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In Vivo Antihypercholesterolemic Potential of *Uncaria cordata* (Lour.) Merr as Ethanolic Extract

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ABSTRACT

The present investigation aims to evaluate the antihypercholesterolemic potential of *Uncaria cordata* (Lour.) Merr. as an ethanolic extract in diet-induced hypercholesterolemic male white mice model. In this study, white mice were segregated into 6 groups; all the groups except the normal control group were given a high-fat diet to induce hypercholesterolemia. After induction of cholesterolemia, normal and negative control groups were treated with NaCMC, the positive control group was treated with atorvastatin, and the remaining three groups received ethanolic extract *Uncaria cordata* (Lour.) Merr. in three doses (100, 200, and 400 mg/Kg BW) for a treatment period of 29 days. Measurement of cholesterol levels was performed on days 0, 15, 22, and 29 using EasyTouch® GCU digital devices. The results were analyzed by one way ANOVA test and ANOVA Repeated test. The results showed that the ethanol extract of the *Uncaria cordata* (Lour.) Merr. root plant dose 100, 200, and 400 mg/Kg BW effect in lowering total cholesterol in male white mice significantly ($P < 0.05$). Ethanol extract from *Uncaria cordata* (Lour.) Merr. a dose of 200 mg/Kg BW showed a better decrease in cholesterol levels on day 29 compared to day 22.
Keywords: *Cholesterol; Flavonoid; Uncaria cordata* (Lour.) *Merr*.

INTRODUCTION

Cholesterol is one of the plasma lipid compounds found in tissues in the form of plasma lipoproteins. Cholesterol can be in a state of free cholesterol or combined with long-chain fatty acids, forming cholesterol esters. These compounds have an important role in the plasma membrane and also as precursors of adrenal cortex hormones and vitamin D and bile acids (Murray *et al*, 2009).

Hypercholesterolemia is a condition that signed with Increased cholesterol levels in the blood and this is the main factor of atherosclerosis associated with coronary heart disease which can lead to many cases of death (Muhtadi *et al*, 2013).

Normally, the body produces the right amount of cholesterol, but the tendency to consume animal foods with high fat can trigger excess cholesterol in the blood (Bertram, 2010).

Coronary heart disease (CHD) is a dysfunction of the heart due to a heart muscle lack of blood due to narrowing of the coronary arteries. Based on Basic Health Research (RisKesDas) in 2018, coronary heart disease was ranked as the seventh non-communicable disease in Indonesia and it is estimated that in 2020 CHD became the first common killer of 36% of all world deaths (DepKes, 2006; Kemenkes, 2019) and because of that, we need antihypercholesterolemic.

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The use of modern or synthetic drugs in the statin class is now often used for cholesterol treatment. However, the disadvantage of the statin group is that they have side effects in the form of gastrointestinal disorders, myopathy, allergic reactions, and rhabdomyolysis (Liman and Hartadi, 2002). Thus, people today prefer natural medicine because natural medicine is believed to be safer, cheaper, and easily found in the community around synthetic drugs (Muhtadi *et al*, 2013). Based on this, research on herbal medicines used as antihypercholesterolemic still needs to be developed (Azhari *et al.,* 2017).

The genus Uncaria has the potential to decrease blood cholesterol levels. Based on research conducted by Frinanda (2014), the best dose of Uncaria gambir in inhibiting the increase in total cholesterol and stabilizing the blood value of mice is 50 mg/kg BW. In Asia, the popular genus Uncaria is used for antidiabetic, immune system stimulants, hypo cholesterol agents and to reduce the risk of strokes, heart attacks, and hypertension (Heizment *et al*, 2005).

Kaik-kaik Root Plant *(Uncaria cordata* (Lour.) Merr.) is the genus Uncaria found in Indonesia, especially in the Indigenous Prohibition Forest of Kampar Regency, Riau Province. The leaves of Kaik-kaik Root (*Uncaria cordata* (Lour) Merr.) Contain secondary metabolites in the form of flavonoids, saponins, terpenoids, steroids, and phenolics (Sefralisa, 2015). Flavonoid compounds play a role in reducing blood cholesterol levels.

