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The Effectiveness of *Moringa Oleifera* Leaf Extract on Hepatotoxic Case Reviewing from Cadmium, SGOT and SGPT Blood Levels in White Rats *(Rattus Norvegicus*) Induced with Cadmium (*Cd*)

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ABSTRACT Moringa leaf (*Moringa oleifera* Lamk.) is part of herbal plants that have been known for their good natural chelating agent and hepatoprotective properties, especially in hepatotoxic events due to cadmium (Cd) exposure, where flavonoid compounds are contained in the form of quercetin and kaempferol, vitamin A, vitamin C and several phenol components that can inhibit the accumulation of cadmium metals and neutralize free radicals by lowering *Serum Glutamic Oxaloacetic Transaminase* (SGOT) and *Serum Glutamic Pyruvic Transaminase* (SGPT) levels, so this study aimed to determine the effectiveness of moringa leaf extract (*Moringa oleifera* Lamk.) against hepatotoxic in white rats (*Rattus norvegicus*) induced with cadmium. This study was expected to provide potential of Moringa leaves at dose 400 mg/kgbw, 500 mg/kgbw and 600 mg/kgbw in white rats to follow up the effective dosage (500 mg/kgbw) on previous hepatotoxic case in short treatment period. This research was an experimental study with quantitative analysis conducted at the Faculty of Veterinary Medicine, Airlangga University, Surabaya Health Laboratory Center, and Bakti Analisa Laboratory in November 2021 – July 2022. The independent variable was the dose of Moringa leaf extract and the dependent variables were levels of cadmium, SGOT and SGPT in white rat blood. Analysis of blood cadmium levels using an atomic absorption spectrophotometer and analysis of SGOT SGPT levels using the BS-200 Chemistry Analyzer. The results showed that the average blood cadmium, SGOT and SGPT levels in treatment group 1 were 0.0605 µg/dL, 260.175 U/L, and 143.85 U/L; in treatment group 2 were 0.075 µg/dL, 250.925 U/L and 77.575 U/L; in treatment group 3 were 0.08125 µg/dL, 171.475 U/L and 66.075 U/L. Based on statistical analysis of One-way Anova test, sig. p value > 0.05, there was no significant difference in cadmium and SGOT levels. Then, the results of the Kruskal wallis test on SGPT levels showed sig. p value > 0.05, there was no significant difference in all treatments. Moringa leaf extract at a dose of 600 mg/kgbw/day had the best effect as chelating agent and hepatoprotective compared to doses of 400 mg/kgbw/day and 500 mg/kgbw/day at cadmium induction for 10 days. Eventhough, there was no differencies in blood cadmium, SGOT and SGPT levels at those group that were given effective dose of moringa leaf extract (*Moringa oleifera* Lamk.) against hepatotoxic in white rats induced with cadmium.

INDEX TERMS Moringa Leaves, Cadmium, SGOT, SGPT, Rattus norvegicus, Atomic Absorption Spectrophotometer, Chemistry Analyzer

I. INTRODUCTION

In providing the food needs of the Indonesian people who continue to experience dynamic development in their population, it is necessary to increase crop yields in the agricultural sector. The agricultural soils, especially in vegetables, can be contaminated with cadmium metal in high concentrations due to the intensive use of phosphate fertilizers[1]. Researched by Park et. al (2021) shows a total cadmium concentration of 251 µg/kg exceeding the maximum limit set by the Korean Food and Drug Safety Agency (200

µg/kg) as a result of using phosphate fertilizers (NPK) on rice farmland[2]. Food (cereals, vegetables, rice) fruit juices can be the main source of cadmium metal pollution in humans due to soil pollution[3][4]. In addition, the consumption of tobacco cigarettes (nicotiana species) releases cadmium metal content from polluted soils[5].

Cadmium is a toxic metal that is dispersed in waters and terrestrial environments, where cadmium metal has the ability to be present in the environment for a long period, is non-degradable and toxic in nature[6]. Cadmium entering the human body can bind to metallothionein in the blood and will accumulate in the kidneys and liver, where there is no efficient mechanism in the process of cadmium excretion from the body[7]. Cadmium can induce lipid peroxidation by stimulating the production of superoxide anions, inhibiting antioxidants (glutase peroxidase and superoxide) and forming free radicals that cause cell damage and the occurrence of chronic diseases[8]. Stress-oxidative from free radicals produced by some chemical procedures in damaging proteins, lipids, DNA can cause poisoning of the liver organs (Hepatotoxic). Some of the impacts on hepatotoxic events are the onset of rheumatoid disease, cancer, degenerative aging processes, cardiovascular diseases and arthritis[9].

Several antioxidant components in natural resources can effectively reduce stress-oxidative due to cadmium toxicity, one of which is the Moringa oleifera plant[10]. Moringa leaves or *Moringa oleifera Lamk*. belonging to the family Moringaceae is known by people in Southeast Asia as a magical plant[11]. The plant M. oleifera is called a miracle plant because on the part of the flower, leaves, seeds it is clinically beneficial[12]. Moringa oleifera is known to have antioxidant, antiiflammatory, hypolipidemia, antihyperglycemia, anticancer, antihypertensive and hepatoprotective ability activities[13]. Flavonoid compounds (Quercetin and kaempferol), vitamin A and ascorbic acid contained in Moringa leaves (Moringa oleifera) have hepatoprotective activity[14]. Quercetin has been reported to have strong free radical collection activity and chelating capacity of metals, especially for iron and cadmium[10]. Antioxidant activity and hepatoprotective potential by Moringa leaves are related to the presence of total phenolics and flavonoids in isolated active extracts or constituents—β-sitosterol, quercetin and kaempferol which have hydroxyl groups that will easily donate electrons to free radicals and neutralize them effectively. The hydroxyl group also increases its antioxidant potential through intermolecular hydrogen bonds involving the -SH group of non-protein thiols and enzymes resulting in an improvement of the antioxidant system against oxidative damage to mammalian liver tissue[11]. Khalofat et. al research (2020) showed an increase in non-enzymatic antioxidants such as glutathione (GSH), ascorbic acid (ASA) and antioxidant activity (superoxide dismutase, catalase, peroxidase) in the cadmium-induced treatment group after administration of Moringa leaf extract[15].

