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The Effect of Ketapang Leaf Extracts (*Terminalia catappa* L.) on the Cholesterol Levels of Male Mice (*Mus musculus* L.) Hypercholesterolmia

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Abstract— *Cholesterol is a useful substance for the body to regulate chemical processes such as building cell membranes, producing vitamin D, and forming steroid hormones. Hyper-cholesterol is a condition where cholesterol levels increase over the normal range (>200 mg/dL). One of the herbs which can be used as a cholesterol-lowering substance is Ketapang leaf. The leaf has several compounds which function as antioxidants. Based on Phytochemical test, this leaf contains flavonoid, saponin, tritepen, diterpen, phenolic, and tannin. Those compounds function to inhibit cell damage. This study was aimed to determine the effect of Ketapang leaf extracts (Terminalia catapa L.) to decrease cholesterol levels of hypercholesterolemia of male mice (Mus musculus) which are induced with lard. The study employed experimental design involving 25 male mice, aged at 2 monthsold and weighing 20-30 grams. These mice were divided into 5 groups, comprising of treatment 1 (2 mg/ 20 grams of weight), treatment 2 (4 mg/ 20 grams of weight), treatment 3 (8mg/ 20 grams of weight), positive control (simvastatin 0,26 mg/kg of weight), and negative control (CMC Na 1%). The extract was given for 14 days with 1% volume of weight. The measurement of blood cholesterol levels was done on day 8 as the preliminary data, day 14 after hypercholesterolemia of male mice, day 21 and day 28 after giving Ketapang leaf extracts. The dosage of Ketapang leaves extracts which was given during the treatments significantly affected (p=0,0043) the average of blood cholesterol levels of male mice. Giving the leaf extracts for two months of treatments was found successful in decreasing blood cholesterol 157,62 ± 17,54; 341,55 ± 12,73; 196 ± 16,32; 196,17 ± 16,93 mg/dL.*

Keywords— Cholesterol, Hypercholesterolemia, Ketapang Leaf Extracts, Male Mice.

I. INTRODUCTION

Cholesterol is a useful substance for the body to regulate chemical processes such as building cell membranes, producing vitamin D, and forming steroid hormones. However, for some of cardiovascular cases, such as coronary heart, the blood cholesterol levels are crucialin the emergence of such disease[1]. Changing of lifestyles in society refers to fast foods, fast services, flavored spice foods, and chosen food varieties has influential bearing on cholesterol-related disease. Those foods actually do not contain high nutrients for the body, but its contentshave high cholesterol instead[2]. Modern dietary habit containing more cholesterol which complemented by high intensity of eatings and stresses pressing duringthe whole day also cause uncontrolled levels of cholesterol on blood[3].

Based on the data from The World Health Organization (WHO) in 2002**,** 16.7 million people died due to cardiovascular cases. The number of cases reached 17.3 million people in 2008, and the estimated number is projected to increase to 23.3 million people in 2030[4]. The case of cardiovascular and blood-vessel diseasesis influenced by many factors, one of which is hypercholesterolemia. It is the condition where blood cholesterol levels rise over normal range (>200 mg/dL)[5]. The total Cholesterol levels can be affected by nutrient intake, specifically foods containing high fat. Besidethe high fat consumtion, the hyper-cholesterol condition due to heredity, less exercises, and smoking habit factors[6]. Accordingly, the availability of herbal alternative medicine for lowering the cholesterol levels is necessary.

Baluran National Park, East Java, Indonesia, is a rich natural conservation area of various flora and fauna resources. It has various kinds of forest ecosystems in a same area: season forest, evergreen forest, coastal forest, ecotone, mangrove forest, and grassland. Evergreen forest is rain forest of tropical lowlands which are normally foundin such areasas coastalregions to mountainous ones. The plant soriginating from this kind of areasdonot possess too hightrunk, but very hard wood. Evergreen forest provides various plants havingrich substances which are usefulin health domains. One of these plant is Ketapang (*Termaliacatappa L.*) [7].

