism disorder characterized by increased blood cholesterol levels. The alternative functional drinks that can reduce cholesterol levels are beverage ingredients that contain flavonoids, such as red dragon fruit and soybeans. Flavonoids can inhibit cholesterol synthesis by inhibiting the HMG-CoA reductase enzyme. Soybeans are also have a hypocholesterolemic effect because they contain isoflavones. This study aimed to determine the potential of soybean and dragon fruit peel powder on the cholesterol levels of hypercholesterolemic Sprague Dawley rats. This study was a true-experimental design with a pretest-posttest with control group. The samples were 27 male Sprague Dawley rats, weighing 150-250 grams and aged 2-3 months. The samples were divided into a control group and a treatment group that was given soybeans dragon fruit peel powder drink at a dose of 12.8 ml/kg BB/day for 14 days. Total cholesterol levels were measured using the CHOD-PAP method. Data were analyzed using the One Way Anova test, Post Hoc or Man Whitney test and Paired T-test. The results showed that there was a significant difference in total cholesterol levels in mice before and after the intervention (p = 0.003). Giving soybeans dragon fruit peels powder drink can significantly reduce total cholesterol levels in the treatment group (P) by 18.45% (p = 0.044).

Keywords: Hypercholesterolemia, Soybeans-Dragon Fruit Peel Powder, Total Cholesterol

1. Introduction

Hypercholesterolemia or commonly called dyslipidemia is defined as a disorder of lipid metabolism which is characterized by an increased and decrease in levels of lipid fractions in the plasma. The main lipid fraction abnormalities are increased of the level of total cholesterol, LDL, triglycerides, and the decrease of HDL [1,2]. The prevalence of dyslipidemia can be identified through a high level of total cholesterol among the residents aged more than >15 which was as big as 21.2%, low HDL by 13.8%, high borderline LDL by 24.9% while the highest category reached 3.4%. This was also indicated by a high borderline level of triglycerides by 13% and the category from high to the highest reached 13.85 [3].

The abnormal lipid level can disturb the metabolism of cholesterol which further may cause the accumulation of cholesterol in hepatocytes. This kind of buildup will be difficult to be transferred by lipoprotein to liver and arteries. The continued cholesterol accumulation will boost the cholesterol synthesis [4]. Hypercholesterolemia can be the main factor of degenerative diseases, one of which is Coronary Heart Disease (CHD)[2].
Someone suffering from hypercholesterolemia have 6.479 times bigger risks of being suffered from CHD than those who do not suffer from dyslipidemia [5].

Dyslipidemia can be tackled by giving a pharmacology and non-pharmacology treatments. Pharmacological therapy was given through drugs, while non-pharmacological therapy is through controlling intake, such as giving an antioxidant. This compound is believed to be able to lower a high level cholesterol or the so-called hypercholesterolemia [6]. Antioxidant is such a free-radical scavenger which can be found in soy [7].

Soy is one beans which is worth to process and produce various kinds of foods and drinks consumed by people in general since the soy commodity in East Java is considered high as big as 35.82% [8]. Soy is proven to be able to decrease the level of total cholesterol since it contains high isoflavones, an antioxidant content, as big as 213.5 mg/100 gr. The isoflavones works as an antioxidant with hypercholesterolemia effects. Besides, it can lower the level of triglycerides as well as increase the HDL level [9]. Soy is one of food ingredients which has the potential of lowering the level of total cholesterol [10].

Flavonoid is one of phenolic compound which is part of anthocyanin antioxidant originally from plant pigments which can protect human body from free radical attacks [11,12]. Flavonoid compound can be found in fruits with red purplish color pigment such as dragon fruit. Dragon fruit is popular among Indonesians today. This can be proven through the increasing number of consumption level of dragon fruit among Indonesians [13]. The increased numbers of consumption of red dragon fruit will badly affect the environment due to the wastes of the peels. Thus, the recycling and processing of dragon fruit peel becomes functional food ingredients needs to be maximized in order to decrease the number of the waste [14].