Studies with animal models with oxidative stress markers and correlations with antioxidant properties in vivo and in vitro have been reported, though not extensively. In in vivo studies, the antioxidant ability of leaf extract has the potential to improve antioxidant status and reduce lipid peroxidation at certain doses. While in vitro showed high antioxidants, it showed a protective effect on the incidence of Reactive Oxygen Species (ROS) [16]. Damage caused by stress-oxidative can significantly increase aspartate aminotransferase (AST/SGOT) and alanine aminotransferase (ALT/SGPT) which are used as indicators of cell damage, particularly in liver and heart cells [17]. Saleh's research (2019) showed that cadmium administration increased levels of SGOT, SGPT, creatinine, urea significantly. Meanwhile, in the treatment group with M. oleifera extract, there was a decrease in superoxide dismutase[18]. Research by Toppo et. al (2015) showed oral administration of cadmium-chloride 200 ppm/kg for 28 days, there was a significant increase in the production of SGOT and SGPT, while in the administration of Moringa oleifera 500 mg/kgBB there was a significant decrease in SGOT and SGPT[14].

Some of these studies have shown the hepatoprotective ability of *Moringa oleifera* Lamk. by reviewing from SGOT and SGPT as indicators of damage in liver cells. The oral administration of Moringa oleifera extract can have drawbacks where at too much dose it can give discomfort. Thus, it is necessary to conduct research in determining the effective dose of *Moringa oleifera* extract in reducing hepatotoxic case after 7 days treatment, it was shorter than the treatment period in previous study due to exposure to cadmium through white rat (Rattus norvegicus) as an experiment animal. So, we can find the weight of *Moringa oleifera* leaf that can be consumed by human daily as a metal chelating agent and natural additive antioxidants (hepatoprotective properties) in treating acute liver damage.

II. MATERIALS AND METHOD

The type of research was an experimental study with quantitative analysis, which aimed to analyze the effectiveness of giving Moringa leaf extract (*Moringa oleifera*) to hepatotoxic by reviewing blood cadmium, SGOT and SGPT levels in white rats induced with cadmium. The study used male white rats (*Rattus norvegicus*) with body weight of about 150-200 g. The rats used came from a white rat farm on Jl. Kendalsari IV, Lowokwaru District, Malang. The sample of this research were 28 white rats as an experiment animals, which would be randomly divided into 7 groups. The Moringa leaf extraction process was carried out at the Toxicology laboratory, Campus, Department of Medical Laboratory Technology, Jl. Karang Menjangan no. 18A, Surabaya city. The research place for giving the treatment of animals was carried out at the Faculty of Veterinary Medicine, Universitas Airlangga Campus C Mulyorejo Surabaya. The examination of blood cadmium in animals was tried to be carried out at the Surabaya Health Laboratory Center, Jalan Karang Menjangan No. 18, Surabaya City. The SGOT SGPT determination of animal

serum was carried out at the Bakti Analisa Laboratory, Jl. Joyoboyo No. 50, Wonokromo District, Surabaya City. This research was conducted in November 2021 – July 2022.

A. EXPERIMENTAL ANIMALS GROUPING

The Placebo Group was a group of white rats induced with CMC. Na as much as 2 mL/day. The Negative Control Group was a group of white rats without cadmium induction and given distilled water. The Positive Control Group was a group of white rats induced with 3 mg/kgbw of cadmium chloride ($CdCl_2$). The Gold Standar Group was a group of white rats induced with 3 mg/kgbw of cadmium chloride ($CdCl_2$) and 9 mg/kgbw of vitamin C. The Treatment Group 1 was a group of white rats induced with 3 mg/kgbw of cadmium chloride ($CdCl_2$) and given Moringa leaf extract at dose of 400 mg/kgbw/day. The Treatment Group 2 was a group of white rats induced with 3 mg/kgbw of cadmium chloride ($CdCl_2$) and given Moringa leaf extract at dose of 500 mg/kgbw/day. The Treatment Group 3 was a group of white rats induced with 3 mg/kgbw of cadmium chloride ($CdCl_2$) and given Moringa leaf extract at dose of 600 mg/kgbw/day.

B. PREPARATION OF MORINGA LEAF WITH EXTRACTION

The moringa leaf extraction process uses the maceration method with a 96% ethanol solvent. Moringa leaves are collected and disorted until green Moringa leaves are obtained and in good condition. Then it is washed with water until clean. Furthermore, drying is carried out by drying at room temperature (30 - 35°C). Dried Moringa leaves are mashed using a blender until they are powdered. Moringa leaf powder is separated from the still rough part. A total of 100 grams of Moringa leaf powder was weighed and put in a maceration jar. Furthermore, 96% ethanol solvent of 1000 mL is added to the maceration jar. The maceration jar is tightly closed and sheathed with aluminum foil. Furthermore, it is allowed to stand for 48 hours. The result of maceration is filtered using filter paper to obtain residue. Then remaseration is carried out on the residue with the same procedure until a clear maceration result was obtained. The extraction results in the form of liquid were evaporated using a rotary evaporator found at the Faculty of Animals, Universitas Airlangga until a pure Moringa leaf extract was obtained [19].