The flavonoids are thought to work by inhibiting the work of the enzyme HMG-CoA reductase which functions as a catalyst in the formation of cholesterol (Sekhon, 2012).

Based on the above description, the researchers were interested in conducting a study on the effect of ethanol extract of leaves of Kaik-kaik Root plants at doses of 100, 200, and 400 mg/kg BW in reducing levels of the total cholesterol of male white mice *(Mus musculus* L.). The method used in this study is POCT (Point Of Care Testing), using an Easy Touch® tool equipped with a blood cholesterol test strip. This method is easy for daily use and still gives accurate data. To increase cholesterol levels of mice, quail 10 mL/kg BW was used (Kusuma *et al* ., 2016). The selection of ethanol solvents for extraction in this study is based on the fact that besides being non-toxic, ethanol is a universal solvent so that it can attract polar and nonpolar compounds, the compounds expected to be attracted by these solvents are flavonoids (Marjoni, 2016).

METHODOLOGY

Materials

The tools used in this study are a set of distillation equipment, vacuum rotary evaporator, analytical scales (Shimadzu Auw 220), dark bottles, animal scales, animal cages, filter paper, aluminum foil, funnels, oral sonde, hot plates, surgical scissors, glasses measuring, alarm, watch glass, drip pipette, mortar and buffer, vial, parchment, test tube (Pyrex Iwaki®), drip plate, measuring device for total cholesterol levels and strip test (Easy Touch®).

The material used in this study was 96% ethanol, mice food (standard feed No. 552), high-fat feed (quail egg yolk), swab alcohol, Sodium Carboxymethyl Cellulose (Na CMC), atorvastatin 10mg, aqua dest, chloroform, ammonia, 2 N sulfuric acid, Mayer reagent, concentrated hydrochloric acid, 1% iron (III) chloride, magnesium metal, norit, anhydrous acetic acid, concentrated sulfuric acid.

Methods

Fresh leaves of kaik-kaik root plant 1 kg cleaned and dried air. The dried samples obtained 450 g were sorted to remove dirt that was still left behind during the drying process. The extraction process is done by maceration or immersion with 96% ethanol solvent which has been distilled. A sample of 250 g which had been dried and chopped before was put into a dark bottle and then immersed in ethanol solvent which had been distilled until the sample was completely submerged. Sample containers are stored at room temperature and protected from direct sunlight. The immersion process lasts for five days while stirring occasionally. The ethanol extract was filtered using filter paper and the pulp was macerated in the same way for 5 days to three repetitions. The results of maceration were collected, then concentrated with a vacuum rotary evaporator.

Animal Test Preparation

Before the experiment, the animals were acclimatized for about 7 days to adapt to the environment. The test animals used were male white mice (*Mus musculus* L.) with an average weight above 20 grams of body weight. Animals are grouped into four groups, namely the control group (Na CMC 1%), doses of 100, 200, and 400 mg/Kg BW. Grouping was done randomly in such a way that the distribution of body weight was evenly distributed for all groups with variations in body weight not more than 20% of the average body weight. Animals are weighed every day to determine the volume of test preparation to be given

Na CMC powder was weighed 0.05 mg. Then sprinkled on hot water 20 times (1 mL) in the hot mortar and left for 15 minutes. Then crushed until homogeneous, then added little by little the ethanol extract of leaves of kaik-kaik root plant that have been weighed according to the planned dose, then crushed to homogeneous and added aqua dest to a volume of 5 mL. The route for giving the test preparation is given orally. This treatment is given every day once a day for 28 days and measurements are taken on days 0, 15, 22, and 29.

Measurement of Total Cholesterol Levels

Measurements were made with a cholesterol measuring device, namely easy touch® GCU. The tool is calibrated first with the code number corresponding to the test strip. The test strip is inserted in a special place on the device, then on the screen, a picture of blood droplets will appear indicating the device is ready for use. Before being measured, mice have fasted for 12-14 hours. According to Murray *et al* (2009), this fasting process aims to reduce the activity of HMG-CoA reductase. After the mice's tail was disinfected with 70% ethanol the tail end was cut until the blood came out and enough to use, the first blood droplet was removed to avoid the wrong results, the next droplet was applied to the test strip until it sounded and 150 seconds will appear on the screen cholesterol with mg / dL. The cholesterol of the experimental animals was measured on the day after the 8th day of acclimatization (T0), day 22 (T15) after 14 days of induction, day 29 (T22),

Figure 1. leaves of Kaik-kaik Root plants

and day 36 (T29) after treatment. Contain a brief but sufficiently complete description of procedures and materials to allow the experiment to be repeated. Only new procedures should be described. Previously published procedures should be referenced. Significance materials should be described in detail.

