Jurnal Teknokes
International: Rapid Review: Open Access Journal **Vol. 15, No. 3, September 2022, pp. 128-136** **ISSN: 2407-896**

RESEARCH ARTICLE **OPEN ACCESS**

Manuscript received July 26, 2022; revised August 23, 2022; accepted August 27, 2022; date of publication September 30, 2022
Digital Object Identifier (**DOI**): https://doi.org/10.35882/teknokes.v15i3.268

How to cite Vernanda Arsya Nabilla, Indah Lestari, Christ Kartika Rahayuningsih, and Juliana Christyaningsih, "Effectiveness Of Moringa Leaf Extract (Moringa oleifera) on Cadmium and LDL (Low-Density Lipoprotein) Cholesterol Levels in Blood as Indicators of Atherosclerosis in Cadmium (Cd) Induced White Rats (Rattus norvegicus) With Atomic Absorption Spectrophotometry Method (AAS)", Jurnal Teknokes, vol. 15, no. 3, pp. 128–136, September. 2022.

Effectiveness Of Moringa Leaf Extract *(Moringa oleifera)* on Cadmium and LDL (Low-Density Lipoprotein) Cholesterol Levels in Blood as Indicators of Atherosclerosis in Cadmium (Cd) Induced White Rats *(Rattus norvegicus)* With Atomic Absorption Spectrophotometry Method (AAS)

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ABSTRACT Moringa leaves (*Moringa oleifera)* have chelating agent properties that act as natural antioxidants in binding heavy metals and free radical scavengers, with cadmium being one of the heavy metals free radicals having toxic effects on the body. Exposure to cadmium can reduce the activity of lipoprotein lipase enzymes (LPL) which function in the triglycerides and free fatty acids catabolism process, and can increase cholesterol and triglyceride levels in the blood. This study aimed to determine the effectiveness of Moringa (*Moringa oleifera)* on Cadmium chloride-induced white rats (*Rattus norvegicus)* using an experimental research design with a quantitative analysis technique. All authors contributed and discussed the results and final draft of the manuscript. This study used the level of cadmium and LDL cholesterol for the dependent variable and the dose of Moringa leaf extract (*Moringa oleifera*) for its independent variable. The samples used were white rats (*Rattus norvegicus*) aged 8-12 weeks with a weight of 150-200 grams, along with blood drawn from the heart for the specimen. The researcher conducted the study at the Faculty of Veterinary Medicine, Airlangga University; Surabaya Health Laboratory Center; and Surabaya Bakti Analysis Laboratory from October 2021 to June 2022 using Atomic Absorption Spectrophotometry (AAS) and Chemical Analyzer BS-200. The results showed that the average value of cadmium and LDL cholesterol levels in white rats in treatment group 1 were 0.605 g/dL and 8.25 mg/dL, group 2 with 0.075 g/dL & 18.5 mg/dL; and group 3 were 0.08125 g/dL and 12 mg/dL. Based on the statistical analysis of the One-Way ANOVA test, the sig. *p-value* was > 0.05. Thus, the average value of cadmium and LDL cholesterol had no significant difference. Moringa leaf extract dose 600 mg/kgBW has good effectiveness as a chelating agent that can reduce LDL cholesterol due to exposure to heavy metal cadmium in *Rattus norvegicus*. This study recommends consuming fresh Moringa leaves (*Moringa oleifera)* as much as 66.522 grams per human/day as an additional antioxidant and chelating agent. In future research, moringa oleifera fresh leaves can be used as medicine according to the dose.

INDEX TERMS Atomic Absorption Spectrophotometry (AAS), Cadmium, Chemical Analyzer, LDL Cholesterol, *Moringa oleifera*, White Rats.

I. INTRODUCTION

Indonesia has entered the fourth industrial revolution era, which means an effort to transform towards integration by improving the online world and industrial production. In the industrial sector, heavy metal cadmium (Cd) is widely used as raw material or supporting material, with 75% of cadmium metal used for the battery industry, ceramics, paint, metal coating, and metal welding. Symptoms resulting from exposure to heavy metals are experienced subjective complaints including fatigue, dizziness, visual disturbances, and respiratory problems, which showed that most of the respondents experienced poisoning from the workplace in the industrial sector. Exposure to cadmium in the workplace can also increase ECG and blood pressure because it can damage the workers' cardiovascular system [1].