The peels of red dragon fruit have stronger antioxidant activities than white dragon fruit. The antioxidant activities from the two types of dragon fruits was evident from IC50 score of the red dragon fruit (76.19 µg/mL) which is lower than the IC50 score of white dragon (101.75 76.19 µg/mL) [15]. The peels of red dragon fruit has bigger antioxidant activities than the flesh so that the peels of the red dragon fruit can be used as a therapy materials to block the oxidation reaction of free radicals which are potentials in decreasing the high level of total cholesterol [16].

According to the previous research [17], the making of soymilk added by the extract of red dragon fruit (Hylocereus polyrhizus) will affect the antioxidant from the soymilk. The more the extract of red dragon fruit added in the milk, the higher the antioxidant content in the soymilk. Besides, adding soybean powder which was then extracted for 4.5 g/kgBB/day for 14 days can lower the level of total cholesterol of Wistar rats which had been induced by MSG although it was not significant [18]. The product combining the powder of soybeans red dragon fruit and soybeans can create a functional drink with high fiber and antioxidant. The more the powder of red dragon fruit added in the combination formula, the higher the fiber and antioxidant content in the product [19].

Based on the analysis of the previous studies, the researcher intended to conduct a research on the effect of giving a combination functional drink made of soymilk combined with the powder of red dragon fruit peels towards the level of total cholesterol of male Sprague dawley rats with dyslipidemia. The soymilk is needed to be combined with the powder of red dragon fruit peels to improve the color of the products that will be produced. The novelty of this research is the innovation of a powdered drink product that combines soybeans with dragon fruit peels. It is expected that the powder of red dragon fruit peels added in the products will make it more appealing besides its functional advantages which is rich with the antioxidants. The product of this study will be processed in the form of powder. It aims to minimize the level of water content so that it can make the shelf life longer [20,21].
2. Materials and Methods

This is a true experimental research. The research design used was Pretest-Posttest with control group design with random sampling. The research samples were male Sprague Dawley rats. Rats were divided into three groups randomly, namely negative control group (K-), positive control group (K+) and treatment group (P). Furthermore, an initial observation of total cholesterol levels (pre test) was carried out in the three groups, followed by giving Rat Bio standard feed intervention of 20 grams in the negative control group (K-), high fat feed 2ml/day and Propilthouracil 10 mL/kgBW/day in the positive control group (K) and high fat feed 2ml/day, Propylthouracil 10 mL/kgBW/day and powdered drink made from soybeans and dragon fruit peel as much as 12.8ml/kgBW/day in the treatment group (P). After a few days, the final examination of total cholesterol levels (post-test) was carried out in the three groups as shown in Figure 1.

![Figure 1](https://via.placeholder.com/150)

**Figure 1.** Experimental research with pre test-post test design with control group

This research was conducted in 2020 at the Biomedical Laboratory of Faculty of Dentistry of Jember University (a place for experimental care and use), Jember Prosenda Laboratory (a place for testing the analysis of total cholesterol level), and Food Processing Laboratory of Health Department, Jember State Polytechnic (a place for producing the powder drink of soybeans and dragon fruit peels. There were 27 rats in the research sample and had to meet the inclusion criteria, namely 2-3 months old, weighing 150-250 grams, being male and having normal cholesterol levels <90 mg/dl. The observation parameter is the total cholesterol level of the Sprague dawley rats which was measured by CHOD-PAP method from 27 rats in total which were interfered by soybeans dragon fruit peels powder as much as 12.8 ml/kgBW/day for 14 days. The analysis result from measuring the total cholesterol level was delivered in the form of table. The data analysis was conducted by ratio data scale with SPSS v16.0 by using normality test. If the data were normally distributed, it was continued with One Way Annova test with significant level \( \alpha = 0.05 \). If there was a significant different, it was continued with Post Hock Test. Mann-Whitney and Paired T-Test. If the data were not normally distributed, it was continued with Kruskall-Wallis test with significant level \( \alpha = 0.05 \). If there was a significant difference, the Mann-Whitney test is continued and the Paired T-test is carried out.