RESULT AND DISCUSSION

One of the genus Uncaria which is thought to have the potential to reduce cholesterol levels is Kaik-kaik root plant. In this study, Kaik-kaik root plants were taken from the Rumbio Traditional Ban Forest of Kampar Regency, Riau Province. Taking plants in the forest to match the sampling sites in the study of the previous kaik-kaik root plants. The part of the plant used is the part of the leaf (figure 1).

Experimental mice were made hypercholesterolemia by feeding a high-fat diet in the form of quail egg yolk as much as 10 ml/Kg BW for 28 days. In this study, we only investigated feeding.

Taking blood of mice is carried out through the tail vein (Lateralis). The blood is taken by cutting a bit of the tip of the mice which has been given 70% alcohol, then removing the first drop of blood, and then the blood is dripped onto a cholesterol test strip that is installed on the device. This cholesterol level measurement uses the Point of Care Testing (POCT) method. POCT is a simple laboratory examination using a small amount of blood sample and the results can be known quickly because without using sample preparation. The principle of inspection with Point of Care Testing (POCT) uses biosensor technology that produces electric charges from chemical interactions between certain substances in the blood and strip electrodes. Changes in electrical potential that occur as a result of the reaction of these two substances will be measured and converted to a number that corresponds to the amount of electric charge produced. The numbers produced in the examination are considered to be equivalent to the levels of substances measured in the blood (Kemenkes, 2010).

The observational data was processed using one-way Analysis of Variance (ANOVA) statistical analysis consisting of one dependent variable (cholesterol level) and one independent variable (treatment), while to find out the differences in the measurement time of cholesterol levels in each group used statistical analysis repeated ANOVA. Statistical methods ANOVA is a comparative analysis technique to find significant differences from the mean (average) data that is more than two variables or groups (Dahlan, 2019).

Based on the results of measurements made, the average total cholesterol level of male white mice (*Mus musculus* L.) in the normal control group, negative controls, positive controls, doses of 100, 200, and 400 mg/kg BW respectively at T0 is 116.8; 166.6; 156.6; 142.4; 149 and 151.6 mg / dL. These levels are appropriate when compared with the normal total cholesterol level of male mice (*Mus musculus* L.), which is 90-170 mg / dL. The average total cholesterol level in T15 is 121; 206.4; 219.4; 174.2; 183 and 196.6 mg / dL. The average total cholesterol level in T22 is 116; 223.6; 143.4; 151,6; 138; 149.8 mg / dL and at T29 is 121.2; 228.6; 137.2; 145; 131.8 and 143.6 mg / dL (see table I) and we can see in Figure 2, that there was an increase in cholesterol levels in all treatment groups on day 15 due to the induction of high-fat feed, and began to experience a decrease in cholesterol levels on days 22 and 29 in the positive control group and the treatment group except for the negative control group.

Based on the subset of homogeneity of the results of the Tukey Post Hoc test, the difference in total cholesterol levels on the 22nd day to day 15 and 29 to day 15, in the negative control group showed significantly different results ($p < 0.05$) with all groups. It can be said that the cholesterol level of the negative control group increased because it was induced with high-fat feed.

