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The Potential of *Cnidoscolus chayamansa* Alchoholic Leaves Extract as Hypolipidemia Agent

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Abstract. Cardiovascular disease caused by hyperlipidemia is the highest case in term of fatality of non-infectious disease in Indonesia. *Cnidoscolus chayamansa* is well known as a herb that has nutraceutical potential for medicine and likely as a hypolipidemia agent. Therefore, this study aimed to identify the optimal dose of *C. chayamansa* as a candidate to treat cardiovascular diseases. A total of 30 white rats were acclimatized in the laboratory for 14 days. After that, the rats were grouped into six groups, randomly, which including a healthy control group (K0), a negative control group or hyperlipidemic rats (K-), a positive control group or hyperlipidemia-induced rats that was supplemented with atorvastatin (K+), then a hyperlipidemic rats treated with alcoholic extract of *C. chayamansa* leaves at doses of 100 mg /kgBB /day for K1, 200 mg/ kgBB/ day for K2 and 400 mg/ KgBB/ day for K3. The results showed that K3 treatment is the most effective and optimum dose for lowering total cholesterol level at 76.81±1.10 mg/dl, triglycerides 72.39±1.66 mg/dl and LDL-C at 21.47±0.58 mg/dl. The aortic histology assessment also showed that the K- group had putative thrombus or plaque in intima, and it was not found in other groups. This research focused on the optimum dose and new usage of *C. chayamansa*, as an anti-inflammatory in atherosclerosis. By understanding the optimum dose of *C. chayamansa*, the community can apply and control the herb consumption for their own therapeutic properties. For future application, the herb is potentially developed as anti-atherogenic medicine.

Key words: *Cnidoscolus chayamansa*, leaves extract, hyperlipidemia, hypolipidemia

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INTRODUCTION

Cardiovascular disease is the first killer disease in Indonesia. In 2013, more than 1.5% or ±3.7 million people aged ≥ 15 years experienced coronary heart disease (CHD) both diagnosed and symptomatic of the disease (KEMENKESRI, 2018). In addition, stroke and hypertension cases increase 1.9% nationally from 2007 to 2013, with the highest percentage occurred in North Sulawesi province which reached 15.2% (KEMENKES RI, 2018).

Various factors contribute to trigger the emergence of cardiovascular disease. Cardiovascular disease occurs due to the condition of hyperlipidemia and hypercholesterolemia which make an aortic disease such as atherosclerosis (Sanchis-Gomar, Perez-Quilis, Leischik, & Lucia, 2016). These conditions result in high levels of lipid group compounds, especially circulated cholesterol or very low-density lipoprotein-cholesterol (VLDL-C) and low-density lipoprotein-cholesterol (LDL-C). When the condition occurrs continuously in long term period (chronic), the cholesterol accumulates in the tunica or blood-vessel wall. High cholesterol content in the tunica gets oxidized and produces free radicals that damages the endothelial cells and induces smooth myocytes migration (Ecker et al, 2006). It leads to severe condition called atherosclerosis. The atherosclerosis involves immune cells such us macrophages and neutrophile that penetrate into subendothelial intima and release the inflammatory cytokine (Ooi et al., 2017). Then, the oxidized LDLs (oxLDLs) release free radicals into the circulatory system and triggers subendothelial damage or lesions (Otsuka et al., 2016). Damaged endothelium secretes adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and chemokines stimulate other leukocytes that increase the inflammation (Fan et al., 2015; Taleb, 2016).

High nutritious foods containing antioxidants and fiber are well known to able to be used as an agent of recovering hyperlipidemia condition. One of the potential food that contains high antioxidants and potentially used as a hypolipidemia agent is *Cnidoscolus chayamansa* (Miranda-Velasquez et al., 2010). This plant is traditionally known as a traditional herbal medicine for cancer, diabetes mellitus, high blood pressure, ulcers, weight loss, and kidney disorders. The administration of *C. chayamansa* leaves extract significantly reduces lipid and cholesterol levels or can act as hypocholesterolemia (García-Rodríguez et al., 2014). Other than that, *C. chayamansa* is also known as an anti-inflammatory because of its potential in binding free radicals. However, research on alcoholic extract of *C. chayamansa* leaves as a hypo-

lipidemia agent has not been much studied. In addition, confirmation is still needed to find out the right concentration as a reference in providing effective doses in reducing blood lipid levels. Therefore, this study aimed to identify the optimal dose of *C. chayamansa* as a candidate to treat cardiovascular diseases. By understanding the optimum dose of *C. chayamansa*, the community can apply and control the herb consumption for their own therapeutic properties. For future application, the herb is potentially developed as anti-atherogenic medicine.

