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Moringa Leaf Powder Improves Lipid Profiles and Aortic Thickness in Wistar Rats Model of Prediabetes Mellitus

Pemberian Serbuk Daun Kelor terhadap Perbaikan Profil Lipid dan Ketebalan Aorta pada Tikus Model Prediabetes Melitus

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ABSTRACT

Background: Consumption of beverages and processed foods with high fructose content has increased recently. This condition increases the risk of metabolic syndrome, which include insulin resistance, impaired lipid profile, and elevated blood pressure. Moringa leaves (Moringa Oleifera), with their bioactive compounds that function as antioxidants, are known to improve fat metabolism and blood glucose.
Objectives: The purpose of this study is to analyze the effect of Moringa Oleifera leaf powder and quercetin in controlling metabolic syndrome conditions by improving lipid profiles and aortic thickness in prediabetes mellitus (pre DM).
Methods: This experimental research used a post-test-only control group design approach. A total of 35 male Wistar rats were divided into 5 groups such as group K0 (standard feed), E1 (66% fructose probe for 16 weeks), E2 (66% fructose probe for 12 weeks), E3 (66% fructose probe + quercetin 50 mg/kgBW), and E4 (66% fructose probe + Moringa leaf powder 500 mg/kgBW). The examination method for lipid profiles involved serum extraction and enzymatic tests, weighing for white fat tissue mass, while the aorta was examined using a light microscope and histological image analysis. Data analysis used the One-Way ANOVA test.
Results: This research showed there are no difference in triglycerides, Low-Density Lipoprotein (LDL) cholesterol, and white adipose tissue significantly between the treatment groups. Level of High-Density Lipoprotein (HDL) cholesterol, total cholesterol, and aortic thickness between the treatment groups are significant differences, with the best results sequentially in groups E2, E1, and K0.
Conclusions: The administration of Moringa leaf powder for 4 weeks showed improvements in lipid profiles and aortic thickness in prediabetes mellitus conditions.

INTRODUCTION

Globalization and urbanization have profound influences on all aspects of life, particularly altering dietary preferences and lifestyles. This phenomenon correlates with the increasing prevalence of obesity within society. In Indonesia, the surge in obesity is evident across communities with diverse economic statuses, as a consequence of shifting dietary patterns and preferences from traditional foods to processed products, which frequently contain elevated levels of fat and sugar[1].

In Indonesia, a substantial 40.1% of individuals aged over 3 years has developed the habit of consuming sweet foods more than once a day, and a significant portion of the population, comprising 61.27%, has adopted a routine of consuming sweet drinks more than once a day[2]. As highlighted by Lumbuun and Kodim (2017), elevated fructose intake has been associated with adverse effects, such as decreased HDL, increased LDL, hyperuricemia, hypertriglyceridemia, hypertension, and insulin resistance, potentially leading to Impaired Glucose Tolerance (IGT) [3]. The carbon atoms provided by fructose for glycerol and acyl-CoA can trigger de novo lipogenesis (DNL), resulting in triglyceride synthesis and increased fat levels in the liver and ultimately leading to decreased insulin sensitivity[4]. This suggests that frequent consumption of sweet drinks poses an increased risk of developing prediabetes and, if left unaddressed, may progress to Type 2 Diabetes Mellitus (T2DM).

Diabetes Mellitus (DM) is a degenerative disease marked by hyperglycemia and impacts the metabolism of carbohydrates, fats, and proteins[5]. Diabetes Mellitus encompasses various types, including type 1, type 2, and gestational; however, 95% of all diabetes cases

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worldwide are attributed to Type 2 Diabetes Mellitus (T2DM)[6]. In Indonesia, the prevalence of DM among the population aged 15 years, as indicated by Basic Health Research data, has risen to 2%[2]. The initial stage of DM symptoms is termed pre-diabetes mellitus (pre-DM) or Impaired Glucose Regulation (IGR), characterized by elevated blood glucose levels but do not meet the threshold for diabetes. This includes fasting blood glucose levels of 100 mg/dL to 125 mg/dL, glucose tolerance of 140 mg/dL to 199 mg/dL, and/or HbA1C levels of 5.7% to 6.4%[7-9].