C. PREPARATION OF MORINGA LEAF EXTRACT FOR THE TREATMENT

Before making Moringa leaf extract (MO), it is necessary to measure bodyweight of rats in each treatment group. This study used Moringa leaf extract at a dose of 400 mg/kgbw, 500 mg/kgbw, 600 mg/kgbw. Then the calculation of the pure Moringa leaf (MO) extract mass will be given according to the treatment group used the following formula (1):

$$M\ \ (g) = M\ \ d\ \ \left(\frac{m}{k}\right) \times r_i\ \ b\ \ (g) \times \Sigma\, r_i\ \ \times d_i\ \ (d\ \ ) \quad (1)$$

MO dose: was the dose of Moringa oleifera extract (mg/kgbw) that given in each treatment

Rats bw : indicated the average body weight of rats in each group (gram).

$\Sigma\, r_i$: indicated the number of rats in each group.

Duration : was the period of giving the treatment (day).

The pure Moringa leaf extract that have been weighed was dissolved with CMC Na. 0.5% before giving to experimental animals. The calculation of Moringa leaf extract uses a percent administration of 1% (1 mL/ 100 g bw) which is commonly used for oral administration of extracts. The concentration of Moringa leaf ethanol extract was given orally using a gastric sonde volume of 2 mL which did not exceed the maximum volume of the rat stomach, which was 3-5 mL. Meanwhile, the calculation of the dose of vitamin C as the gold standard in treating hepatotoxic was based on the recommended dose to humans for consumption and then converted to rats. The dose of vitamin C in humans to against physiological disorders in organs was 500 mg/day[20]. So that the dose of vitamin C for each rat was 9 mg/day based on conversion factor from human to white rat.

D. TREATMENT OF EXPERIMENTAL RATS

Experimental animals was treated in food and environment adaptation for 10 days. On 11th day, each group was given a 3 mg/kgbw $CdCl_2$ solution orally for 3 days, except the negative control group and the placebo group. Then the negative control was not given treatment, the positive control group was still given $CdCl_2$ 3 mg/kgbw and treatment groups 1, 2 and 3 that had been induced $CdCl_2$ 3 mg/kgbw were given Moringa leaf extract orally according to the prescribed dose for 7 days. Placebo group was given CMC-Na treatment. The group that became the gold standard was given vitamin C as an antioxidant at dose 9 mg/day for 7 days.

E. BLOOD SAMPLING OF EXPERIMENTAL RATS

Blood samples were collected into EDTA tubes and anti-coagulant tubes after the treatment period was complete from the heart puncture. Blood samples were taken directly from the left ventricle of the heart after anesthesia by inhalation using isofluran through the drop jar. The collected blood samples were stored at room temperature for 30 minutes. In obtaining serum, centrifugation is carried out at 5000 rpm for 15 minutes, then the SGPT SGOT enzyme examination could be carried out[21].

F. PREPARATION OF BLOOD WITH WET DESTRUCTION METHOD

Picked up 1 mL of blood sample and put into nessler tube. Furthermore added 10 mL HNO_3 concentrated as the solvent. Then put back in the microwave to warm the temperature below 160°C, heating time adjust the sample. Left overnight to dissolve perfectly. Once the solution was completely dissolved, removed it from the microwave and added aquades as much as 10 mL. Then it was poured on the nessler tube and added metal-free aquadest up to the 50 mL mark. Furthermore, absorbance measurement is carried out using the

Atom Absorption Spectrophotometer.

G. SOLUTION PREPARATION FOR BLOOD CADMIUM ANALYSIS

Solution preparation in conducting the blood cadmium analysis consists of the preparation the standard solution and blank solution.

1. Preparation of Standard Solution (0.02 µg/L; 0.04 µg/L; 0.06 µg / L; 0.08 µg/L; 0.1 µg/L)

The standard solution used comes from the Merck brand cadmium (Cd) master solution of 1000 ppm, then dilution with aquadest is obtained until the desired concentration is obtained. At the atomic absorption spectrophotometer measurement with carbon furnace atomization method uses standard solutions with concentration series of 0.02 µg/L; 0.04 µg/L; 0.06 µg/L; 0.08 µg/L; 0.1 µg/L in accordance with SNI 06-6989.38 – 2005.

2. Preparation of Blank Solution (0 µg/L)

Blank solution used in cadmium metal measurements using metal-free aquadest[22].

H. DETERMINATION OF CADMIUM LEVELS IN BLOOD USING ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS)

Determine the maximum wavelength first by ensuring that the cadmium hollow cathode lamp was installed and then turning on the power button on the AAS Thermo Scientific, adjusting the lamp according to the metal analyzed through the SSA software system. The wavelength of 228.8 nm was used for measuring the cadmium absorbance. The standard solution measurement against calibration blanks resulting in a calibrations curve. The wavelength of the curve was used to measure the concentration of cadmium level in the samples. Next, we aspirated the samples and determined the concentration using calibration curve with three AAS replicate measurements based on SNI 06-6989.38 – 2005[23].