Sources of heavy metals are cadmium (Cd) derived from copper, smelting, nickel refining, fossil fuels, and phosphate fertilizers. Most absorption is through the respiratory tract and a lesser extent, through the gastrointestinal tract; the absorption is through the skin [2]. Cadmium is absorbed in the blood to bind Metallothionein (MT) and transported to the liver, heart, and kidneys, causing disease due to heavy metal exposure. Then, the bioaccumulation of cadmium that enters the body will be absorbed into the blood vessels and cause integrated endothelial dysfunction, loosening the endothelial bond. The endothelium will experience a release in the blood vessels called the Circulating Endothelial Cell (CEC). Endothelial cells are fat derived from cholesterol that forms lipoproteins which affect LDL and triglycerides against atherosclerosis [3]. A previous study Borné et al. (2017) stated that 34.5% of respondents who indicated atherosclerosis had significantly higher cadmium levels with an average ± SD: 0.53 ± 0.58 g/L

Exposure to cadmium can reduce the activity of the lipoprotein lipase (LPL) enzyme, which functions in the catabolism of triglycerides and free fatty acids, so that it can increase cholesterol and triglyceride levels in the blood. The results of the previous study Samarghandian et al. (2015) stated that cadmium administration could significantly increase triglycerides and LDL cholesterol serum levels with a decrease in HDL cholesterol. Low cadmium concentrations can also affect lipid profiles through lipid peroxidation.

If cadmium enters the body, it can potentially become toxic and cause potential dangers to the tissues and organs of the human body. Examples are the narrowing of blood vessels (atherosclerosis), blood pressure disorders (hypertension), red blood cell disorders (anemia), damage to the kidneys (creatinine), liver (SGOT-SGPT), heart (Coronary Heart Disease), and can cause death [6]. Exposure to cadmium in the body could increase the risk of coronary heart disease and blood cholesterol and *Circulating Endothelial Cells* (CEC).

Adapted from Siyu, Zhang, and Cheng (2021) showed that levels of cadmium were positively correlated with the blood, and can risk of cholesterol among other triglycerides and total cholesterol, which indicated that elevated levels may play an important in Cd-related CDV. The purpose of this research is to determine the effectiveness of Moringa (*Moringa oleifera)* on Cadmium chloride-induced white rats (*Rattus norvegicus)* using an experimental research design with a quantitative analysis technique.

Cadmium is a heavy metal that can enter the blood vessels and increase free cholesterol so that the plasma can increase the production of ROS (*Reactive Oxygen Species*). Increased ROS in the body can lead to reduced antioxidants as cell defenses and cause oxidative stress, which can cause inflammatory diseases. Antioxidants derived from animals and plants can counteract ROS and neutralize oxidants. The content of antioxidants are flavonoids, carotenoids, phenolics, phenolic acids, and minerals which can reduce oxidative stress on cells in the body [8].

One of the treatments for toxicity in the body is to utilize natural resources as antioxidants against heavy metals entering the body. Antioxidants are compounds that can counteract free radicals that enter the body, obtained from the body's metabolism, food and drink contamination, and air and environmental pollution. A study Renfan and Yang (2020) stated that green tea is an effective antioxidant in experimental animals and humans to reduce cholesterol levels in the blood.

(*Moringa oleifera*) is one of the natural resources of herbal plants found in Indonesia that has many benefits and is rich in nutrients. One of the most prominent parts of the Moringa plant is the leaves which contain antioxidant compounds [10]. Based on the phytochemical test, the antioxidant consists of tannins, flavonoids, saponins, alkaloids, and anthraquinone. Leaves (*Moringa oleifera*) have a high protein value, which is ten times higher in vitamin than carrots, seven times higher in vitamin C than oranges, 17 times higher in calcium than milk, and 15 times higher than bananas [11].

Utilization of antioxidants derived from Moringa leaves can be done with the Extraction method which is a process of separating a chemical compound using an appropriate solvent [12]. Moringa leaves (*Moringa oleifera*) contain 0.09% β-sitosterol, reducing cholesterol levels and LDL concentrations in serum plasma, and the most effective reduction in cholesterol value was by giving a combination of bay leaf extract and Moringa leaf in a 1:1 ratio due to the presence of flavonoid compounds.

Continuous exposure to cadmium in the body can have a toxic effect on health. Moringa leaves can act as antioxidants in counteracting free radicals of heavy metal cadmium and reduce cholesterol levels in the body. Thus, it is necessary to research and analyze the effectiveness of Moringa leaf (*Moringa oleifera*) against LDL cholesterol as an indicator of atherosclerosis in cadmium-induced white rats (*Rattus norvegicus*) using the AAS (Atomic Absorption

Spectrophotometer) method. This study aimed to determine the effectiveness of Moringa (*Moringa oleifera)* on Cadmium chloride-induced white rats (*Rattus norvegicus)* using an experimental research design with a quantitative analysis technique.

