# Anti-Hyperlipidemic Effect of 70% Ethanol Extract from *Mesona palustris* Blume Leaves on Male Hamsters

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Abstract: In Indonesia, *Mesona palustris* Blume is known as the black *cincau* (black grass jelly). This plant has been used for various health condition. This study aims to determine the effectiveness of 70% ethanol extract from black *cincau* leaves as anti-hyperlipidemic on hyperlipidemic hamsters. The dried leaves were extracted using 70% ethanol by maceration method. Syrian hamsters were divided into 7 groups of 4 hamsters), i.e. normal control group, positive control group (fenofibrate 1.235 mg/Kg body weight), positive control group (atorvastatin 2.4 mg/Kg body weight), negative control group, test groups with 3 dose variations (780, 1560, and 3120 mg/Kg body weight each day). All groups except normal control group were induced with high-fat diet for 28 days. Parameters measured were decreased total blood cholesterol, LDL, triglycerides level, and increased blood HDL level. Treatment was performed for 14 days. The 70% ethanol extract of black *cincau* with a dose of 3120 mg/Kg body weight was able to decrease blood total cholesterol level by 51.49%, blood LDL level by 49.92%, and blood triglyceride level by 45.70%. Extract with the dose is also able to increase blood HDL level by 29.30%. Fenofibrate was able to decrease blood triglyceride level by 50.62% and increase blood HDL level by 34.82%, whereas atorvastatin was able to decrease blood total cholesterol level by 54.34% and blood LDL level by 51.13%. It could be concluded that the 70% ethanol extract from black *cincau* leaves is effective as an anti-hyperlipidemic agent in hyperlipidemic hamster equivalent to positive control group.



## 1 INTRODUCTION

The highest prevalence of cardiovascular disease in Indonesia is coronary heart disease, which is 1.5%, with the highest rate is in East Nusa Tenggara province (4.4%) and the lowest in Riau Province (0.3%) (Ministry of Health of Republic of Indonesia, 2013). One of the causes of coronary heart disease is atherosclerosis - an accumulation of lipids and fibrous tissue in the coronary arteries that narrows the lumen of the blood vessels. When the lumen narrows the resistance to blood flow increases (Price and Wilson, 2006).

Hyperlipidemia is a condition when one or more of the fat components such as cholesterol, phospholipids or triglycerides, increase. The hyperlipidemic condition is characterized by an increase concentration of lipoprotein - a substance for fat transport in plasma (Priyanto, 2009). An increase in the concentration of lipoproteins are characterized by increased total cholesterol, triglycerides, Low Density Lipoprotein (LDL) levels, and decreased of High-Density Lipoprotein (HDL) level (Price and Wilson 2006). Cholesterol is a component of fat or lipids, which is continuously formed or synthesized in the liver. Approximately 70% of cholesterol in the blood is synthetized in the liver, while the rest comes from food intake (Anies, 2015).

An increase in the lipid levels in the blood can occur due to poor lifestyle, such as eating foods high in fat (Hardman and Limbird, 2012). This condition can be overcome by improving lifestyle and consuming anti-hyperlipidemic drugs, which are used to treat the hyperlipidemic condition by reducing the total cholesterol, triglycerides, LDL, or increasing HDL levels. Unfortunately, drugs such as fibrates and statin groups generally cause harmful side effects such as muscle aches, itchy skin, rashes, and other effects such as visual impairment, and peripheral neuritis. Fibrate groups can also aggravate the condition of liver function disorders so that they cannot be given to patients with liver failure. Statins such as simvastatin can cause undesirable side



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effects such as muscle pain, muscle weakness (myopathy) and chest pain (Junaedi, 2012). The use of natural ingredients becomes an alternative to avoid the side effects arising from synthetic drugs. Plants that have been shown to have a lipid-lowering and antioxidant activity are black *cincau* or black grass jelly (*Mesona palustris* Blume) (Amelia and Tri, 2014).