Dyslipidemia is a condition characterized by elevated levels of fat and cholesterol in the blood resulting from abnormal lipid metabolism. Dyslipidemia is marked by a decrease in HDL levels and an increase in LDL, total cholesterol, and triglycerides[10]. Individuals with Type 2 Diabetes Mellitus (T2DM) experiencing dyslipidemia are identified as a predictive risk factor for cardiovascular disease (CVD) in diabetes[6]. The metabolic disruptions triggered by T2DM lead to increased lipolysis and lipogenesis, consequently elevating the production of Very Low-Density Lipoprotein (VLDL) which circulates in the bloodstream, leading to elevated triglyceride levels[11]. According to Rantung et al. (2014), metabolic disturbances in T2DM conditions cause an increase in the flow of free fatty acids in the liver, subsequently inducing triglyceride production[12]. These triglycerides are then synthesized into VLDL, contributing to elevated LDL levels in the bloodstream. This escalation poses an increased risk of aortic wall thickening and atherosclerotic disease[13].

The management of prediabetes, T2DM, and associated risk factors such as lipid profile disorders can be addressed through medication and lifestyle modifications. Lately, many individuals have relied on synthetic anti-diabetic drugs for treatment. However, these medications may pose side effects for patients. As an alternative, functional foods offer a relatively safe and cost-effective option. Locally available functional foods like Moringa leaves (Moringa Oleifera) are abundant in Indonesia. Moringa is recognized for its ability to improve blood pressure levels, blood glucose, and triglyceride profiles[14]. Furthermore, the antioxidant and antidiabetic bioactive compounds found in Moringa leaves are known to improve conditions of hyperglycemia and hypercholesterolemia. Previous research by Monika and Lestariyana (2014) revealed that the quercetin content in Moringa leaves reduces insulin resistance by increasing the levels of adiponectin hormone produced by adipose tissue in diabetic animal models[15]. Moringa plants have high antioxidant activity and contain active compounds, particularly in their leaves. Research conducted by Putra et al. (2016) indicated that Moringa leaves contain various antioxidants, including alkaloids, saponins, steroids/triterpenoids, tannins, phenolics, and flavonoids[16]. Additionally, findings from Aborhyem et al. (2016) demonstrated that Moringa leaves can enhance the lipid profile in hyperlipidemic rats by lowering total cholesterol, LDL, and triglyceride levels while elevating HDL cholesterol levels, thus improving the atherogenic index[17].

Although previous studies have explored the effects of various parts of the moringa plant, such as seeds, fruit, roots, water fractions, and leaf extract, on lipid profiles and aortic thickness in male Wistar rats, research specifically focusing on the administration of Moringa leaf powder to improve these parameters in rats subjected to a high fructose diet has never been carried out. Therefore, from this background and context, the objective of this research is to evaluate the efficacy of Moringa Oleifera leaf powder and quercetin in improving the lipid profile and aortic thickness in male Wistar rats induced with a high fructose diet.

METHODS

This research employed a true experimental design with an in vivo post-test-only control group approach. The study was conducted at the Experimental Animal Research Laboratory and Clinical Pathology Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang for the management and surgical procedures involving experimental animals, as well as for lipid profile measurements. The research was conducted from September 2021 to February 2022, following approval from the ethics commission of the Faculty of Medicine, University of Mataram, under approval number 182/UN18.F7/ETIK/2021.

The research sample was divided into five groups, each consisting of five rats, with an additional reserve of two rats per group. The sample size was calculated using the Frederer (1991) formula. The treatment groups included K0 (Normal Control Group), E1 (T2DM Control Group), E2 (Prediabetes Control Group), E3 (Quercetin Intervention Group), and E4 (Moringa Leaf Powder Intervention Group). After a 7-day adaptation period, the samples were randomized and fasted for 12 hours to measure blood glucose levels before induction on a high fructose diet. Subsequently, groups E1, E2, E3, and E4 were administered a standard AIN-93 M feed along with a 66% high fructose diet via probe. Prior to surgery, blood glucose levels were measured to ensure that rats in group E1 were in the diabetic state and those in group E2 were in the prediabetic state.