I. DETERMINATION OF SGOT AND SGPT LEVELS IN BLOOD OF EXPERIMENTAL ANIMAL

Ensure that the BS – 200 Chemistry Analyzer instrument had been checked with SGOT SGPT serum control and showed good maintenance results. Then input the sample identity in the BS – 200 Chemistry Analyzer software. Furthermore, it examined the parameters of SGOT (AST) and SGPT (ALT) on the sample serum[14].

J. STATICTICAL ANALYSIS OF RESEARCH DATA

Data analysis in this study was carried out by testing the normality by the Shapiro-Wilk method and the homogeneity by the Levene method. When the distributed data was normal and homogeneous, continued with One Way Anova to find out the significant differences between treatment groups. Statistical testing was carried out at a confidence degree of 95%. If there were differences, a follow-up Post Hoc Tukey HSD test was carried out to analyze where the significant differences were found in the data. However, when the data was not normally distributed and inhomogeneous, a non-parametric test of the Kruskal-Wallis method and the Mann Whitney test determined that there were differences between groups.

III RESULTS

This study was conducted on 28 white rats (Rattus norvegicus) as experimental animals obtained from white rat farms, Jl. Kendalsari IV, Lowokwatu District, Malang City with certain criteria. Before grouping white rats, it was necessary to examine weight rats for determining the dose of treatment given after the adaptation period was over. Then the treatment was carried out for 10 days on experimental animals and carried out a determination of blood cadmium, SGOT and SGPT levels. Research data on cadmium (Cd) levels in white rats (*Rattus norvegicus*) were obtained from treatments in the form of cadmium chloride ($CdCl_2$) induction and Moringa leaf extract (Moringa oleifera) at a dose of 400 mg/kgbw, 500 mg/kgbw, 600 mg /kgbw and Vitamin C according to the treatment of each group. Based on the examination of cadmium (Cd) levels in white rat blood specimens using an atomic absorption spectrophotometer at the Surabaya Health Laboratory Center, the mean results of cadmium (Cd) levels in the blood of white rats were obtained which were presented in the Table 1.

TABLE 1
Blood Cadmium (Cd) Levels in White Rats Blood

Sample Groups	Mean of Cadmium Levels (µg/dL)
Placebo (PL)	0,068
Control Negative (N)	0,06725
Control Positive (KP)	0,10272
Gold Standar (GS)	0,0925
Treatment 1 (P1)	0,0605
Treatment 2 (P2)	0,075
Treatment 3 (P3)	0,08125

Based on the results of cadmium determination in Table 1, it could be seen the average of blood cadmium (Cd) levels of white rats in each treatment group based on the treatment of vitamin C and Moringa leaf extract. The average cadmium level in the blood of white rats as a Negative Control (N) was 0.06725 µg/dL interpreting the normal value of cadmium levels in white rats. Average cadmium levels in the Placebo (PL) group with CMC. Na orally sonde 2 mL/day was 0.068 µg/dL, which was above the negative control mean as a normal group. The highest average cadmium blood level after treatment was 0.10275 µg/dL, which was found in the treatment group as a Positive Control (KP).

The average blood cadmium level in the Gold Standard (GS) group was 0.0925 µg/dL with vitamin C treatment being above the negative control average as a normal group. The lowest average result of cadmium levels in the blood after treatment was 0.0605 µg/dL, which was found in treatment group 1 (P1) with moringa leaf extract administration of 400 mg/kgbw/day and these levels were below the negative control average as a normal group. The average level of cadmium in the blood of Treatment Group 2 (P2) was 0.075

µg/dL with Moringa leaf extract treatment of 500 mg/kgbw/day being above the average of the Negative Control group as a normal group. The average level of cadmium in the blood of treatment group 3 (P3) was 0.08125 µg/dL with Moringa leaf extract treatment of 600 mg/kgbw per day. In the Table 1 showed the differences in average blood cadmium levels in each treatment group of Moringa leaf extract (P1, P2, and P3) with dose variations of 400 mg/kgbw, 500 mg/kgbw and 600 mg/kgbw. The average cadmium levels in each extract treatment group at doses of 400 mg/kgbw, 500 mg/kgbw and 600 mg/kgbw were below the average cadmium levels of the Gold Standard group with vitamin C treatment.

Research data on SGOT and SGPT levels in white *rats (Rattus norvegicus)* were obtained from the treatment in the form of Moringa leaf extract (*Moringa oleifera*) at a dose of 400 mg/kgbw, 500 mg/kgbw, 600 mg/kgbw and vitamin C according to the treatment of each group after being induced with $CdCl_2$. Based on the results of the examination of SGOT and SGPT levels as indicators of hepatotoxic events in the blood serum of white rats using the BS-200 *Chemistry Analyzer* at the Bakti Analisa Laboratory, the average results of SGOT and SGPT levels can be obtained which can be seen in Table 2 and Fig. 1 as follows.