Black *cincau* is a traditional food that contains antioxidants agents and has been used empirically for various health conditions. Black *cincau* contains phenolic components such as protocatechuic acid, p-hydroxybenzoic, vanillic acid, and syringic acid, flavonoids, polyphenols, saponin glycosides, terpenoids and steroids and gel-forming components that are natural polysaccharides (Maslukhah *et al*., 2016). The antioxidants in the phenolic compounds enable black *cincau* to reduce blood fat (Fauzziyah *et al*., 2016). Previous research has shown that black jelly leaves extract with a dose of 130 mg/200 g body weight can decrease cholesterol level by 22.44%, triglyceride by 26.95%, LDL by 42.39% and the increase of HDL by 27.62% in white rats (Amelia and Tri, 2014). Seventy percent ethanol extract of black *cincau* leaves has higher in vitro antioxidant activity compared to water extract (Widyaningsih, 2013).

This study aims to determine the activity of 70% ethanol extract of black *cincau* leaves with three different dose variations in male hamsters with hyperlipidemic condition. Fenofibrate and atorvastatin are used as positive controls. Observations were performed on total cholesterol, triglycerides, LDL, and HDL levels.

# 2 MATERIALS AND METHODS

## 2.1 Extraction of Black cincau Leaves

The dried black *cincau* leaves obtained from Balai Penelitian Tanaman Obat dan Aromatik (BALITTRO), Bogor in March 2017. It was extracted by maceration method using 70% ethanol solvent. The filtrate was evaporated using vacuum rotary evaporator (EYELA) until extract could be obtained. The quality of the extract was determined by identification of organoleptic, water content, and phytochemical screening.

## 2.2 Animal Preparation

Twenty-four male Syrian hamsters (*Mesocricetus auratus*) aged 3-4 months around 50-100 g were obtained from Research Animal Breeder, Bekasi. This study used six groups with each group consisting of four animals. Before treatment, the animals were acclimatized for seven days. At this stage, the animals were given standard drink and feed. Except normal control groups, all the other groups were made into hyperlipidemic conditions with high-fat feeding (40% quail yolk, 10% vegetable oil, and standard feed for hamsters' ad 100%) for 28 days. The protocol no. 17-05-0488 was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia no. 459/UN2.F1/ETIK/2017.

## 2.3 Animal Test Treatment

All groups were treated for 14 days. Group I (normal control group) was given standard feed each day, group II (negative control group) was given Na-CMC 0.5% each day, group III (positive control group) was given fenofibrate at 1235 mg/Kg body weight each day, group IV (positive control group) was given atorvastatin at 2.4 mg/Kg body weight each day, group V (dosage 1 test group) was given the black *cincau* leaves extract at 780 mg/Kg body weight each day, group VI (dosage 2 test group) was given the black *cincau* leaves extract at 1560 mg/Kg body weight each day, group VII (dosage 3 test group) was given the black *cincau* leaves extract at 3120 mg/Kg body weight each day. On day 29 and day 44, the animals were fasted and then anaesthetized with an injection of ketamine with a dose of 74 mg/Kg body weight. Blood sample was taken through the orbital sinus. Blood sample was collected in a microtube, then centrifuged for 15 minutes at 4000 rpm to obtain the blood serum for measurement of total cholesterol, triglycerides, LDL and HDL levels by enzymatic methods.

## 2.4 Measurement of Total Cholesterol Level

Ten µl of serum was added with 1000 µl of enzyme reagent, then homogenized using vortex and incubated for 5 minutes at 37°C and analyzed with a Microlab-300 clinical spectrophotometer.