II. MATERIALS AND METHOD

This study was an experimental research with a quantitative analysis technique. The dependent variables wcadmium and LDL cholesterol level rol and the dose of Moringa leaf extract (*Moringa oleifera*) for its independent variable. The samples were white rats (*Rattus norvegicus*) aged 8-12 weeks, weighing 150-200 grams. The researcher used 24 white rats (*Rattus norvegicus*) from Federer's calculation formula for the research samples, divided into seven treatment groups. This study utilized blood drawn from the heart and conducted research at the Faculty of Veterinary Medicine, Airlangga University, Surabaya Health Laboratory Center, and Surabaya Bakti Analysis Laboratory from October 2021 to June 2022 using the Atomic Absorption Spectrophotometry (SSA) Chemical Analysis BS-200. The researcher also applied primary data obtained directly after conducting research in the laboratory. In collecting the data, the researcher collected and inspected the test materials.

A. EXPERIMENTAL ANIMALS GROUPING

- Placebo Group: A group of white rats induced with 2mL/day of CMC-Na.
- Negative Control Group: A group of white rats not induced with 3 mg/kgBW of cadmium chloride ($CdCl_2$) and given distilled water.
- Positive Control Group: Group of white rats induced by 3 mg/kgBW of cadmium chloride ($CdCl_2$).
- Gold Standard Group: Group of white rats induced with 3 mg/kgBW of cadmium chloride ($CdCl_2$) and given 9 mg/kgBW of Vitamin C.
- Treatment Group 1: White rats induced with 3 mg/kgBW cadmium chloride ($CdCl_2$) and given Moringa leaf extract (*moringa oleifera*) at a dose of 400 mg/kgBW.
-Treatment Group 2: White rats induced with 3 mg/kgBW of cadmium chloride ($CdCl_2$) and given Moringa leaf extract (*moringa oleifera*) at a dose of 500 mg/kgBW.
-Treatment Group 3: White rats induced with 3 mg/kgBW of cadmium chloride ($CdCl_2$) and given Moringa leaf extract (*moringa oleifera*) at a dose of 600 mg/kgBW.

B. INSTRUMENTATION

This research uses instrumentation in the form of Rotatory Evaporator, Atomic Absorption Spectroscopy (AAS) and Chemical Analyzer BS-200.

C. MORINGA LEAF EXTRACT

The leaves were cleaned using running water to clean and remove dirt on the leaves, dried in a way without being exposed to sunlight, and aerated at room temperature. After drying, the Moringa leaves were grounded into powder and then sieved to ensure that the sample is completely smooth. The extraction process used the maceration method. Then, the moringa leaf powder was macerated using 96% ethanol solution and put in a jar for 2 days, in a closed room and with no sunlight. The maceration results were filtered using Whatman filter paper. The residue was macerated for two days until the maceration extract looked colorless or transparent. The liquid extract was then evaporated using a water bath with a 40 degrees temperature to form a thick preparation, obtaining a constant weight.

D.VITAMIN C DOSAGE DETERMINATION

Orally, humans can only consume 500 mg/day of Vitamin C, with an average body weight of 70kg [13]. In this study, the researcher converted the dose of Vitamin C to match a rat weighing 200g. The Vitamin C given to white rats is 9mg of Vit C/200gBW rats/day.

E. THE 0,5% NA-CMC SOLUTION PREPARATION

The 0.5% CMC-Na solution preparation is by dissolving 50 mg of C with 10 mL of distilled water and stirring gently until homogeneous. Heat for 15 minutes until the solution is clear and gel-like, and add up to 100 mL of distilled water in a volumetric flask [14].

F. MORINGA LEAF EXTRACT PREPARATION

The researcher gave the concentration of Moringa leaf extract orally using a gastric probe with a volume of 2 mL but did not exceed the maximum limit of 3-5 mL. If the Moringa leaf extract given exceeds the limit, complications will occur. The worst possibility is that the experimental animal dies.

G. CADMIUM CHLORIDE PREPARATION

The cadmium induction in white rats used a solution of cadmium chloride ($CdCl_2$) at a dose of 3 mg/kgBW given for seven days. The researcher gave cadmium chloride to the white rats with an oral gastric probe which can reduce the activity of SOD (*Superoxide Dismutase*), an antioxidant that can reduce free radicals and cadmium chloride exposure [15].

H. EXPERIMENTAL ANIMALS TREATMENT

After the white rats adapted to the new environment, on the 8th day, the white rats were grouped randomly with four white rats in each group. The Normal group was not induced with cadmium but given distilled water. The researcher gave the placebo group 2 mL/day of CMC-Na, the positive group with 3 mg/kg BW of cadmium chloride ($CdCl_2$), and the Gold Standard group induced with 3 mg/kgBW of cadmium chloride ($CdCl_2$) and given a 9 mg/kgBW dose of vitamin C as an antioxidant. The three treatment groups were induced with cadmium using 3 mg/kgBW of cadmium chloride ($CdCl_2$), then given Moringa leaf extract with doses of 400 mg/kgBW,
500 mg/kgBW, and 600 mg/kgBW for seven days.

I. EXPERIMENTAL ANIMALS EUTHANASIA

After experimenting, on day 18, the white rats would be fasted for at least 8 hours before surgery. On the 19th day,

the researcher conducted the euthanasia process by killing the animals using humanely acceptable techniques [16].