TABLE 2
SGOT and SGPT Levels in White Rats Blood

Sample Groups	SGOT (U/L)	SGPT (U/L)
Placebo (PL)	275.075	71.575
Control Negative (N)	264.95	91.95
Control Positive (KP)	235.85	60.4
Gold Standar (GS)	186.55	74.85
Treatment 1 (P1)	260.175	143.85
Treatment 2 (P2)	250.925	77.575
Treatment 3 (P3)	171.475	66.075

In Table 2, it showed the differences average SGOT and SGPT levels of each treatment group. The highest average blood level of SGOT was 275,075 U/L found in the Placebo (P) group with CMC.Na treatment every day. The lowest average level of SGOT in the blood was 171.475 U/L, which was found in the Treatment 3 (P3) group with Moringa leaf extract treatment at a dose of 600 mg/kgBB. The average SGOT level in the Negative Control (N) group was 264.95 U/L acting as a normal group without any treatment. The average SGOT level of the Positive Control Group (KP) was 235.85 U/L with $cdcl_2$ administration of 3 mg/kgBB being below the average value of the normal group. The average SGOT level of the Gold Standard (GS) group was 186.66 U/L with vitamin C treatment being below the normal group average. The difference in the average of SGOT levels in Treatment 1 and Treatment 2 (P1 and P2) was 260.175 U/L and 250.925 U/L with the treatment dose of Moringa leaf extract 400 mg/kgbw and 500 mg/kgbw.

From Table 2, it could be seen that the highest average result of SGPT levels in the blood was 143.85 U/L, which was found in the Treatment group 1 (P1) with a dose of Moringa leaf extract of 400 mg/kgbw. The lowest average blood SGPT level was 60.4 U/L in the Positive Control group (KP) with $CdCl_2$ administration. The average result of SGPT levels in the blood of the Negative Control Group (KN) was 91.95 U/L as a normal state of white rats without treatment. The average SGPT content of the Placebo (P) group was 71.575 U/L with CMC.Na treatment. The average level of SGPT in the blood of the Gold Standard (GS) group was 74.85 U/L with the treatment of vit C after being given the $CdCl_2$ treatment was below the average value of the negative control group. The average results of SGPT levels in the treatment of Moringa leaf extract (*Moringa oleifera*) at several doses were different. The average SGPT levels in treatment groups 2 and 3 (P2 and P3) were 77.575 U/L and 66.075 U/L with Moringa leaf extract treatment of 500 mg/kgbw and 600 mg/kgbw.

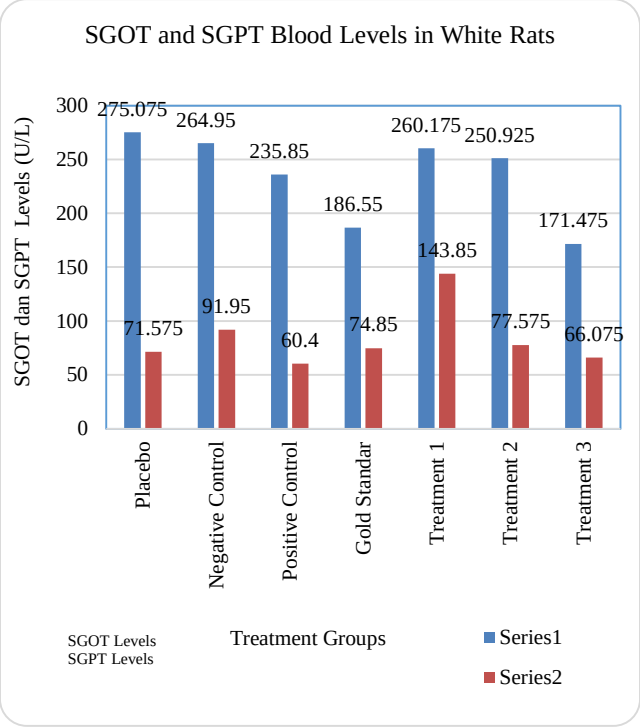

FIGURE 1. SGOT And SGPT Blood Levels in White Rats

In the treatment group with Moringa leaf extract in Fig. 1 showerd that all of them were below the average value of SGOT levels in the negative control group. Then, the average SGPT levels in Treatment group 3 were lower than the average SGPT levels in Treatment 1 and Treatment 2 groups.

A. STATISTICAL ANALYSIS

1) SAPHIRO WILK NORMALITY TEST

The data test of cadmium levels in white rat conducted normality test using Saphiro wilk test to find out the normality distribution of the data. In SPSS program version 16.0

obtained test results that normality tests on cadmium (Cd) levels in the blood of white rats had a signification p values > 0.05 in each treatment group, then a null hypothesis (H0) was accepted. It had the meaning of the value of Cd levels in the blood of white rats of each group was normally distributed. The normality tests on SGOT and SGPT levels in the blood of white rats had a signification p values > 0.05 in each treatment group, then a null hypothesis (H0) was accepted. So that it had the meaning of the value of SGOT and SGPT levels in the blood of white rats of each group were normally distributed.

2) LEVENE HOMOGENEITY TEST

In SPSS program version 16.0 obtained test results that homogeneity test at blood cadmium (Cd) levels of white rats had signification p value of 0.137 > 0.05, then a null hypothesis (H0) was accepted. It had the meaning of the value of cadmium (Cd) levels in the blood of white rats was homogeneous. The homogeneity test on SGOT levels in the blood of white rats has a signification p value of 0.257 > 0.05, then a null hypothesis (H0) was accepted. It had the meaning of the value of SGOT levels in the blood of white rats was homogeneous. The homogeneity test on SGPT levels in the blood of white rats had a signification p value of 0.001 < 0.05, then the null hypothesis (H0) was rejected. It meant the value of SGPT levels in the blood of white rats was not homogeneous and the data was non-parametric, it was necessary to test the difference with the Kruskal wallis test.