The dose of fructose administered was 9 g/kgBW/day with a concentration of 66%. The dose of Moringa leaf powder was 500 mg/kgBW, and the dose of quercetin was 50 mg/kgBW with a concentration of 4%. According to research by Romero-Nava (2017), the administration of 66% fructose leads to elevated levels of blood glucose, cholesterol, LDL, and triglycerides[18]. Additionally, research by Villarruel-Lopez (2018) suggests that administering 500 mg/kgBW of Moringa leaf powder to diabetic rats reduces blood glucose levels[19]. Since Moringa leaf powder was administered via a probe, prior dissolution in distilled water was required before being given to experimental animals.

The Moringa leaves utilized were sourced from Moringa plantations in Selaparang District, Mataram City, Lombok, West Nusa Tenggara province, which were declared pest-free and underwent classification at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, University of Mataram. Following this, the Moringa leaves underwent phytochemical testing at the Integrated Research Laboratory, Faculty of Mathematics and Natural Sciences, Udayana University, Bali. Based on the results of the phytochemical tests, the Moringa leaf

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powder used contains alkaloids, flavonoids, tannins, and phenols, with polyphenols exhibiting the highest content at an average of 864.09 ± 8.91 mgGAE/100 g, and quercetin at 30.33 ± 4.82 mg/g.

The materials utilized for caring the experimental animals included AIN-93M feed, unrestricted access to water, 66% fructose solution, and bedding. Additionally, materials for examination were quercetin obtained from Sigma and Moringa leaf powder sourced from West Nusa Tenggara province, along with additional items for animal maintenance such as gloves, alcohol, and dry tissue. Lipid profile examination materials were reagents and blood serum. Tools employed for experimental animal care and induction included animal cages equipped with drinking bottles, digital scales (Tanita-KD 100), and gastric probes for administering fructose and Moringa solutions. For the preparation of Moringa leaf powder, digital balances with a precision of 0.001 mg, a food blender, and sieves with a size of 104-mesh were utilized. Blood samples were collected using red cap serum tubes, hematocrit tubes, and various laboratory support tools such as glucometers, spectrophotometers, centrifuges, test tubes, scalpels, micropipettes, tips, water baths, and 5 cc syringes.

Initially, determined Moringa leaves from West Nusa Tenggara province were selected as intervention materials, followed by the preparation of Moringa leaf powder accompanied by calculating the water content, ash content, and conducting phytochemical tests for flavonoids and amino acid content. In the second stage, male Wistar rats were fed a high fructose 66% diet at a dose of 9 g/kgBW for 12 weeks to induce a prediabetes model. Prediabetes in rats was confirmed by assessing Fasting Blood Glucose (FBG) and 2-Hour Postprandial Blood Glucose (2hPPBG) levels using the Point of Care Test (POCT) method by taking blood samples from the lateral vein of the rats' tail, and the results were expressed in mg/dL. The third stage involved administering a high fructose diet to induce Type 2 Diabetes Mellitus (T2DM) in rats (Group E1), followed by a 4-week intervention with Moringa leaf powder (Group E4) and quercetin (Group E3) for gold standard. The final stage entailed testing dependent variable parameters to evaluate the effects of Moringa leaf powder and quercetin interventions.

Data on the lipid profile levels of rats were obtained by anesthetizing the animals with ketamine and collecting blood samples from the heart using a syringe. These blood samples were then placed into yellow top Vacutainers and centrifuged at 5000 rpm for 10 minutes to obtain serum, which served as the test material for assessing lipid profile parameters. Additionally, the mass of white adipose tissue and the thickness of the aortic organ were measured post-euthanasia. The measurement of white fat tissue mass involved the collection of visceral white fat (epididymal, perirenal, and omental fat) as well as subcutaneous fat (inguinal fat). Each part was then weighed in a petri dish using a balance, and the total white fat mass was determined by summing the weights of the visceral and subcutaneous fat. For the measurement of aortic thickness, the rat's aortic organ was taken, fixed in formalin solution for 24 hours, processed into histological preparations, and stained with Hematoxylin-Eosin (HE) following standard procedures conducted at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Brawijaya.

Histological examination of the aorta's appearance, utilizing a micrometer, was conducted to observe thickening of the aortic wall. Preparations were examined under a light microscope at 400 times magnification linked to Image Pro software. Measurement of aortic wall thickness involved drawing a perpendicular line from the innermost layer of the intima to the outermost layer of the adventitia. The acquired data underwent analysis using Statistical Product and Service Solutions (SPSS) version 22. The Shapiro-Wilk test was executed to assess normality, succeeded by a test for homogeneity of variance. Subsequently, the One-Way ANOVA test was followed by Post Hoc Tukey to ascertain differences between treatment groups.