J. BLOOD SAMPLING

The researcher attained the blood from the rat's heart after being anesthetized using an ether compound placed on a cotton swab and put in a closed container [16]. Around 2-4 mL of blood was taken and placed in two different tubes. The first tube contained an EDTA anticoagulant to check the blood cadmium levels. The second tube didn't have an anticoagulant in order to check the LDL cholesterol levels in the blood.

J. WET DESTRUCTION SAMPLES PREPARATION

Prepare the blood samples contained in the vacutainer tube and homogenize them by inverting the tube. Afterward, add 1 mL of concentrated nitric acid (HNO_3) to the homogeneous pipette. Then put it in the microwave with a temperature below 150 ° C for about 20 minutes. When the solution is clear, add heavy metal-free distilled water until the 50 mL mark, then read in SSA.

K. SSA STANDARD SOLUTION MEASUREMENT

To achieve the maximum wavelength, install a cadmium hollow cathode, turn on the SSA power button, and adjust the SSA lamp to match the metal analyzed. Set at a wavelength of 228.8 nm. To determine the metallic cadmium concentration present in the sample, the researcher used the wavelength obtained from the standard curve.

L. SSA SAMPLE EXAMINATION

Select the cathode lamp according to the analysis, then set the parameters to be analyzed. Enter the blank using Standard 1 ppm; 2 ppm; 3 ppm; 4 ppm; 5 ppm; 6 ppm; 7 ppm; and 8 ppm. Insert the sample until the curve rises, and perform a sample check on the next one by reinserting the blank. Then the tool will read and record the absorbance.

M. LDL CHOLESTEROL LEVELS EXAMINATION

The LDL cholesterol levels examination used the direct *homogenous enzymatic colorimetric assay* on serum in the blood. Serum samples were mixed with reagents, incubated at 37 degrees for 5 minutes and added a second reagent. Then incubated for another 5 minutes and read using a spectrophotometer at a wavelength of 600 nm [17].

N. ANALYSIS TECHNIQUE

The data analysis used in this study is normality and homogeneity tests. If the data is distributed and homogeneous, it will be continued with the One-Way ANOVA test to determine the significant difference between the two data. With a p-*value* of 0.05. If there are differences in the data, it is continued with *Post Hoc Test* to see which groups experience differences. If not distributed normally, then it is continued with the Kruskal Walis test to find out the differences in the groups. This study used the level of cadmium and LDL cholesterol for the dependent variable and the dose of Moringa leaf extract (*Moringa oleifera*) for its independent variable.

III. RESULTS

Research on the effectiveness of Moringa leaf extract (*Moringa oleifera*) on levels of cadmium and LDL cholesterol in blood as an indicator of atherosclerosis in cadmium chloride-induced white rats (*Rattus norvgicus*). The white rats were obtained from rat farms Jl. Kendalsari IV, Mojolangu, Lowokwaru, Malang, East Java. The experimental aimals used in this study were 28 white rats (Rattus norvegicus) which were grouped into 7 groups including Placebo Group (PL), Negative Control (N), Positive Control (KP), Treatment 1 (T1), Treatment 2 (T2) and Treatment 3 (T3), in each group there were 4 white rats. Based on the result of examination of cadmium levels in the blood of white rats cadmium chloride-induced used the Atomic Absorption Spechtrophotometer method can be seen in Table 1.

TABLE 1
Cadmium Levels in White Rats Blood

Sample Groups	Mean of Cadmium Levels µg/dL
Placebo	0,068
Negative Control	0,6725
Positive Control	0,10275
Gold Standard	0,0925
Treatment 1	0,0605
Treatment 2	0,075
Treatment 3	0,08125

Based on the results of the examination contained in Table 1 show the average levels of cadmium in blood of rats after being given treament. From the overall data obtained, the average value of cadmium levels in cadmium chloride-induced white rats based on the treatment group given Moringa leaf extract (*Moringa oleifera*) is shown in Figure 1.

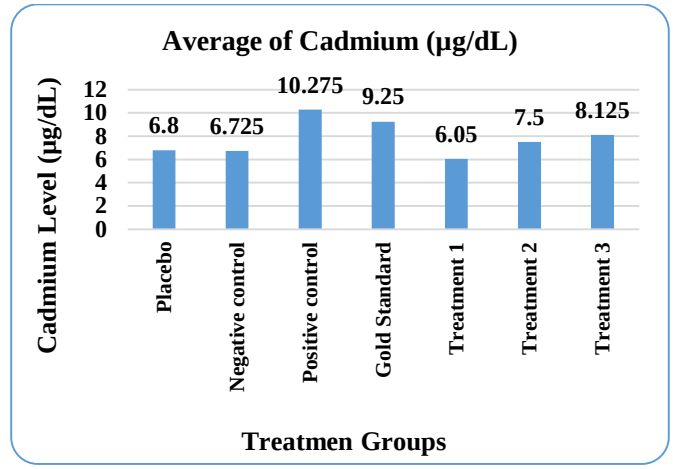

FIGURE 1 **Average levels of cadmium in the blood of White rats**

Figure 1 shows that the highest average cadmium level in the blood of white rats is in the positive control group with 0.10275 µg/dl and the lowest average is treatment 1 group with 0.605 µg/dl.