3) ANOVA ONE WAY TEST

After the research data was known to be normally distributed and homogeneous, namely in the results of the examination of cadmium (Cd) levels and SGOT levels, it can be continued with the One Way Anova test to find out significant differences in the examination results of each treatment group. In SPSS program version 16.0 obtained test results that The One-way Anova test on cadmium (Cd) levels in the blood of white rats had a signification p value of 0.749 > 0.05, then the null hypothesis (H0) was accepted. It meant that there was no significant difference in blood cadmium (Cd) levels of white rats in each treatment group. There was no need to continue the Post Hoc test.

The One-way Anova test on SGOT levels in the blood of white rats had a signification p value of 0.209 > 0.05, then the null hypothesis (H0) was accepted. So that means that there was no significant difference in SGOT levels in the blood of white rats in each treatment group. There was no need to continue the Post Hoc test.

4) KRUSKAL WALLIS TEST

In the results of the SGPT level examination that was distributed normally and inhomogeneously, so it could be continued with the Kruskal wallis test to find out significant differences in the results of the SGPT level examination of each treatment group. In SPSS program version 16.0 obtained test results SGPT levels in the blood of white rats had a signification p value of 0.594 > 0.05, then a null hypothesis (H0) was accepted. It showed that there was no significant difference in SGPT levels in the blood of white rats in each treatment group.

IV. DISCUSSION

This study aimed to determine the effectiveness of Moringa leaf extract (*Moringa oleifera)* against the cadmium exposure that causes hepatotoxicity for 7 days of treatment using white rat experimental animals (*Rattus norvegicus*). Previously, adaptations have been carried out for 10 days in white rat experimental animals for adjustment of feed and the environment. Furthermore, the weight of white rats was carried out which would be used in determining the treatment of each group according to the dosage. The research treatment was given to white rat experimental animals in the form of cadmium chloride ($CdCl_2$) induction at a dose of 3mg/kgbw was carried out by orally sonde after adaptation was completed. The administration of $CdCl_2$ dose treatment in this study was in line with the research of Abdelaziz, Elhabiby and Ashour (2013), showing that the exposure of $CdCl_2$ doses of 3 mg/kgbw for 72 hours in rabbit species caused an increase in SGOT and SGPT levels by 49.5% and 73.3% compared to the control group in the absence of treatment[24]. Thus, the administration of $CdCl_2$ at this dose had intended to have a toxic effect on the liver organs (hepatotoxic) of experimental animals for 10 days of treatment.

Cadmium metal has the ability to accumulate in the body which can cause anemia, hypertension, heart failure, kidney dysfunction to osteoporosis[25]. Several studies have shown damage to some organs in the body due to exposure to cadmium, especially to the liver. Cadmium induces apoptosis and or necrosis on observations in vitro in cells as well as in vivo. Exposure to cadmium acutely increases the accumulation of cadmium in the liver[26].

The increase in cadmium levels occurred in the Positive Control group after $CdCl_2$ induction with the highest blood cadmium levels of 0.10275 µg/dL compared to other treatment groups, it showed that the administration of treatment through gastric sonde had succeeded in increasing blood cadmium levels. In the negative control group with an average blood cadmium level of 0.06725 µg/dL, the body's cadmium levels were normal because they were not cadmium-induced treatment. Meanwhile, the placebo group with an average blood cadmium level of 0.068 µg/dL showed that the treatment of CMC.Na did not affect cadmium level in the blood. The results of cadmium examination in the Positive Control group were compared with the Negative Control group according to the study of Toppo *et. al* (2015), where there was an increase in cadmium levels after induction of $CdCl_2$ doses of 200 ppm in white rats for 28 days[14].

The cadmium induction in experimental animals *in vivo* has shown a hepatotoxic effect, where cadmium has the ability to bind to *sulfhydryl* groups that play a role in mitochondrial molecules, inactivate *sulfhydryl* enzymes and

cause oxidative stress, thus leading to cell death related to apoptosis and autophagy [27][28]. Cell death (apoptosis, autophagocytosis or necrosis) in the liver can increase in SGOT and SGPT levels due to an increase in the permeability of cell membranes in releasing transaminase enzymes in the blood[14]. Increased levels of SGOT and SGPT as enzymes of liver function activity are the main hepatotoxic markers of cadmium toxicity[29].

In the examination of SGOT and SGPT levels, after the administration of $CdCl_2$ 3 mg/kgbw in the Positive Control group had SGOT levels of 235.85 U/L and SGPT levels of 60.4 U/L, which showed the lowest SGOT levels and SGPT levels when compared to the Negative Control group and the Placebo group. However, the differences in the results of the SGOT and SGPT levels in the three groups did not show significant differences after statistical analysis using the *One-way Anova* test. This study is in accordance with the study of Zou *et. al* (2021) about determination of SGPT levels, that no significant difference was found in the group without treatment with the cadmium induction treatment group at a dose of 50 mg/L in rats for 90 days[27]. However, there were still significant differences in the SGOT levels of the study group. There was a discrepancy in the study of Toppo *et. al* (2015), that in the mice group with a $CdCl_2$ treatment of 200 ppm daily had significantly higher SGOT and SGPT levels compared to the mice group as a control group[14]. The discrepancy in the results of the SGOT and SGPT levels in this study can be influenced by the duration of cadmium induction treatment where the study was 28 days. The research of Juliati *et. al* (2016) showed a significant difference in the duration of exposure of $CdSO_4$ metal doses of 3 mg/L to malodialdehyde (MDA) levels as a biological marker of damage to white rat liver tissue due to oxidative stress at exposure to 2 weeks, 4 weeks and 6 weeks[30]. The timing of the study can affect the cadmium toxokinetic and the biological markers of liver damage levels.