RESULTS AND DISCUSSION

Rat food intake was determined using the food weighing method, where the food provided to each rat per day was initially weighed and recorded. Subsequently, any remaining food (leftovers) was weighed again and recorded, and the difference in intake was calculated. Data on weight gain were obtained from the difference in body weight at the end and beginning of the treatment period. Table 1 presents the mean feed intake and changes in body weight of the experimental animals.

Table 1. Mean Feed Intake and Weight Gain of Experimental Animals for Each Treatment Group

Treatment Group	Feed Intake (g)	Fructose (ml)	Total Energy (kcal)	Weight Gain (g)
K0	23.96 ± 4.75	0.00 ± 0.00	73.14 ± 14.50	17.83 ± 25.16
E1	28.09 ± 5.25	3.26 ± 0.20	94.58 ± 16.47	52.17 ± 22.33
E2	23.60 ± 8.13	3.35 ± 0.52	81.09 ± 25.99	31.17 ± 37.12
E3	25.03 ± 4.14	3.04 ± 0.14	85.47 ± 12.86	46.17 ± 23.03
E4	26.80 ± 5.03	3.07 ± 0.17	90.12 ± 15.77	48.33 ± 28.22
p-value*	0.611	0.000	0.294	0.211

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K0: Standard feed; E1: Standard feed, fructose 66% 16 weeks; E2: Standard feed, fructose 66% 12 weeks; E3: Standard feed, fructose 66% 16 weeks, quercetin 4 weeks; E4: Standard feed, fructose 66% 16 weeks, Moringa leaf powder 4 weeks, *One Way ANOVA statistical test

Table 1 reveals that the highest mean food and total energy intake were observed in treatment E1, while the lowest was recorded in group E2. The results of the One-Way ANOVA test indicated a p-value of 0.611 (p>0.05), suggesting no significant differences in mean feed intake between the treatment groups. Similarly, there was no significant difference in mean total energy intake among the treatment groups. Although the feed intake in the diabetes group or the treatment group with a 66% high fructose diet (E1) was higher compared to the control group (K0), this difference was not statistically significant. These findings indicate that the treatment involving a high fructose diet with the dose administered to group E1 influenced the food intake of the experimental animals, although insignificantly. Additionally, the addition of fructose induction in group E1 led to a higher total energy intake compared to group K0. Providing a high fructose diet has physiological effects and influences metabolic damage such as increased insulin, triglycerides, and the development of fatty liver[20]. Previous study has shown that administering 66% fructose for 2 weeks resulted in significantly lower insulin receptor mRNA levels in skeletal muscle and liver compared to rats fed a standard chow diet[21]. Therefore, in this study, a high fructose 66% diet was employed. The results of this study indicated that the average fasting blood glucose level in pre-DM rats was 124 ± 8 mg/dL, and the GDP level in pre-DM rats was 298.67 ± 23.86 mg/dL, while the GD2JPP level in the pre-DM rat group was 152 ± 18.36 mg/dL, and the GD2JPP level in the T2DM rat group was 325.17 ± 33.98 mg/dL. These blood glucose levels significantly differed from those of the healthy rats not subjected to a high fructose diet, with the GDP level of healthy rats measured at 79.67 ± 7.37 mg/dL and GD2JPP at 102.33 ± 4.32 mg/dL.

The feed utilized contains protein, fat, and carbohydrate nutritional contents of 5.65%, 2.12%, and 65.89%, respectively. Based on these proportions, it is evident that more than 50% of the feed consists of carbohydrates. Excessive carbohydrate intake can lead to various health problems, one of which is T2DM. This is because an excess of carbohydrates can elevate glucose levels in the body, as carbohydrates are absorbed in the form of glucose, thereby increasing the secretion of the hormone insulin. Consuming excessive amounts of carbohydrates can result in the conversion of carbohydrates into fat in the liver. The excess fat intake can be stored as triglycerides and subsequently broken down into glycerol and free fatty acids, contributing to weight gain[22].