Based on the result of the examination of LDL Cholesterol levels in the blood of white rats cadmium chloride-induced used the Enzymatic Colorimetri method with BS-200 Chemistry Analyzer can be seen in Table 2

TABLE 2
LDL Cholesterol Levels in White Rats Blood

Sample Groups	Mean of LDL Cholesterol Levels mg/dL
Placebo	19
Negative Control	18,5
Positive Control	17,25
Gold Standard	21,75
Treatment 1	18,25
Treatment 2	18,5
Treatment 3	12

The average value of LDL cholesterol levels in cadmium chloride-induced white rats based on the treatment group given Moringa leaf extract (*Moringa oleifera*) is shown in Figure 2.

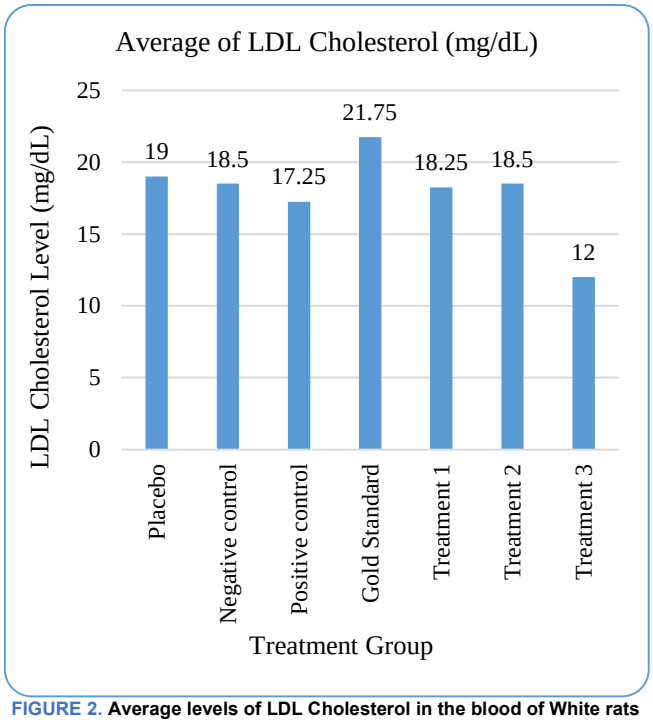

FIGURE 2. Average levels of LDL Cholesterol in the blood of White rats

Figure 2 shows that the highest average level of LDL choleterol in the blood of white rats is in the Gold standard group with 21.75 mg/dl and the lowest average is in treatment 3 group with 12 mg/dL.

A. ANALYSIS TECHNIQUE

This study used the level of cadmium and LDL cholesterol for the dependent variable and the dose of Moringa leaf extract (*Moringa oleifera*) for its independent variable.

1) NORMALITY TEST *SHAPIRO-WILK*

Test was used to determine whether the data obtained were normally distributed or not. The data obtained is the value of cadmium levels and LDL cholesterol levels in white rats induced by cadmium chloride and administration of Moringa*oleifera*. The normality test used is *Shapiro-Wilk* because the data used are 28 where <50. Based on the results output SPSS , a significant value was obtained on the examination of cdmium levels in each group having a significant value (p) *value* > 0.05, proving that Ho was accepted and Hi was rejected, which means the data obtained were normally distributed.

2) HOMOGENITY TEST *LEVENE*

Test Homogeneity test is used to determine whether several variants of the population have similarity values or not. Homogeneity test is a requirement in *One-Way ANOVA*. Based on the results *output*showed Homogeneity Test of Cadmium Levels and LDL Cholesterol Levels. The significant value of cadmium was 0.137 and the significant value of LDL cholesterol was 0.693. In both the homogeneity test results, the value (p) *value* > 0.05, which means that the variants of the seven treatment groups being compared have similarities or are homogeneous. Thus, the assumption of homogeneity in the *One-Way ANOVA* fulfilled and can be continued in the *One-Way ANOVA*

3) *ONE-WAY ANOVA*

Test *One-way ANOVA* used to compare the mean of the population to determine the significant difference between data groups. Based on *output* the SPSS *One-Way ANOVA* on cadmium levels obtained a significant value of 0.749 and on LDL cholesterol levels a significant value obtained was 0.297. In both the *One-Way ANOVA* the (p) *value* > 0.05, then the null hypothesis Ho was accepted, and Hi was rejected, which means that in the seven treatment groups there was no significant difference in the levels of cadmium and LDL cholesterol in the blood of white rats. From the data above, it is not continued with the *Turkey Post Hoc Test.*