METHODS

This study was designed using a randomized post-test only control group design. The *C. chayamansa* extraction was carried out at the Biochemical Laboratory, while the treatments of rat were conducted at the Animal Physiology Laboratory, Department of Biology Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang. A total of 30 Wistar-male rats, 180-200 gr of body weight, were maintained with standard *ad libitum* pellet feeds. Rats were randomly divided into six groups which including a healthy control group (K0), a negative control group or hyperlipidemic rats (K-), a positive control group or hyperlipidemia-induced rats that was supplemented with atorvastatin (K+), then a hyperlipidemic rats treated with alcoholic extract of *C. chayamansa* leaves at doses of 100 mg /kgBB /day for K1, 200 mg/ kgBB/ day for K2 and 400 mg/ KgBB/ day for K3.

C. chayamansa Extraction

To obtain the alcoholic extract, 2 kg of *C. chayamansa* leaves obtained from third leaves from the upper shoot were washed under water, then dried. The leaves were cut into small pieces and dried at 40-50ºC for 3 days or until dry. Dried leaves were ground into fine powder. The fine powder was then weighed as much as 50 gr, put into a dark bottle, and added with 500 ml of 96% alcohol solvent. The mixture was macerated at room temperature for two days. At the first 6 hours, the bottle was occasionally shaken, then allowed the mixture to re-macerate for the next 18 hours. The maceration results were then filtered using sterile gauze and sterilized filter paper. The remaining pulp was then re-macerated for 24 hours until an extra solution was obtained. The extract solution was put into a round-bottom flask and attached to a rotary vacuum evaporator. The distilled water was added to the water container to normal limits. The vacuum pump and rotary vacuum evaporator were set at 50ºC, 20 Psi pressure and 120 rpm rotation. Evaporation process was stopped when thick lines of the extract solution appear at bottom part in the bottle and the color turn into greenish brown. The extract solution was dried to dry at 50ºC. Then, the dried extract was weighed and dissolved into olive oil to be given orally.

Hyperlipidemia Induction and *C. chayamansa'*s Alcoholic Leaves Extract

To make hyperlipidemia rats, 2 ml of duck egg yolks and 2 mg of cholesterol were delivered to the gastric using oral gavage method and feeded using standard feed for 14 days. The standard feed in this study was pellet 594 (PT Japfa Confeed Indonesia). The admintration of atorvastatin dose of 20 mg/ person was converted into 0.38 mg/ rat. Both atorvastatin and alcoholic extract of *C. chayamansa* leaves was given at day 15^{th} to 30^{th} every morning, according to the dose per each group.

Blood Collection and Serum Preparation

On the 31^{th} day, rat's blood was collected through the retroorbital plexus (eye corner) using microhematocrit. The blood that comes out was collected in 1.5 ml tubes that has been added with EDTA before. After being taken, the blood sample was incubated at room temperature for 30 minutes and centrifuged at 8000 rpm for 5 minutes, to get the serum (yellowish clear liquid). After centrifuge, the serum will separate from blood clots or pellet. The serum fluid was then separated, and put in another 1.5 ml tube, then stored at -4ºC. The fat profile analysis including total cholesterol, triglyceride, HDL-C, LDL-C were conducted using DiaSys EmBh (Germany), following manufacturer procedure. Comparison of cholesterol ratio and atherogenic index were obtained with LDL/ HDL formula and Log10 (Triglyceride / HDL-C) (Dobiášová, 2004), then the mice were sacrificed and dissected to take their aorta.