In overcoming the occurrence of hepatotoxic due to cadmium induction which causes damage to its cells, treatment was given in the form of Moringa leaf extract in the treatment group with 3 dose variations (mg/kgbw). Moringa leaf extract was obtained by maceration method with a 96% ethanol solvent. The maceration method was carried out for 2 days by soaking 300 g of Moringa leaves simplisia in a suitable solvent to extract flavonoid compounds in Moringa leaves, then re-remaceration was carried out for 2 days. The procedure corresponded to the research of Bennour *et. al.* (2021) which used the remaseration method with the appropriate solvent by showing the highest quercetin content [31]. Then to get a pure Moringa leaf extract without any residual solvent in the extract, evaporation is carried out with the IKA RV 10 *Rotary Evaporator* with a rotational speed of 55 rpm, for 3x6 hours. In the extraction process, the weight of pure Moringa leaf extract was obtained by 50,504 grams.

In other treatment groups as an effort to intoxicate after being induced $CdCl_2$, then white rats were given Moringa leaf extract at a dose of 400 mg/kgbw in treatment of group 1, 500 mg/kgbw in treatment of group 2, and 600 mg/kgbw in treatment of group 3 by orally sonde. In this study, Moringa leaf extract was given using CMC. Na 0.5%. This was in accordance with the study by Aristianti *et. al* (2021) related to the addition of CMC.Na 0.5% as *a suspension agent* at the three concentrations in ethanol leaves extract of *M. oleifera* for their development into safe natural drug candidates [32].

From the results of the blood cadmium determination in Table 1, it could be seen that the average blood cadmium of the entire Moringa leaf extract treatment groups were lower than the average cadmium level in the Positive Control group which was only given $CdCl_2$ treatment. Blood cadmium levels after being given Moringa leaf extract in Treatment 1, Treatment 2 and Treatment 3 (0.0605 µg/dL; 0.075 µg/dL; 0.08125 µg/dL) were lower than those of the Gold Standard group (0.0925 µg/dL), it showed that Moringa leaf extract *(Moringa oleifera*) acted as a *chelating agent* better than vitamin C. Quercetin has strong free radical picking activity and *chelating* capacity of metals, especially for cadmium metals[10]. Inhibition of cadmium metal accumulation in tissues is due to the presence of carboxyl and hydroxyl groups as a good *chelating agent* in *Moringa oleifera* leaf extract[33].

There was an increase in the average blood cadmium levels in the Moringa leaf extract treatment group sequentially although not significantly in statictic analysis, it showed that the administration of Moringa leaf extract had not been maximized in terms of the length of treatment time. This is the first study to examine blood cadmum levels compared to each treatment group of Moringa leaf extract (*Moringa oleifera*) at doses of 400 mg/kgbw, 500 mg/kgbw and 600 mg/kgbw with the treatment time of 7 days.

The blood cadmium examination in this study was intended to determine the degree of acute exposure of cadmium due to the time of treatment given to experimental animals after cadmium induction. Blood cadmium levels are not recommended to be used as a support for the evaluation process of disease severity, because cadmium has a relatively long half-life in the blood [34]. Thus, there is no relationship between the cadmium levels of the blood of experimental animals and the levels of SGOT and SGPT as an evaluation of hepatotoxic severity.

Based on the research results, the average levels of SGOT and SGPT in each treatment group of Moringa leaf extract (*Moringa oleifera)* at doses of 400 mg/kgbw, 500 mg/kgbw and 600 mg/kgbw decreased sequentially. The average SGOT and SGPT levels of Treatment group 1 were 260.175 U/L and 143.85 U/L, in Treatment group 2 were 250.925 U/L and 77.575 U/L, in Treatment group 3 171.475 U/L and 66.075 U/L. The average of SGOT and SGPT levels in the Gold Standard group were 186.55 U/L and 74.85 U/L, while in the Positive Control group were 235.85 U/L and 60.4 U/L. From the data on the average levels of SGOT and SGPT, it showed that the Treatment 3 group had lower SGOT and SGPT levels compared to the Gold Standard group, Treatment 1 and Treatment 2. The treatment of Group 3 with Moringa leaf

extract at dose 600 mg/kgbw had lower average SGOT levels compared to the Positive Control group.

The lower levels of SGOT and SGPT in the blood of experimental animals were caused by the hepatoprotective ability of Moringa leaves. Moringa leaves are known to have a high content of flavonoids, vitamin A, vitamin C and several phenol components, so they can prevent oxidative damage to cell membranes[29]. *Moringa oleifera* also has flavonoid compounds in the form of *quercetin* and *kaempferol* which are related to hepatoprotective potential, which in that group can neutralize free radicals due to cadmium induction, besides that the hydroxyl group also increases the improvement of the antioxidant system against oxidative stress in mammalian liver tissue[11][35][10]. The research by Kou *et. al* (2018) also stated that Moringa leaf extract can restore *glutathione* levels and prevent lipid peroxidation in the liver, especially in oral administration which was significant to hepatoprotective ability to liver damage[36].