The administration of ethanol extract of leaves of kaik-kaik root at a dose of 100 mg / Kg BW showed significantly different results ($p < 0.05$) with the negative control group, positive control,

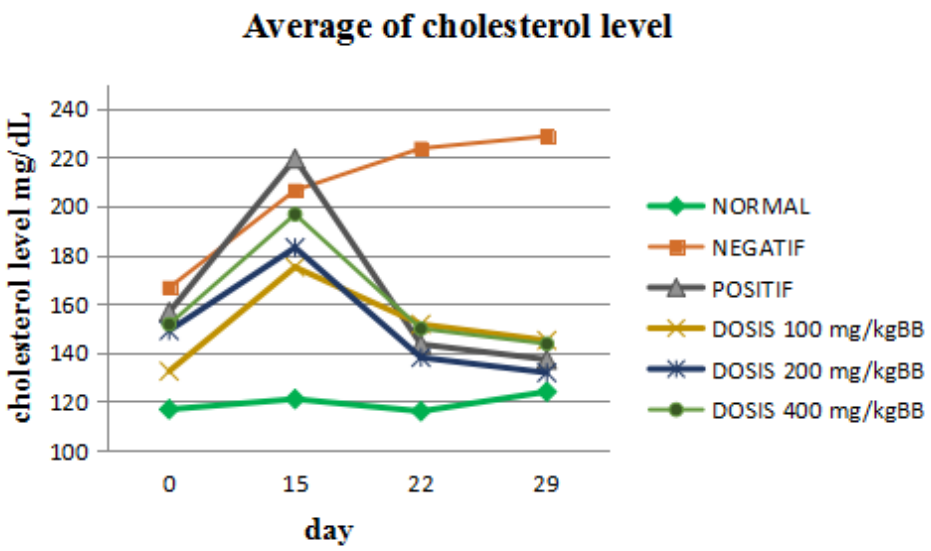

Figure 2. Average of cholesterol levels

normal control, 200 and 400 mg kg BW doses. It can be said that on the 22nd and 29th-day ethanol extract of leaves of kaik-kaik root plant dose of 100 mg / Kg BW was able to reduce total cholesterol levels because it was significantly different ($p < 0.05$) with a negative control group but had not been able to reduce comparable cholesterol levels with a positive control group.

The administration of ethanol extract of leaves of kaik-kaik root plant 200 mg/kg BW showed results that did not differ significantly ($p > 0.05$) with a group dose of 400 mg/kg BW and showed significantly different results ($p < 0.05$) with a negative control group, positive control, normal control and dose of 100 mg/kg BW. This can be interpreted that on the 22nd day and 29th day the ethanol extract of leaves of kaik-kaik root plant at a dose of 200 mg/Kg BW had activity in reducing total cholesterol levels which were comparable to the group dose of 400 mg/Kg BW but not comparable with the positive control group given atorvastatin.

The administration of ethanol extract of leaves of kaik-kaik root at a dose of 400 mg/kg BW showed significantly different results ($p < 0.05$) with the negative control group and positive controls. This can be interpreted that the ethanol extract of leaves of Kaik-kaik root plant at a dose of 400 mg/kg BW can reduce total cholesterol levels but is not comparable with the positive control group given atorvastatin.

In this present study, we found based on the results of the repeated ANOVA test, that the total cholesterol levels of all groups at T0, T22, and T29 were significantly different ($p < 0.05$) with cholesterol levels in T15. This shows that in T15 there was an increase in mice cholesterol after an induced high-fat feed, but in the normal control group, there was no significant difference ($p > 0.05$) because in the normal control group only Na CMC was given without induction of high-fat feed, so that cholesterol levels same as T15. Total cholesterol levels of mice given ethanol extract of kaik-kaik root leaves, doses of 100 and 400 mg/Kg BW showed results that were not significantly different ($p > 0.05$) at T22 with T29. This can be interpreted that the administration of ethanol extract of leaves of kaik-kaik root of doses of 100 and 400 mg/Kg BW was not able to reduce total cholesterol levels in T29, but in the 200 mg/Kg BW dose group there were significant differences ($p < 0.05$) on T22 with T29. This shows that the ethanol extract of leaves of kaik-kaik root plant dose of 200 mg/Kg BW is still able to reduce total cholesterol levels in T29.