Histopathological Examination of the Aorta and the Number of Foam Cells

The rat's aorta was separated from other organs, then used for histological observation. The organ was prepared using embedding (paraffin block) with H&E staining (Truong, Harper, & Tanguay, 2011). Organs taken from the aortic vessels from the heart to the abdomen. The part used for preparations was along from thorax to kidney (abdominal aorta). The preparations were observed at the Multimedia Laboratory, Department of Biology (Unnes), for their aortic histopathology with 100x and 400x magnificancy and described.

Data Analysis

Every parameters data (total cholesterol, triglyceride, HDL, LDL and LDL / HDL ratio) were tabulated. The data normality analysis was conducted using

Kolmogorov-Smirnov test. Hypothesis testing used One-way ANOVA, and significantly analysis was performed using Least Significant Difference (LSD) test. Statistical analysis was assisted with SPSS for Windows version 13. The significance value in this study was if the analyzed variables had P <0.05.

RESULT AND DISCUSSION

The treatment results showed that administration of egg yolks and cholesterin increase lipid levels in rat's blood plasma. This condition was characterized by increased levels of total cholesterol, triglycerides and LDL-C. High cholesterol diet has triggered the formation of a hypercholesterolemia condition characterized by a high ratio of LDL-C to HDL-C in treatment group.

Hypercholesterolemia as a manifestation of hyperlipidemia directly stimulates endothelial dysfunction which is characterized by accumulation of cholesterol in blood vessels wall. It was shown in K- with high concentration of total cholesterol (115.38±1.54 mg/dl), triglyceride (162.60±1.68 mg/dl), and LDL-cholesterol (33.98±1.30 mg/dl) with low concentration of HDL-cholesterol (14.91±0.66 mg/dl) (Tabel 1). K- group used as lipid level threshold for hyperlipidemia and hypercholesterolemia, It mean, all groups that has no significant or significantly different but higher on cholesterol, LDL-C and triglyceride levels will be grouped as hypercholesterolemia rats. The cholesterol is distributed throughout body by LDL-C and accumulated in smooth muscle cells (Hoekstra & Van Berkel, 2016; Steyers & Miller, 2014). High cholesterol concentrations can also be observed in myocyte cells that have been weakened and seen damaged (Figure 2-K-). It was caused by cholesterol that enters and deposits in tunica media triggering myocytes turn into foam cells.

The cholesterol influx into myocyte cells, pushes the nucleus to cell's edge and triggers macrophage migration until inflammation occurrs. In the condition of hypercholesterolemia, LDL-C oxidation, and macrophage activity, there is an increase in free radicals. High level of free radicals increases the activity of macrophages, thus triggering inflammation and increasing the number of free radicals. The oxidative stress condition triggers the release of cas-9 by the mitochondria which accelerates the occurrence of cell apoptosis (Zampetaki et al.,2013).

Leaves extract from *C. chayamansa* (Figure 1) has high content of phenolic compounds including protocatechuic acid and rutine which act as antioxidants (Loarca-Piña et al., 2010). In fact, *C. chayamansa* extract showed hypoglycemic effect after 120 minutes of administration. The antioxidant contents contained in *C. chayamansa* are flavonoids, triterpene, coumarin and cinnamic acid derivatives (de Oliveira-Júnior et al., 2018), isoquercetin, campesterol, eupafolin, and hispidulin (Sanchez-Hernandez, Barragan-alvarez, Torres-, & Padilla-camberos, 2017). The administration of *C. chayamansa* alcoholic leaves extract showed significantly different results in parameters profile among groups (Table 1).

Figure 1. *C. chayamansa* tree has palmately leaves with deep lobate notch

High intake of *C. chayamansa* leaves extract was in line with decrease of total cholesterol, triglyceride and LDL-C along with an increase in HDL-C. The K- group had the highest levels of triglycerides, total cholesterol and LDL-C compared to the other groups with 162.60±1.68 mg/ dl, 115.38±1.54 mg/ dl, and 33.98±1.30 mg/ dl respectively. This lipid parameters in this group was considered as hyperlipidemia threshold. It is mean, when the rats has same value of lipid parameter (not significantly different) or higher with the rats in K- group, they will be categorized as hyperlipidemic rats. Based on the treatment dose, the administration of low dose *C. chayamansa* leaves extract in K1 group, was reducing triglyceride level (116.38±1.95 mg/dl) even the titer is still high. In the group that was given *C. chayamansa* leaves extract, the most effective optimum dose that can decrease lipid concentration was in the K3 group, where all lipid biomarkers decreased, whereas HDL-C had the most significant increase of 26.32±0.80 mg/ dl.