III. DISCUSSION

This study aims to analyze the effectiveness of Moringa leaves (*Moringa oleifera)* with various concentrations used as thick extracts at doses of 400 mg/kgBW, 500 mg/kgBW, and 600 mg/kgBW on Cadmium and LDL Cholesterol levels as an indicator of atherosclerosis which is narrowing of the arterial walls. Due to lipoprotein accumulation, in white rats (*Rattus norvegicus)* cadmium-induced. In this study, the experimental animal was a White Rat (*Rattus norvegicus)* which was adapted to a new environment for 10 days by being fed and drinking 2 times a day before being given treatment. The conduction of white rats treatment was to see the effectiveness of Moringa leaf extract. The researcher

induced the white rats with 3 mg/kgBW of cadmium chloride (CdCl2) to increase the LDL cholesterol levels above the usual value or a hyperlipidemic state. It was to observe the effectiveness of Moringa leaf extract more clearly. Cadmium exposure can reduce the activity of the lipoprotein lipase (LPL) enzyme that functions in the triglycerides and free fatty acids catabolism, increasing cholesterol and triglyceride levels in the blood. Study Tinkov, (2018) stated that exposure to cadmium in the body increases the risk of coronary heart disease by increasing blood cholesterol and *Circulating Endothelial Cell* (CEC), thereby triggering atherosclerosis.

In the Moringa leaf extract production, the solvent used was the 0.5% CMC-Na which acted as a stabilizer and binder to the extract used. Based on a study Saryanti, Setiawan and Daryanto (2020) , CMC-Na as a *gelling agent* that has stability and is anionic can increase the concentration of gel preparations. The results in Fig. 1 show that the cadmium levels in each group of white rats induced with 3 mg/kgBW of cadmium chloride $CdCl_2$ have clinical differences. The average value of the Negative Control group was 0.06725 µg/dL which was the interpretation of the normal value of cadmium levels in white rats. The average value of the Placebo group treated with 2 mL/day of 0.5% CMC-Na was 0.068 µg/dL. The average value of cadmium in the Positive Control Group was 0.10275 µg/dL which had the highest value compared to other groups. In the Positive Control group, the treatment was the induction of 3mg/kgBW of cadmium chloride for 10 days with the oral sonde method. However, the average value of cadmium levels in the placebo and the negative control groups were not much different because these groups were no cadmium chloride induction treatments. Cadmium chloride is a compound of water-soluble cadmium salts, and is a hydrate mixture with a purity level of 95% to 99%. It is also a carcinogenic agent in animals. Cadmium induces lipid peroxides by stimulating the production of superoxide anions and inhibiting antioxidants such as Glutathionin Peroxide and Superoxide Dismutase, resulting in the accumulation of free radicals in the body, which causes disturbances in lipid metabolism, including LDL cholesterol in the blood and the body [20].

Based on the results of LDL cholesterol levels in the Placebo group, the level of LDL cholesterol was 19 mg/dL. The Negative Control group has a value of 18.5 mg/dL. The researcher could not compare the two groups with the treatment group because the treatments were different. The Placebo group's treatment was 2 mL /day of CMC-Na 0.5%. The Treatment group was given 3 mg/kgBW of cadmium chloride and Moringa leaf extract with doses of 400 mg/kgBW, 500 mg/kgBW, and 600 mg/kgBW. The Positive control group had 3 mg/kgBW of cadmium chloride with a value of 17.25 mg/dL, which was lower than the placebo and the negative control groups. It is due to the high level of consumption in the placebo and negative control groups compared to the positive control group. The food consumed by the white rats was the Pur Ayam 511, with nutritional content of 21-23% crude protein, 5-10% fat, and 3-5% crude fiber. The protein in the food is included in animal protein. The higher the level of animal protein consumption, the higher the LDL cholesterol will be, which can cause atherosclerosis [21]. Protein and fat contained in the food broke down into energy, free fatty acids, cholesterol, triglycerides and phospholipids in the intestine. Then it will enter the blood and will go to the liver. In the liver, cholesterol will be transported by LDL lipoproteins and distributed to body cells that need it. LDL contains cholesterol esters with high concentrations and is atherogenic. These results were per previous research Soliman, (2020) that stated that the higher the intake of food in the form of protein and fat that is used as energy, the higher the value of LDL cholesterol levels in the blood will be.