3. Results and Discussion

This research involved 27 normal rats which were divided into three groups, negative control group (K-), positive control group (K+) and treatment group (P). The negative control group (K-) were only given standard Rat Bio food. Meanwhile, the positive control group (K+) and treatment group (P) were induced with high-fat diet and Propylthouracil (PTU), resulting in a hypercholesterolemia rats model. Furthermore, the treatment group (P) was given a drink of soybean powder and dragon fruit peel to reduce total cholesterol
levels. Total cholesterol levels were observed three times, namely before induction (T0), before intervention (pre-test) and after intervention (post-test).

3.1. Analysis of Total Cholesterol Level before Induction (T0)

The result of normality test revealed that the data were normally distributed. Thus a parametric test was conducted by employing One Way Anova Test resulting in p = 0.612 (p > 0.05) which can be seen through Table 1.

Table 1. Total Cholesterol Level before Induction (T0).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD (mg/dl)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (K-)</td>
<td>80.5 ± 9.47</td>
<td></td>
</tr>
<tr>
<td>Positive control (K+)</td>
<td>74.8 ± 9.92</td>
<td>0.612</td>
</tr>
<tr>
<td>Treatment (P)</td>
<td>78.2 ± 13.93</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Based on the result of One Way Anova test, there was no significant different between the groups (p = 0.612) implying that the rats used as the sample of this research were healthy or possessing a normal level of total cholesterol that was < 90 mg/dl [22].

3.2. Analysis of Total Cholesterol Level before Intervention (Pre-Test)

The result of normality test of the pre-test of total cholesterol level was normally distributed (sig p > 0.05) so that it could continue to One Way Anova Test with significant level p < 0.05. The test result of One Way Anova can be seen on Table 2.

Table 2. Total Cholesterol Level before Intervention (Pre-Test).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD (mg/dl)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (K-)</td>
<td>92.87 ± 22.44</td>
<td>0.022*</td>
</tr>
<tr>
<td>Positive control (K+)</td>
<td>90.25 ± 19.09</td>
<td></td>
</tr>
<tr>
<td>Treatment (P)</td>
<td>113.88 ± 17.66</td>
<td></td>
</tr>
</tbody>
</table>

*One way anova, significant p<0.05.

Table 2 showed that the average total cholesterol level in the treatment group (P) was higher than the negative control group (K-) and the positive control group (K+). The results of one way ANOVA statistical analysis showed that there were differences in pretest total cholesterol levels in the three groups (p < 0.05). Furthermore, based on the result of the Post Hoc test, it was revealed that a group which had a significant difference was the negative control group (K-) with treatment group (P). Another significant different was also found on the treatment group (P) and positive control group (K+).

Based on the observation result of the total cholesterol level before given the intervention, the negative control group (K-) has a total cholesterol level which was higher than the positive control group (K+). This was unexpected as the negative control group (K-) was only given rat food pellets that is Rat Bio “Citra Feed”. 20 grams of Rat Bio consist of 12 g protein and 4 g fat. 4 grams fat in it is considered as a high fat food for rats as the normal content of high fat food for rats contains 3.5 grams fat [23], [24]. High fat consisted in Rat Bio was indicated as the main factor of the increased total cholesterol level among the rats in the negative control group (K-).

Stress factor also becomes the indication of the high level of total cholesterol in the negative control group (K-). Stress can increase free fatty acids and VLDL secretion which further may impact to the increase of total cholesterol level [25]. This becomes the reason of the irrelevant result between the negative (K-) and positive (K+) control groups. Similar
results were also found on the difference between the positive control (K+) and experimental (P) groups. The total cholesterol level of the rats in each group before the treatment showed that both groups had high total cholesterol level that was >90 mg/dl. This is in line with the expected outcomes by the researcher where a high fat diet and PTU induction were given for the consecutive 28 days until finally made the rats hypercholesterolemia.