The low concentration of serum lipid may cause by bioactive compound in the *C. chayamansa* extract. Based on the result, the LDL-C decrease, while HDL-C increase in line with administration of the *C. chayamansa* leaves extract. It is related with previous research that flavonoid content in the *C. chayamansa* leaves extract potentially plays a role as an antioxidants to cure hypocholesterolemic condition (Cárde-

nas-Ibarra et al, 2017; Moura et al., 2019). This is supported by research showing that *C. chayamansa* leaves extract can inhibit cholesterol synthesis and degradation of LDL cholesterol (Nhoek et al., 2018). Flavonoid levels in blood plasma derived from dietary flavonoids may inhibits free radicals in the early stages of atherosclerosis (Treml & Šmejkal, 2016). High plasma flavonoid levels are associated with a lower risk of cardiovascular disease in women (Cárdenas-Ibarra et al., 2017). The flavonoids mostly can be found or bounded in low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) fraction in the blood serum by it's lipophilic properties. Based on these properties, in daily diet plan, consuming fat must follow by flavonoids-contained-food.

Table 1. Atherosclerosis biomarker after *C. chayamansa* alchoholic extract supplementation

Group	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)
K0	51.65±1.75 [a]	59.06±0.89 [a]	17.33±0.74 [a]	29.41±1.16 [a]
K-	115.38±1.54 [b]	162.60±1.68 [b]	33.98±1.30 [b]	14.91±0.66 [b]
K+	74.61±1.40 [c]	84.90±1.77 [c]	23.01±0.74 [c]	24.70±0.42 [c]
K1	94.85±1.22 [d]	116.38±1.95 [d]	28.23±0.58 [d]	20.20±0.49 [d]
K2	85.83±0.94 [e]	98.40±1.07 [e]	25.08±0.71 [cd]	23.26±1.25 [c]
K3	76.81±1.10 [c]	72.39±1.66 [f]	21.47±0.58 [c]	26.32±0.80 [c]

Note: different superscript letters (a-f) indicate significantly different value among groups.

In the *C. chayamansa* leaves extract treatment group, all lipid concentration decrease except the HDL-C. The optimum dose was obtained from K3 group the extract is able to act as hypolipidemia agent effectively even in low concentration. The results obtained from extract administration for 15 days were significantly different compared with the K0 group. The decreasing concentration of the total cholesterol, triglyceride and LDL-C along with an increase in HDL-C level indicates that *C. chayamansa* alcoholic leaves extract administration affects lipid metabolism in liver and may contribute in cholesterol biosynthesis inhibition. Furthermore, the atherogenic index also showed that given *C. chayamansa* leaves extract of 400 mg/ KgBW did not differ from the normal group (Table 2).

Some of the flavonoids found from *C. chayamansa* are aromadendrin, naringenin and apigenin (Galati, Sabzevari, Wilson, & O'Brien, 2002). Where the flavonoid compounds have a C ring structure that can bind hydrogen oxide (•OH) (hydroxylation C-ring) produced by macrophages in cholesterol oxidation during foam cell formation (Treml & Šmejkal, 2016). The hydrogen oxide released by macrophages, decreasing proinflammatory cytokines and chemokines, which secreted by endothelial cells (Steyers & Miller, 2014). This results in a decrease in the rate of penetration of macrophages into the tunica media and reduces the risk of thrombus plaque formation which triggers blood vessel blockage. In addition, the possibility of flavonoid from *C. chayamansa* is also recognized in intervention of lipid metabolism in the liver and LDL-C degradation which causes a decrease in circulation and accumulation of choleserol in the body

Table 2. Atherogenic index after *C. chayamansa* alchoholic extract supplementation

Group	LDL-c/HDL-c	Atherogenic Index
K0	0.54 [a]	0.303 [a]
K-	2.60 [b]	1.038 [b]
K+	0.92 [c]	0.536 [ce]
K1	1.46 [d]	0.761 [d]
K2	1.08 [c]	0.626 [e]
K3	0.82 [c]	0.439 [ac]

The alcoholic leaves extract of *C. chayamansa* is identified containing high level of tannins, saponins, alkaloids, anthraquinone steroids, phlorotannins, and oxalates (Iwuji & Nwafor, 2015; John & Opeyemi, 2015; Obichi, Monago, & Belonwu, 2015). Analysis of *C. chayamansa* revealed high enough fiber and protein content in leavess and low in carbohydrates. In addition, *C. chayamansa* leavess also contain bioactive compounds such as vitamin C, carotenoids, and vitamin A.