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Ketapang leaves (*Termaliacatappa L.*) contain flavonoid, saponin, tritepen, diterpen, fenolik compound, and tannin. These leaves possess secondary metabolite which has antioxidant compounds[8]. Flavonoid is one of polyphenol compounds having various effects such as antioxidants, antitumor, anti-inflammatory, antibacterial, and anti-virus[9]. This study has revealed that Ketapang leaf extracts can decrease cholesterol levels of the hypercholesterolemia male mice[10].

II. RESEARCH AND METHOD

Mice which were used in the study were male mice, aged 56-70 days oldand weighing 20-30 grams. The mice were acclimatizedfor seven days to adaptto the environment.

The creation of hypercholesterolemia conditionon mice was done by inducing lard orally as much as 1% of the mice weight. For example, a mouse weighing 30g was given 0,3ml lard, and it was given for seven days from day 8 to day 14.

In the production of Ketapang leaf extracts (*Termaliacatappa L.*), the samples were taken from Baluran National Park, Situbondo, East Java, Indonesia. The selected leaves were dried, and then they were crushed using a blender to generate Ketapang powder (*Termaliacatappa L.*). The powder was then extracted by using 96% ethanol and further the Ketapang extract was given to the mice for 14 days: from day 14 to 28.

The medications for decreasing cholesterol levels in this study used10 mg simvastatin. The dosage for adults (70kg) was 10mg/day; hence, the simvastatin dosage for the mice (20 grams) was 10 x 0.026 = 0.26/mg/day/20g of weight (0.026 was the conversion factor of human to mice)[11]. Simvastatin was the positive control which was given for14 days, especially on day 14-28[12].

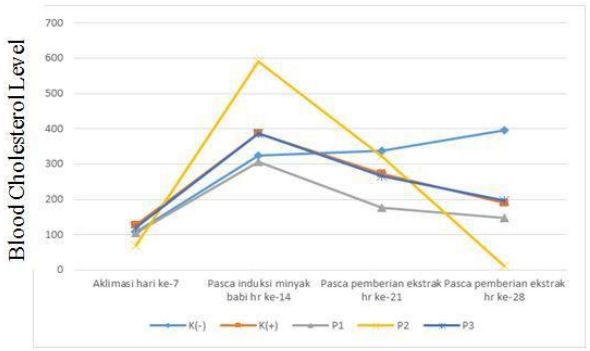
The measurement of cholesterol levels was done by using cholesterol-level measuring device, namely glucometer. The measuring was completed by making an injury on the point of mice's tail, and then the blood was dripped on cholesterol strips and glucometer would show thenumeric scale indicatingthe cholesterol level. This measuring was done on day 8, 14, 21, and 28.

The data analysis was executed by using one-way anova with a credibility level of 95%, and it was followedby duncan test at 5% significant level.

III. RESEARCH RESULT

The Measurement Result of Cholesterol Level of Male Mice (*Termaliacatappa L.*)

The research result revealed that the measurement of cholesterol levels for the all treatments decreased, except cholesterol level for the negative control group increased. The research result is drawn on Picture 1.



Picture 1. The Graph of Average Decrease on Cholesterol Level

Note:

C (-) = 1% CMCNA; C (+) 0.26 mg/20 g body weight Simvastatin;

T1 = 2 mg/20 g body weight extract of Ketapang leave; T2 = 4 mg/20 g body weight extract of Ketapang leave; T3 = 8 mg/20 g body weight extract of Ketapang leave.

The research execution indicated an average decrease of the cholesterol levels in each treatment. The various average decrease of cholesterol levels are put in Table 1.

Table.1: The Average and Standard Deviation of Decrease on Cholesterol Level

Treatment	Average ± Standard Deviation of Decrease (mg/dL)
C (-)	-73.33 ± 05.47
C (+)	196.17 ± 16.93
T1	157.62 ± 17.54
T2	341.55 ± 12.73
T3	196.00 ± 16.32

Note:

C (-) 1% CMCNA

C (+) 0.26 mg/kg body weight Simvastatin

T1 = 2 mg/20 g body weight extract of Ketapang leave

T2 = 4 mg/20 g body weight extract of Ketapang leave

T3 = 8 mg/20 g body weight extract of Ketapang leave.