This decrease in cholesterol levels is thought to be due to the presence of secondary metabolites as flavonoids, phenolics, and saponins contained in the extract. According to Sekhon (2012), flavonoids can reduce levels of cholesterol by inhibiting the activity of the enzyme HMG-CoA reductase, flavonoids are also thought to inhibit ACAT activity (Acyl-CoA Cholesterol Acyltransferase) in the liver. HMG-CoA reductase enzyme has a role in the synthesis of cholesterol so that when the enzyme's work is inhibited, cholesterol synthesis will decrease. The mechanism of inhibition by flavonoid compounds

Table I. Effect of Ethanol Extract of Kaik-kaik Roots (*Uncaria cordata* (Lour.) Merr.) On Total Cholesterol Levels of Male White Mice (*Mus musculus* L.).

Group	Number of Animals	Cholesterol Level (mg/dL)			
		t_0	t_{15}	t_{22}	t_{29}
Normal	**1**	119	120	117	123
	2	119	125	120	129
	3	118	115	112	116
	4	116	120	112	123
	5	112	125	119	129
	average±SD	**116.8±2.949**	**121±4.183**	**116±3.807**	**124±5.385**
Negative	**1**	168	207	224	224
	2	164	204	228	237
	3	166	208	225	230
	4	169	206	221	230
	5	166	207	220	222
	average±SD	**166.6±1.949**	**206.4±1.516**	**223.6±6.949**	**228.6±5.899**
Positive	**1**	155	211	139	137
	2	157	218	142	136
	3	155	222	146	140
	4	157	224	145	137
	5	159	222	145	136
	average±SD	**156.6±1.673**	**219.4±5.176**	**143.4±2.880**	**137.2±1.643**
Dose 100 mg/kg BW	**1**	142	175	151	142
	2	144	174	154	139
	3	139	175	145	146
	4	145	177	157	150
	5	142	174	151	148
	average±SD	**142.4±2.302**	**175±1.224**	**151.6±4.449**	**145±4.472**
Dose 200 mg/kg BW	**1**	150	183	139	132
	2	148	184	137	132
	3	151	186	138	136
	4	150	182	139	131
	5	146	180	137	128
	average±SD	**149±2**	**183±2.236**	**138±1**	**131.8±2.863**
Dose 400 mg/kg BW	**1**	152	193	155	140
	2	152	196	150	145
	3	150	197	145	145
	4	150	198	145	140
	5	154	199	154	148
	average±SD	**151.6±1.673**	**196.6±2.302**	**149.8±4.764**	**143.6±3.507**

occurs when an analog of the HMG-CoA reductase enzyme with its substrate, HMG-CoA, is converted into mevalonic acid. This shows that flavonoids act as competitive inhibitors with HMG-CoA so that the HMG-CoA reductase enzyme tends to bind to flavonoids as a result of a decrease in the formation of mevalonic acid which plays a role in cholesterol biosynthesis.

Flavonoids and phenolic compounds can be antioxidants because of their ability to donate hydrogen atoms, free radical scavengers, and metal ion chelating. LDL is a lipoprotein that contains a lot of cholesterol, this cholesterol will be partially taken to the liver, other steroidogenic tissues that have receptors for LDL cholesterol and some will experience oxidation and are captured by the scavenger-A receptor (SR-A) in macrophages and will become foam cells (foam cell), so the more LDL cholesterol levels inside the plasma, the more that will experience oxidation and captured by macrophagic cells which play a role in the formation of atherosclerosis (Sudoyo *et al*, 2009). It can be said that flavonoids and phenolics as antioxidants can inhibit the oxidation of cholesterol so that foam cells are not formed. Based on research conducted by Rocio *et al* (2013)

Saponin compounds reduce plasma cholesterol levels by reducing absorption from cholesterol in the intestine.

CONCLUSION

Based on the research that has been done, it can be concluded that the ethanol extract of leaves of kaik-kaik root plant (*Uncaria cordata* (Lour.) Merr.) For 14 days in male white mice (*Mus musculus* L.) at doses of 100, 200. and 400 mg/Kg BW has an effects on the decrease in cholesterol levels. The ethanol extract of leaves of kaik-kaik root at a dose of 200 mg/Kg BW on the 29th day still reduced cholesterol levels compared to the 22nd day. The group dose of 200 mg/Kg BW on days 22 and 29 can lower cholesterol levels greater than the group dose of 100 and 400 mg/Kg BW.

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