Saponins, tannins and fibers are effective compounds to bind lipids in intestine and reduce. It's amount to enter the body (Ge, Zhu, Peng, Deng, & Li, 2016; Zhao, 2016). This is likely to significantly reduce the circulation of lipids in the body of hypercholesterolemia rats that are accommodated by LDL-C. Based on observations, K- group had higher LDL-C levels and lower HDL-C compared to other groups. Then in other groups treated with *C. chayamansa* showed an increase in HDL-C. It can be assumed, that circulated cholesterol from liver to the body was decreased during treatment (Cárdenas-Ibarra et al., 2017). Furthermore, the decreased LDL-C may also cause by increased cholesterol retake from foam cell or endothelial cells back to the liver.

Other than that, groups of vitamins C, A and carotenoids also play a role in preventing oxidative stress caused by high cholesterol (Iswari & Susanti, 2016). Then, carotenoids have an important role in the proliferation of immune cells and secretion of proinflammatory cytokines. Directly, the carotenoids and vitamin A groups are converted into ready-to-use active retinoid acids which increase the apoptosis of adipose cells and inhibit the metabolism of adipogenesis (Bonet, Ribot, & Palou, 2012; Lobo et al., 2010). Retinoid acid binds to the specific receptor Retinoic acid receptor (RAR) and retinoic X receptor (RXR) to

stimulate the expression of anti-inflammatory genes and suppress lipogenesis (Zapata-Gonzalez et al., 2007).

Based on the aortic histology confirmation, the K- group showed fattening of smooth muscle cells (white cavity) and irregular elastic fibers (Figure 1.K-). This is probably caused by the accumulation of cholesterol in tunica media. Fatty smooth myocyte is not found in other groups, in contrast, elastic fibers are still solid and not destroyed by cell migration. The form of fatty tissue in the aorta does not show to the formation of aortic thrombus or plaque which indicates the progression of atherosclerosis, possibly it is caused by short period in the cholesterol induction.

Various studies have confirmed the presence of sterol compounds and many non-polar compounds such as short chain fatty acids which have potential as antioxidants. Some active compounds from sterol groups such as β-sitosterol, phytosterol, campesterol and various plant sterols are found in various species of Cnidoscolus including *C. chayamansa* (Ribeiro, E Silva, De Assis, Correia, & Damasceno, 2017; Santos et al., 2017). Whereas the types of short chain fatty acids found include butyric acid and palmitate (Orji et al., 2016). Both sterols and short chain fatty acids, have activity in tethering free radicals, especially the oxidant from reactive oxide group [-$^{*}O_2^{--}$].