3.3. Analysis of Total Cholesterol Level after Intervention (Post-Test)

The result of normality test of the total cholesterol level after intervention (post-test) showed that the distribution was normal (sig \( p > 0.05 \)) so that it could be tested by using One Way Annova on the significant level \( p < 0.05 \). The result of One Way Annova test can be seen through the following Table 3.

Table 3. Total Cholesterol Level after Intervention (Post-Test).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD (mg/dl)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (K-)</td>
<td>89.25 ± 11.00</td>
<td></td>
</tr>
<tr>
<td>Positive control (K+)</td>
<td>90.37 ± 11.62</td>
<td>0.801</td>
</tr>
<tr>
<td>Treatment (P)</td>
<td>92.87 ± 10.58</td>
<td></td>
</tr>
</tbody>
</table>

The observation result on the level of total cholesterol after the intervention (post-test) on soybeans dragon fruit peels powder drink by using One Way Annova test obtained a score of \( p=0.801 \) (sig. \( p < 0.05 \)). It is implied that there was no difference on the level of total cholesterol between groups.

The analysis result on the level of total cholesterol after the intervention showed no significant difference between groups since on the negative control group (K-), the total cholesterol level unexpectedly reduced. This was caused by appetite loss among the members in negative control group (K-) during the intervention. The rat food pellets used was Rat Bio given to negative control group (K-) containing 60% of carbohydrate. Food containing high carbohydrate can increase leptin hormones which is then able to reduce the appetite [23],[24].

3.4. Difference Analysis on Total Cholesterol Level on Pre- and Post-Test of Each Group

The observation data on the total cholesterol level on pre- and post-test were analyzed statistically by using Shapiro Wilk normality test. It was obtained a significant value \( p > 0.05 \) which means the average of total cholesterol level before and after the interventions were normally distributed. The test was continued by using Paired T-Test. The test result on the difference of total cholesterol level on pre- and post-test is delivered in Table 4.

Table 4. Difference of Total Cholesterol Level on Pre- and Post-Test of Each Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-Test (mg/dl)</th>
<th>Post-Test (mg/dl)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (K-)</td>
<td>92.87 ± 22.44</td>
<td>89.25 ± 11.00</td>
<td>0.681</td>
</tr>
<tr>
<td>Positive control (K+)</td>
<td>90.25 ± 19.09</td>
<td>90.37 ± 11.62</td>
<td>0.988</td>
</tr>
<tr>
<td>Treatment (P)</td>
<td>113.88 ± 17.66</td>
<td>92.87 ± 10.58</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

*Paired T-Test (Sig < \( \alpha \); \( \alpha = 0.05 \)).

The results of pre- and post-test on the total cholesterol level of all groups were decreasing. On negative control group (K-) the test result revealed that there was no significant difference which was marked with the score of \( p=0.681 \). This result was triggered by the declining total cholesterol level among the rats which was insignificant.
The rats in negative control group were only given the rat food pellets that was Bio Rats during the intervention and not given a high fat diet. On the other hand, the result obtained from positive control group (K+) demonstrated an insignificant difference which was indicated by the score of p=0.988. This result was caused by a diet includes high fat which was delivered in the form of quail egg yolk and PTU, so that the results in Table 4.5 exposed the average total cholesterol level in the pre- and post-test increased from 90.25 mg/dl to 90.37 mg/dl. This was in line with the expected outcomes in which rats in the control group should not perform differently before and after the intervention.

The test result in the experimental group (P) showed that there was a significant difference between the total cholesterol level before and after the intervention of powder drink of soybeans and dragon fruit peels. The score of p=0.003 justifies the significant changes on the total cholesterol level during the intervention of supplying the powder drink of soybeans and dragon fruit peels. This is in line with a previous research, the intervention of soy milk added with the extract of red dragon fruit peels (Hylocereus polyrhizus) which can affect the antioxidant from the soy milk [17]. Besides, giving 4.5 g/kgBB/of the extracted soybean powder for 14 days could lower the total cholesterol level of Wistar rats [18].