As seen in Table 1, it is clear that the highest average decrease on cholesterol level is evident in T2 (4 mg / 20 g body weight extract of Ketapang leave), which reaches 341.55 ± 12.73 mg/dL. Treatment group of T3 (8 mg/20 g body weight extract of Ketapang leave) indicates a figure of 196.00 ± 16.32 mg/dL. This result is almost equal with the positive control group's. Treatment group of T1, 2 mg/20 g body weight extract of Ketapang leave, has the lowest average decrease on cholesterol levelfor about 157.62 ± 17.54 mg/dL. Treatment Group, which indicates an increase in cholesterol level for about -73.33 ± 05.47 mg/dL, is C (-) 1% CMCNA.

The data of normality test evinced extract provision of p = 0.892 evident on the decrease of cholesterol level on day 28. A figure of p = 0.451 was representative of

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homogeneity test. In accordance with the aforementioned data, with the statistical analysis of $p > 0.05$, it indicated that the obtained data could proceed to further analysis by ANOVA test.

Referring to ANOVA test result, the analysis indicated significant value of $p = 0.408$ ($p < 0.05$). Owing to this result, the provision of Ketapang leave extract on high-cholesterol mouse yielded significant influence on the decrease on cholesterol level. The ANOVA test revealed that all treatment groups had significant differenceto negative control. As a result, it allows further analysis by means of Duncan test.

Table.2: The Result Test on The Average Influence of Ketapang Leave Extract on The Decrease on Cholesterol Level

Treatment	Average ± Standard Deviation of Decrease (mg/dL) on day 28
C (-)	-73.33 ± 05.47a
C (+)	196.17 ± 16.93b
T1	157.62 ± 17.54b
T2	341.55 ± 12.73b
T3	196.00 ± 16.32b

Based on Table 2 on Duncan Test, Group C (-) has meaningful different to C (+), T1, T2, and T3. C (+) group shows no meaningful difference to T1, T2, and T3. It proves that C (+), T1, T2, and T3 have nearly similar characteristics in lowering cholesterol level.

IV. DISCUSSION

The research finding indicated that the treatment groups, C (+), T1, T2, and T3 exerted significant influence on the decrease on cholesterol level, except on treatment group C (-). It indicated an opposing result that there was increased cholesterol level in the blood. That increase occurred in as much as there was no provision of cholesterol medicine or Ketapang leave extract.

As regard with the average decrease of sugar level in each treatment, the data indicated the following results: positive control of 196.17 ± 16.93, negative control of 196.17 ± 16.93, treatment 1 of 157.62 ± 17.54, treatment 2 of 341.55 ± 12.73, and treatment 3 of 196.00 ± 16.32. Referring to the aforementioned data, the experiment revealed that, on the highest-to-smallest scale, the average decreases comprised of Ketapang leave extract of 4 mg/20 g body weight in treatment 2, positive control, Ketapang leave extract 8 mg/20 g body weight in treatment 3, and Ketapang leave extract 2 mg/20 g body weight in treatment 1.

This research discovered disclosed that treatment 2 possessed the maximum potential in decreasing cholesterol level compared to treatment 1and treatment 3. Based on the average of cholesterol level measurement, the research indicated that treatment 3had the optimum result inreducting the sugar level. This feature resembled the performance of positive control in decreasing the cholesterol level. This showed that the provision of 8 mg/20 g body weight Ketapang leave extract had the same function as Simvastatin 0.26 mg/kg body weight.

On treatment 2, the mice's cholesterol level was easy to fluctuate. This was due to the presence of steroid hormone which was influential in decreasing cholesterol [16]. The different existence of steroid hormone on every individual triggered the physiological sensitivity in receiving suspensions given during the research. This caused significant increase in mice's cholesterol level when the induction of lard was operated, which was also representative of the provision of Simvastatin, resulting in experimental animal undergoing significant decrease. This finding was also corroborated by the physical features of experimental animal 2, marked by the enlargement of prostate. The research indicated that the instability of steroid hormone in the body had significant influence on volatile cholesterol level. Moreover, that volatility was indicated by enlarged prostate, resulting in prostate cancer[17].