## 2.5 Measurement of LDL Level

One hundred µl serum was added with 1000 µl of precipitation reagent (a mixture of heparin and sodium citrate solution). The mixture was homogenized using a vortex and incubated at 37ºC for 5 minutes, then was centrifuged at 4000 rpm and

was allowed to stand for 1 hour until LDL precipitation was formed. One hundred µl of the supernatant was mixed with 1000 µl of cholesterol enzyme reagent. The mixture was homogenized using a vortex and then incubated for 5 minutes at 37°C and analyzed with a Microlab-300 clinical spectrophotometer.

## 2.6 Measurement of Triglyceride Level

Ten µl of blood serum was mixed with 1000 µl triglyceride reagent kit, then was homogenized with vortex and was incubated for 5 min at 37ºC. Furthermore, it was analyzed using a Microlab-300 clinical spectrophotometer.

## 2.7 Measurement of HDL Level

Two hundred µl of serum was added with 500 µl of precipitation reagent. The mixture was homogenized using a vortex and was incubated at room temperature for 10 minutes. It was centrifuged at 4000 rpm for 10 minutes. 100 µl of supernatant was added with 1000 µl cholesterol reagent then was incubated at 37ºC for 5 minutes. It was analyzed with a Microlab-300 clinical spectrophotometer.

## 2.8 Data Analysis

The data were analyzed for normality and homogeneity then were continued with one-way ANOVA with 95% significance level ($p<0,05$). The Tukey test was conducted to see a significant difference between each group (Priyatno 2010).

# 3 RESULTS AND DISCUSSION

A total of 600 g of dried black *cincau* leaves was extracted with 70% ethanol solvent produced 187.7 g extract. It was blackish-brown, viscous, and had distinctive smell extract. The water content of the extract was 8.7%. The extraction of black *cincau* leaves with 70% ethanol revealed compounds of flavonoids, saponins, phenolics, and triterpenoids/steroids. The results of the chemical content identification can be seen in Table 1.

Table 1: Chemical content of 70% ethanol extract from black *cincau* leaves

| Chemical content | Result |
|---|---|
| Alkaloids | - |
| Flavonoids | + |
| Saponin | + |
| Phenolic | + |
| Triterpenoids/Steroids | + |

Note: (+) = positive; (-) = negative

Based on the test, the 70% ethanol extract of black *cincau* with a dose of 3120 mg/kg body weight could decrease total cholesterol level by 51.49%; LDL level by 49.92%; and triglyceride level by 45.70%. It could also increase HDL level by 29.30% in male hamsters. Fenofibrate was able to decrease triglyceride level by 50.62% and increase HDL level by 34.82%. Atorvastatin was able to decrease total cholesterol level by 54.34% and LDL level by 51.13%. These results can be seen in Figure 1 to Figure 4.

The 70% ethanol extract of black *cincau* leaves contain compounds that could alter the lipid profile

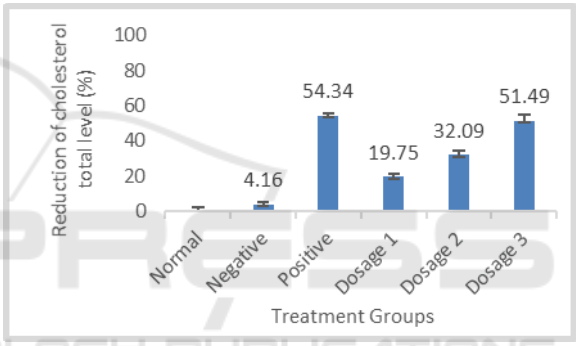


Figure 1: The reduction percentage of cholesterol total level from black *cincau* leaves 70% ethanol extract with dosage variations and control groups. Error bars show the standard deviation from the average of four data.