Moringa plant is one type of plant that has a high protein content. The content of compounds owned by Moringa is rich in nutrients including amino acids, vitamins, potassium, and antioxidants [23]. The researcher obtained the Moringa leaves (*Moringa oleifera*) from the Wonokusumo Tengah area, Semampir District, Surabaya City, where they were a mixture of young and old Moringa leaves. Following the process of taking Moringa leaves was the process of separation between Moringa leaves and stems, then washed using running water, followed by the process of drying them so that they become simplicia in the form of powder. Moringa leaves were dried by aerating under the shade with a room temperature range (25°C-30°C). After drying, it was mashed using *a chopper blender* and then sifted until it became a powder. The researcher used the maceration method to conduct the extraction process because it was easy to use. Moreover, it did not require a heating process, so the flavonoid compounds were not damaged and were thermolabile [24]. The extraction process used 96% ethanol solvent in a ratio of 1:10 because it is included in a non-toxic solvent and had good absorbance so that it could inhibit the growth of microorganisms [25].

Preparation of Moringa leaf extract at a dose of 400 mg/kgBW, 500 mg/kgBW and 600 mg/kgBW was adjusted to the body weight of each rat and the average body weight of each group of white rats was calculated before being given treatment. This is in accordance with research Pitchford and Smith (2018) which stated that all rats were weighed before being tested and the average was determined in each group to determine the dose.

The results of the study showed that the cadmium levels in each group of white rats induced with 3 mg/kgBW of cadmium chloride have clinical differences. In Treatment Group 1 (P1), the average cadmium level was 0.605 µg/dL,

Treatment Group 2 (P2) was 0.075 µg/dL, and Treatment Group 3 (P3) was 0.8125 µg/dL There was a decrease compared to the Positive Control group with 0.10275 µg/dL though it was not that significant. Treatment Group 1's blood cadmium levels average decrease had a slight value difference with the Placebo and Negative Control groups. The insignificant decrease indicated that the administration of Moringa leaf extract was still insufficient. Thus, the cadmium levels decrease was not maximal. Moreover, the highest cadmium levels decrease was in the Treatment group, with the dose of 400 mg/kgBW. An increase in cadmium levels in the treatment group given Moringa leaf extract with three doses of 400 mg/kgBW, 500 mg/kgBW and 600 mg/kgBW could occur due to some factors. One was during the ten days of treatment, where there was no maximization of cadmium metabolism. Previous study Tinkov et al. (2018) showed that the induction of 15 ppm cadmium chloride for 30 days could significantly increase blood cadmium levels. The long half-life of cadmium is 30 years because the accumulation of cadmium in the body is long. Cadmium in the blood can be deposited for 75 to 128 days for 3 to 4 months. Cadmium will be transported into the blood which then binds to red blood cells and proteins with high mul weight in plasma, specifically albumin [27].

Fig. 2 shows the average results of LDL cholesterol levels. The Positive Control Group had an average of 17.25 mg/dL, while Treatment group 1 with an extract dose of 400 mg/kgBB had an average of 18.25 mg/dL, and Treatment group 2 with a dose of 500 mg/kgBW had the average of 18.5 mg/dL. It showed an increase between the Positive Control Group and Treatment Groups 1 and Treatment 2. This increase could occur because of the high-stress level in the treatment group because the experimental animals underwent decoding twice a day compared to the positive control group that had it once. Too much sonde can increase stress in experimental animals and affect LDL cholesterol, causing a high increase in blood cholesterol compared to normal and controlled stress levels. When stressed, the response is by secreting cortisol and epinephrine hormones from their adrenal glands. High epinephrine hormone could cause the secretion of VLDL (*Very Low-Density Lipoprotein*) and LDL(*Low-Density Lipoprotein*) to increase In addition to the decoding factor, cages, maintenance, handling, body weight measurement, and cage cleaning could also contribute to the stress in rats.

Based on the study results, Treatment Group 3 with 600 mg/kgBW of moringa leaf extract had the lowest LDL cholesterol level compared to all groups. Fig. 2 shows that the average value of LDL cholesterol levels in Treatment 3 group was 12 mg/dL. The decrease in LDL cholesterol levels indicated that the administration of 600 mg/kgBW of moringa leaf extracts reduced LDL cholesterol after induced by cadmium chloride. Previous study Lacorte et al. (2022) stated that 300 mg/kgBW of Moringa leaf extract for 90 days could significantly reduce LDL cholesterol. Moringa leaf extract contains antioxidants such as Vitamin C, polyphenols, flavonoids, and carotene. Vitamin C is the highest antioxidant that acts as an inhibitor in inhibiting the oxidation of reactive free radical reactions so that it is relatively stable. Vitamin C and Vitamin E can stop free radical chain reactions, Vitamin E will capture free radicals and convert them into Vitamin E radicals. Then, Vitamin E will combine with Vitamin C to inhibit the process of oxidative reactions and bind Vitamin E when free radicals are damaged. The bounded vitamin E is then converted into free Vitamin E and restores its function as an antioxidant [29]. Beta-carotene in Moringa leaf extract protects lipid membranes from peroxidase and stops free radical chains. Beta-sitosterol content lowers cholesterol levels by decreasing LDL concentrations in plasma and inhibiting cholesterol reabsorption from endogenous sources. The content of flavonoids and polyphenols plays a role in increasing superoxide dismutase and catalase. Thus, the levels peroxidase and cholesterol decrease [30].