Ketapang extract leave had the potential to trigger cholesterol level since the research finding indicated the leave extract could bring down cholesterol level of experimental mice under hypercholesterolemia condition. That was due to the presence of antioxidant compounds preserved in the leave extract. These compounds, which had the potential to reduce cholesterol level, included flavonoid, phenol, tannin, and fenol[8]. In medical realm, sinamaldehid compound, which was the subordinate from phenol compound, was known to possess platelet anti-aggression feature and function as similar vasodilator[12] via invitro. Flavonoid is one of phytochemical division having the same structure known as polinenol. Abundance of research claimed that flavonoid could reduce the risk of cardiovascular disease since it played essential role in lipid metabolism[13]. Flavonoid mechanism in reducing total cholesterol level was germane to decreasing the activity of HMG-KoA reductase, reducing the activity of *cyl-CoA* enzyme cholesterol acyltransferase (ACAT), and lowering cholesterol abortion in digestion system [14]. Tannin was used as antioxidant in lipid and tanning compound could precipitate protein mucosa existing in the small intestine surface which caused reduction on food absorption. As such, tanning compound could aid in reducing food lipid absorption, which as a corollary reduced the work of liver cell in synthesizing lipid [15]. Under such circumstance, tannin, flavonoid, and phenol possibly worked together in controlling cholesterol level in the blood. Accordingly, in hypercholesterolemia condition the mice's cholesterol level decreased.

The positive control employed in this research was Simvastatin. It is was given orally on daily basis during the

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treatments. Simvastatin is a medicine from statin class which has superior in some aspects. First, this medicine is *Generik*-available in Indonesia, meaning it is available and quite cheap. Additionally, the medicine has been reliably consumed in society for more than 20 years. Second, based onresearches documented in Cardiovascular Disease Book called Braunwalds, Simvastatin can decrease cholesterol level by 20% and the risk of blood-vein disease by 24% when given at a do sage of 40mg/day. The mechanism of Simvastatin is reducing lipid by means of inhibiting 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase. HMG-CoA reductase releases the precursor of mevalonic acid cholesterol from coenzyme A. Simvastatin'scompetitive inhibition affect cellular compensation response such as the escalation of HMG-CoA reductase enzyme and Low Density Lipoprotein (LDL) [16] receptor.

In the Duncan test operation, the negative control group had meaningful difference from positive control group, treatment 1, treatment 2, and treatment 3. That difference was due to the escalated cholesterol level on negative control group, while on the other control groups was found decreased cholesterol level. The extract control groups T1, T2, and T 3 had no significant differences as every treatment group had almost same skills and activities to decrease blood cholesterol level. There fore, it can be concluded that T1, T2, and T 3 groups have compounds potential in lowering cholesterol level.

Based on the accomplished research, the effect of *Ketapang* (*Terminalia catappa L.*) leaves extract on cholesterol level was not merely due to the impact of such compounds as tannins, flavonoids and phenols, but also the presence of other compounds which had not been researched. Therefore, a further research is still needed to obtain the other maximum effects of *Ketapang* leaves as one of the effective natural alternative medicines to decrease blood cholesterol level in the hypercholesterolemia condition.

V. CONCLUSION

The provision of *Ketapang* leaves extract with concentrations of 2 mg/20 g weight, 4 mg/20 g body weight, and 8 mg/20 g body weight was influential in decreasing blood cholesterol level in male mice significantly by the negative control.

The extract concentration of 4 mg/20 g body weight had a maximum potential in decreasing blood cholesterol level with an average decrease of 341, 55 ± 12.73 mg/dL. The extract concentration of 8 mg/20 g body weight had the optimum potential in decreasing blood cholesterol level with an average decrease of 196 ± 16.32 mg/dL.

It is essential to carry out a further research on the *Ketapang* leave compound which has crucial affects in decreasing the male micecholesterol level. The intended research using a rat *(Rattus novergicus* L.) in the experiments is still called upon.

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