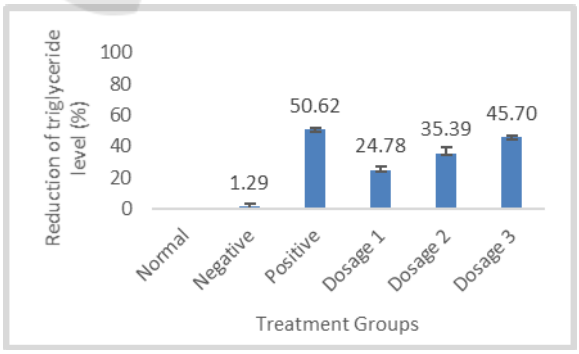


Figure 2: The reduction percentage of triglyceride level from black *cincau* leaves 70% ethanol extract with dosage variations and control groups. Error bars show the standard deviation from the average of four data.

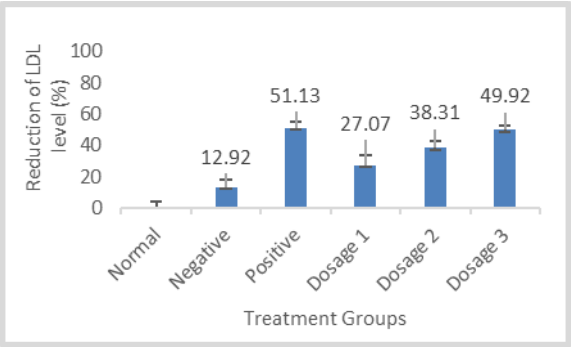


Figure 3: The reduction percentage of LDL level from black cincau leaves 70% ethanol extract with dosage variations and control groups. Error bars show the standard deviation from the average of four data.

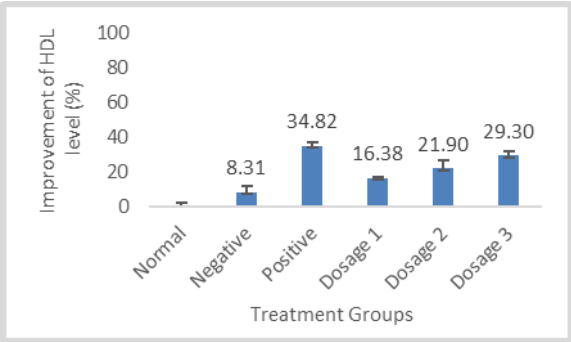


Figure 4: The improvement percentage of HDL level from black cincau leaves 70% ethanol extract with dosage variations and control groups. Error bars show the standard deviation from the average of four data.

by reducing blood total cholesterol, triglycerides, LDL and improving HDL levels. High consumption of flavonoids has previously been reported to contribute to a decreased risk of coronary heart disease by lowering the serum cholesterol and triglycerides in rats. The plasma cholesterol concentration was lowered by rutin (one of flavonoids groups). It has potential biological effect on lowering the plasma cholesterol and hepatic HMG-CoA reductase activity (Park *et al*., 2002). Ruel *et al*., (2006) reported that flavonoid consumption may be cardioprotective, and give a favourable impact on circulating HDL-cholesterol concentrations. Plant components that are known to have the ability to decrease intestinal lipid absorption are polyphenols, saponins and plant sterols. Saponins from dietary *Momordica dioica* powder prevent the development of fatty liver by the inhibition of intestinal lipid absorption as a lipase inhibitor in rats (Sato *et al*., 2011). Triterpenoidal saponins provide inhibition of pancreatic lipase enzymes (Lunagariya *et al*., 2014). Bioactive terpenoids can modulate the activities of ligand-dependent transcription factors, namely, peroxisome proliferator-activated receptors (PPARs). Because PPARs are dietary lipid sensors that control energy homeostasis, daily eating of these terpenoids may be useful for the management of obesity-induced metabolic disorders such as type 2 diabetes, hyperlipidemic, insulin resistance and cardiovascular diseases (Goto *et al*., 2010).

## 4 CONCLUSIONS

Based on the research, 70% ethanol extract of black *cincau* leaves at dose 3120 mg/Kg body weight is able to decrease total cholesterol, triglyceride, LDL and increase HDL level in male hamsters with hyperlipidemic condition comparable to fenofibrate and atorvastatin.

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