The downturn of the total cholesterol level among the rats after given the intervention of powder drink of soybeans and dragon fruit peels was caused by the content of flavonoid in the peels of red dragon fruit and isoflavones in the soybeans. Flavonoid is an antioxidant compound which can lower the total cholesterol level by blocking the activities of HMG-CoA reductase enzyme in changing HMG-CoA to mevalonate [26]. The isoflavones contained in the soybean powder (soya) works as the antioxidant with hypocholesterolemia, lowers the triglyceride level, and increases the HDL level [9]. The content of β-conglycinin protein compound (7S globulin) in the soybean powder could also hinder the absorption of total cholesterol. The protein plays a role in increasing the secretion of bile acid and preventing cholesterol absorption so that the total cholesterol level will cut down. β-conglycinin protein compound (7S globulin) in soybeans can scale down the biosynthesis process of cholesterol by lowering the regulation of HMG-KoA enzyme [27].

### 3.5 Analysis of Differences on Total Cholesterol Levels Pretest and Posttest

The normality test of observation data on total cholesterol level after the intervention employed Shapiro Wilk test. The analysis result of the total cholesterol level after the intervention in the negative control group (K-) (p=0.006), positive control group (K+) (p=0.470), experimental group (P) (p=0.206) showed a datum which was not normally distributed that was in the negative control group (K-). The homogenity test using Lavene test obtained an analysis result of p=0.526 which implies a similarity or homogeneity of data variant in each group. The test result had fulfilled the requisites of non-parametric analysis so that it was continued to be tested by using Kruskall Wallis test with the following results:

<table>
<thead>
<tr>
<th>Group</th>
<th>Difference on Pre- and Post-Tests (mg/dl)</th>
<th>Changes Percentage (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (K-)</td>
<td>3.62</td>
<td>3.89</td>
<td></td>
</tr>
<tr>
<td>Positive control (K+)</td>
<td>-0.12</td>
<td>-0.13</td>
<td>0.044*</td>
</tr>
<tr>
<td>Treatment (P)</td>
<td>21.01</td>
<td>18.45</td>
<td></td>
</tr>
</tbody>
</table>

*Kruskall Wallis-Test (Sig < α; α = 0.05).

According to the test result above, the groups who were significantly different are negative control group (K-) and the experimental group (P). A significant difference was also found on positive control group (K+) and the treatment group (P). Based on the data
it can be revealed that there is a difference gap between the control negative and experimental groups. It is not in accordance with the hypothesis. This shows that the reduction in total cholesterol levels in the treatment group did not match the total cholesterol levels in the negative control group.

The calculation result of the total cholesterol level before the intervention (pre-test) and after the intervention (post-test) obtained a percentage of the decrease of total cholesterol level. The percentage of total cholesterol level implied an unexpected decrease in the negative control group (K-). The decrease and increase of total cholesterol level may be caused by two factors namely endogenous (hormonal) and exogenous factors coming from food supply. The hormonal factor becomes the indication causing the decrease of total cholesterol level on the negative control group (K-). Estrogen hormone is a hormone affecting the cholesterol homeostasis. The increased estrogen hormone can also increase the regulation function of mitochondria in liver functioning in shaping and doing the excretion of lipoprotein. Stress among the experimental animals during the research may become the main reason behind the unexpected decrease and increase of total cholesterol level. Stress can influence the metabolism process of lipid and on rats’ bodies due to the imbalance of glucocorticoids hormones which also plays role in processing lipolysis [28].

4. Conclusions
There was a significant difference in total cholesterol levels before and after the intervention (p<0.05) between the control group, both positive control and negative control, and the treatment group. The percentage change in total cholesterol levels before and after being given the soybean and dragon fruit peels powder drink in the treatment group was 18.45%. Giving soybeans dragon fruit peels powder drink can reduce total cholesterol levels in the treatment group, but the reduction in cholesterol levels has not reached that of the negative control. In conclusion, the soybeans dragon fruit peels powder drink which contains 562 mg/100 grams of flavonoids and 571.6 mg/100 grams of phenols has the potential to reduce total cholesterol levels

6. Acknowledgments
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