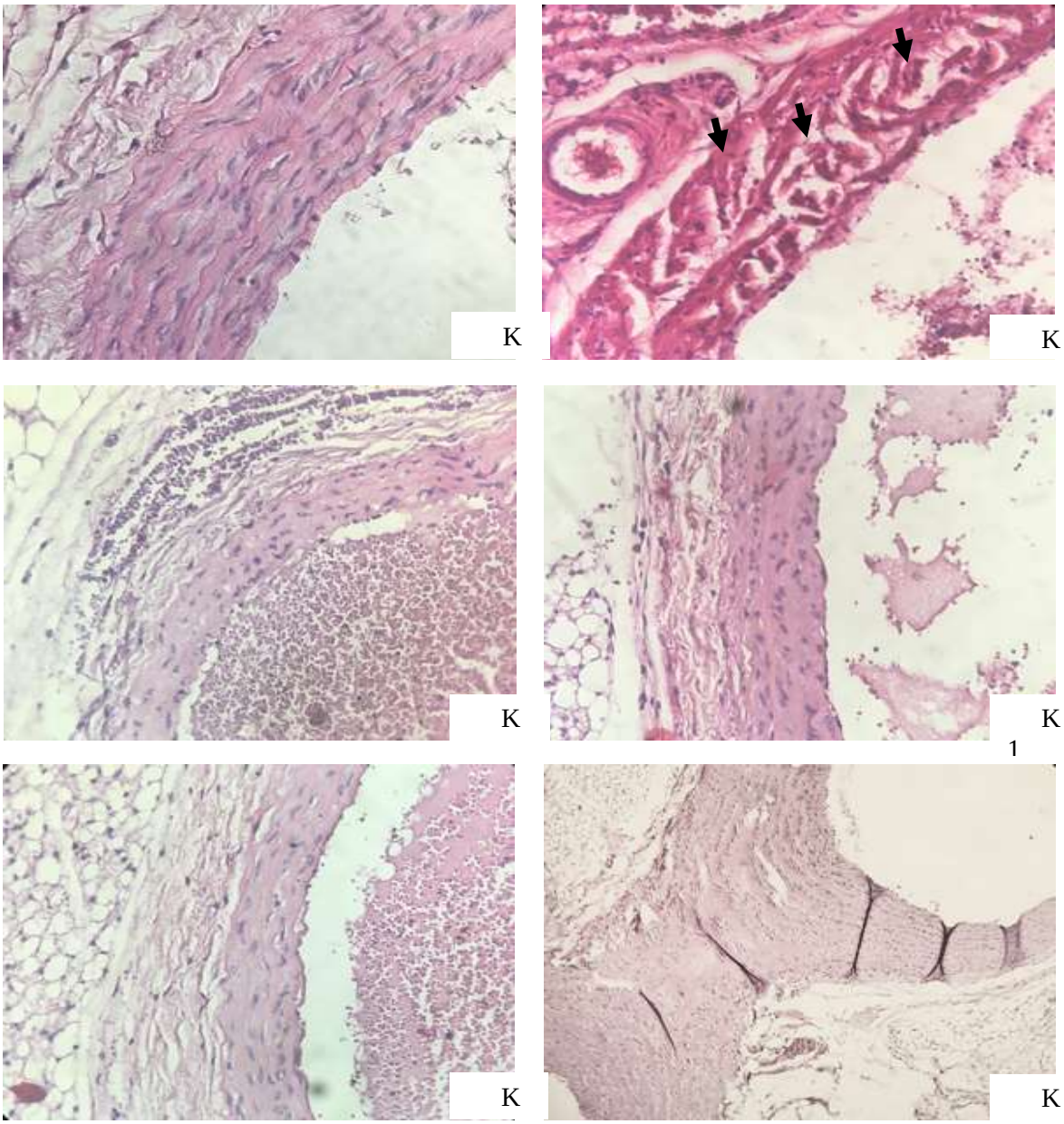

Figure 2. Histological description of aortic rat after 15 days administration of C. chayamansa leaves extract. The condition of weakening of smooth muscle cells is indicated by arrows. (HE staining, K0 and K+ 40x magnification, K-, K1-K3 40x magnification)

In general, administration of *C. chayamansa* leaves extract involves various active compounds as antihipolipidemia. This research focused on the optimum dose and new usage of *C. chayamansa*, from as an anti-inflammatory at the previous invention to be a potentially anti-atherogenic herbs in future. Based on the obtained results, we can understand that the *C chayamansa* potentially can be used as anti-hyperlipidemia and anti-hypercholesterolemia.

CONCLUSION

In this study, the administration of 400 mg/ KgBM/ day of C. chayamansa alcoholic leaves extracts is the most effective decreased total cholesterol, triglycerides, and LDL-C, and were able to increase the concentration of HDL-C in blood plasma. The administra-tion of a dose of 100-400 mg/ KgBB shows that the alcoholic leaves extract of C. chayamansa has a protective effect against excess cholesterol which may destroy the myocytes in the endothelial wall. Therefore, it is necessary to de-velop research to identify specific compounds that are likely to have the most effective therapeutic effect in reducing lipid levels in the blood. Further research also needed to determine how much safe-ty dose of the C. chayamansa leaves extract and its toxicity effects to the body.

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