The normality test results showed that the distribution was normal, with p-*value* > 0.005 for cadmium and LDL cholesterol levels. Then, for the homogeneity test, a significant p-value of 0.137 > 005 for cadmium levels and LDL cholesterol levels was 0.693 > 0.05, indicating that the data are homogeneous. In the One-way ANOVA test, the results obtained significant values for cadmium levels p 0.749 <0.05 and cholesterol levels p 0.292 <0.05. It means that there was no significant difference between the two data. It is due to the ten days of treatment, resulting in the metabolism between cadmium and LDL cholesterol was not maximal. Moringa leaf extract can significantly reduce LDL cholesterol with a 14 days treatment because the steroid component acts as an inhibitor that work and inhibit adipocytes, reducing LDL cholesterol [28].

Based on the study results, a fresh dose of 600 mg/kgBW Moringa (*Moringa oleifera*) leaves utilized for human life reduced LDL cholesterol in cadmium-induced white rats compared to 400 mg/kgBW and 500 mg/kgBW dosage. The production of Moringa leaf extract used 500 grams of fresh leaves which are then macerated and evaporated to produce 50,504 grams of thick extract. In Moringa leaf extract (*moringa oleifera*) treatment, the researcher weighed 600 mg/kgBW of Moringa leaf extract for seven days with four white rats (*Rattus norvegicus*). The total was 2,961 grams of thick extract. Thus, the Moringa leaf extract obtained in one rat was 0.10575 grams/day. If implemented using a dose of fresh Moringa leaves, the weight of Moringa leaf extract was 1.1879 grams/200 grams/rat. The utilization of 600 grams of fresh Moringa leaves in humans is converted to an average human body weight of 70 kg, so the fresh Moringa (*Moringa oleifera)* needed is 66.522 grams per human/day.

The limitation of this research are the treatment time too short so the effectiveness of moringa leaves extract have not seen maximum, because the half-life of cadmium in bllod is about 2-3 months and because the steroid component acts as an inhibitor that works and inhibit adipocytes can't lower LDL cholesterol to the maximum. Thus, these facst may have affected the results of this study. In futere research, moringa oleifera fresh leaves can be used as medicine according to the dose and longer treatment time.

From the discussion above, the researcher concluded that the distribution of Moringa leaf extract at a dose of 600 mg/kgBW successfully reduced LDL cholesterol in cadmium-induced white rats. Although it was not significant in each group, the Moringa leaf (*Moringa oleifera*) acts as an antioxidant in preventing oxidative stress. It has oxidative properties so that free radicals will oxidize antioxidants and protect other molecules from oxidation damage by free radicals and reduce Lipid Peroxide (LPO) [31].

IV. CONCLUSION AND SUGGESTION

This study aimed to determine the effectiveness of Moringa (*Moringa oleifera)* on Cadmium chloride-induced white rats (*Rattus norvegicus)* using an experimental research design with a quantitative analysis technique.The levels of cadmium (Cd) and LDL cholesterol in the blood of white rats at a dose of 400 mg/kgBW were 0.0605 g/dL and 18.25 mg/dL. The levels of cadmium (Cd) and LDL cholesterol in the blood of white rats at a dose of 500 mg/kgBW were 0.075 g/dL and 18.5 mg/dL. The levels of cadmium (Cd) and LDL cholesterol in the blood of white rats at a dose of 600 mg/kgBW were 0.08125 g/dL and 12 mg/dL. At a dose of 600 mg/kgBW, it was best effective in lowering LDL cholesterol compared to doses of 400 mg/kgBW and 500 mg/kgBW. The antioxidant content of Flavonoids and Vitamin C played a role in preventing oxidative stress, which has oxidative properties so that free radicals will oxidize antioxidants and protect other molecules from oxidative damage by free radicals, and can reduce Lipid Peroxide.

The researcher's intention in conducting this study is to determine the functions and benefits of Moringa leaves (Moringa oleifera) and consuming fresh Moringa leaves (Moringa oleifera) as much as 66.522 grams per human/day as an additional antioxidant and chelating agent against the incidence of atherosclerosis in lowering LDL cholesterol from exposure to heavy metal cadmium.

For further researchers, they can develop and conduct further research on the effectiveness of Moringa oleifera with lipid profile parameters, including Total Cholesterol, HDL Cholesterol, LDL Cholesterol, and Triglycerides by a longer treatment time. Institutions can implement community service by educating the public about the functions and benefits of Moringa leaf extract (*Moringa oleifera*) as an additional antioxidant and chelating agent in the body.

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