The results of statistical analysis using *the One-way Anova* Test on cadmium and SGOT levels found no significant differences in all treatment groups. The results of statistical analysis using *the Kruskal Wallis* Test on SGPT levels of all treatment groups also found no significant difference. The results of SGOT levels and SGPT levels in this study were not in accordance with the research of Alshubaily and Soluman Almotairi (2020), which was shown to be a significant decrease in SGOT and SGPT levels after induction of $CdCl_2$ as much as 11 mg/kgbw in the treatment group of aquades extract-Moringa leaves dose 250 mg/kgbw and 500 mg/kgbw for 4 weeks[29]. But, this study was in accordance with the research of Aml Salem Saleh (2018), that showed an increase in CdCl treatment after 30 days intoxication with Moringa leaf at dose 400 mg/kgbw, while a drecrease in the hepatic SOD and GSH levels [18].

In this study, there were limitation, namely the duration of the treatment of giving *Moringa leaf extract (Moringa oleifera*). Differences in the duration of moringa leaf extract can affect the ability in lowering SGOT and SGPT levels in experimental animals as shown in the research of Toppo *et. al* (2015) for 28 days, the decrease in SGOT levels was 14.86% and SGPT levels was 26.032% with Moringa leaf extract of 200 mg/kgbw[14]. In the study of Alshubaily and Soluman Almotairi (2020), for 4 weeks, the decrease in SGOT and SGPT levels of the Moringa leaf extract group at a dose of 250 mg/kgbw was obtained by 28.93% and 21.18%, then in the 500 mg/kgbw dose group obtained by 36.38% and 27.673%[29]. Meanwhile, in this study with a treatment duration of a week, Moringa leaf extract dose 400 mg/kgbw obtained an increase in SGOT levels and SGPT levels of 10.31% and 138.1 %, at a dose of 500 mg/kgbw an increase of 6.39% and 28.42%, at a dose of 600 mg/kgbw a decrease in SGOT levels of 27.29% and an increase in SGPT levels of 9.39% compared to the Positive Control group. Then the duration of treatment can affect the ability of Moringa leaf extract to overcome hepatotoxic. Stress experienced by experimental animals can potentially affect oxidative stress that damages cell integrity[37], where stress occurs in the treatment group due to lack of adaptation to sonde treatment and experimental animals that fight due to unequal *breeding* processes.

From the discussion above, it can be concluded that the administration of Moringa leaf extract at a dose of 600 mg/ kgwb provides a better hepatoprotective effect compared to the dose of Moringa leaf extract of 400 mg/kgbw, 500 mg /kgwb and vitamin C in terms of SGOT and SGPT levels as a marker of liver damage. This study showed that Moringa leaves had the potential to have *chelating agent* and hepatoprotective abilities in cadmium exposure for 10 days with an insignificant decrease in the entire group statictically.

The Moringa leaf extract in this study required 500 g of fresh Moringa leaves, which were then converted into powder for maceration and evaporation until 50,504 g of pure Moringa leaf extract was obtained. In treatment group 3 with Moringa leaf extract dose 600 mg/kgbw required 2,961 g of pure Moringa leaf extract for 4 rats for 7 days. Then, the Moringa leaf extract that needed in a rat weighing of 176.25 g was 0.10575 g/day. The utilization of the results in this study for human, we can use fresh Moringa leaves. Then, the calculation of fresh Moringa leaves that had been adjusted to human body weight (70 kg) was 66,522 g/ day, which was equivalent to Moringa leaf extract dose 600 mg/kgbw in white rats. So, the fresh Moringa leaves as much as 66,522 grams can be given to humans every day to overcome damage of liver due to the acute cadmium exposure.

IV. CONCLUSION

This study aimed to determine the effective dose of moringa leaf extract (at dose 400 mg/kgbw, 500 mg/kgbw and 600 mg/kgbw) against hepatotoxic case in white rats induced with cadmium. From the results of this study, it could be seen that the blood cadmium (Cd) level of white rats was 0.0605 µg / dL, SGOT level was 260.175 U/L, and SGPT level was 143.85 U/L with Moringa leaf extract at a dose of 400 mg/kgbw/day. The blood cadmium (Cd) level of white rats was 0.075 µg/dL, SGOT level was 250.925 U/L, and SGPT level was 77.575 U/L with Moringa leaf extract at a dose of 500 mg/kgBB/day. The blood cadmium (Cd) levels of white rats was 0.08125 µg/dL, the SGOT level was 171.475 U/L, and the SGPT level was 66.075 U/L with Moringa leaf extract at a dose of 600 mg/kgBB/day. So, the treatment with *Moringa oleifera* leaf extract at dose of 600 mg/kgbw/day had the best effect compared to a dose of 400 mg/kgbw/day and 500 mg/kgbw/day in overcoming hepatotoxic due to cadmium exposure. The content of flavonoid compounds in the form of *quercetin* and *kaempferol*, vitamin A, vitamin C and phenol components that are high in Moringa leaves has the potential for *chelating agents* by inhibiting the accumulation of cadmium in the liver and lowering SGOT and SGPT levels as natural antioxidants in acute cadmium-induced in the white rats.

This study was expected to provide knowledge about the use of fresh Moringa leaves (*Moringa oleifera*) as much as

66,522 grams or more can be consumed daily (body weight 70 kg), as a metal chelating agent and natural additive antioxidants (hepatoprotective properties) in treating liver organ damage, especially due to acute exposure to cadmium. This research can be a reference by implementing the ability of *chelating agents* and high antioxidants of Moringa leaves *(Moringa oleifera*) through community service activities, especially in areas with heavy metals exposures on agricultural soils and high smokes exposure. For further study is recommended to analyze with reviewing other parameters such as the body's natural antioxidant enzymes (*GSH*, *GPx*, *CAT*, *SOD*) and liver tissue images with a longer treatment time.

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