Table 1 illustrates that the highest increase in body weight was observed in the E1 group, while the lowest increase was recorded in the K0 group. The normality test yielded a p-value of 0.929 (p>0.05), indicating normal data distribution. Similarly, the homogeneity test yielded a p-value of 0.716 (p>0.05), suggesting homogeneous data. The results of the ANOVA test revealed a p-value of 0.204 (p>0.05), signifying that changes in mouse body weight did not significantly differ between treatment groups. Although changes in body weight between treatment groups were not statistically significant, there was an increase in body weight in all groups by the end of the study. Particularly, the weight gain in the diabetes group or the group administered a 66% high fructose diet (E1) was higher than in the control group or the group not given a 66% high fructose diet (K0). It indicates that a high fructose diet influences the weight gain of experimental animals.

Fructose does not stimulate the production of leptin and insulin, thereby resulting in increased feelings of hunger and a desire to consume additional sources of energy[23]. Consequently, the group subjected to a regular diet experienced a minimal increase in body weight, as their energy intake was lower compared to the high-fructose high-fat diet group. In situations where the body lacks energy, fat and protein stores undergo gluconeogenesis, contributing to their breakdown. Continual depletion of fat and protein reserves leads to a decrease in body weight[24].

Table 2. Mean Levels of Total Cholesterol, Triglyceride, LDL, and HDL Serum of Experimental Animals for Each Treatment Group

Treatment Group	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Total Cholesterol (mg/dL)
K0	37.17 ± 14.40	31.98 ± 3.24^{b}	13.7 ± 4.12	64.00 ± 7.04^{b}
E1	54.83 ± 12.25	13.28 ± 1.63^{a}	18.33 ± 5.32	38.83 ± 11.79^{a}
E2	31.00 ± 22.57	34.22 ± 4.63^{b}	15.03 ± 5.32	50.50 ± 8.12^{a}
E3	51.67 ± 32.46	23.23 ± 7.93^{ab}	17.65 ± 4.39	53.67 ± 11.38^{a}
E4	47.33 ± 13.38	32.95 ± 7.52^{b}	18.32 ± 4.29	49.50 ± 9.83^{a}
p-value*	0.251	0.000	0.153	0.004

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K0: Standard feed, E1: Standard feed, fructose 66% 16 weeks, E2: Standard feed, fructose 66% 12 weeks, E3: Standard feed, fructose 66% 16 weeks, quercetin 4 weeks, E4: Standard feed, fructose 66% 16 weeks, Moringa leaf powder 4 weeks, * One Way ANOVA statistical test, data are the mean ± standard deviation, "a,b,ab" indicates differences in groups ($p < 0.05$)

Table 2 presents the mean values and standard deviations for each parameter using descriptive tests. The results indicate that group E2, the prediabetes control group on a high fructose diet, exhibited the lowest mean triglyceride levels. Conversely, group E1, the T2DM control group on a high fructose diet, displayed the highest mean triglyceride levels. However, the mean triglyceride levels did not exhibit significant differences among the five treatment groups according to the One-Way ANOVA test. Research by Masnunah (2019) yielded consistent results, indicating no differences among the non-diabetes mellitus group, the DM group without Moringa leaf extract, and the DM treatment groups administered 200 mg/kgBW, 400 mg/kgBW, and 600 mg/kgBW of Moringa leaves[25].

The triglyceride levels recorded in this study ranged from 20 mg/dL to 101 mg/dL, all of which were within the normal range. This outcome can be attributed to factors such as energy or fat intake, which contribute to increased serum triglyceride levels. Excessive consumption of saturated fat-rich foods can lead to elevated lipogenesis and free fatty acids, which subsequently combine with glycerol to form triacylglycerol. Increased triglyceride synthesis in the liver occurs in conditions of higher fat intake, resulting in elevated blood triglyceride levels.

The negative control group (K0) exhibited higher values than the positive control group (E2), contrary to the theory suggesting that rats with DM induced by insulin resistance would experience increased triglycerides. Insulin serves as an inhibitor of hormone-sensitive lipase and enhances Lipoprotein Lipase (LPL) activity. In DM, insulin levels decrease, leading to increased hormone-sensitive lipase activity, which promotes lipolysis, while LPL activity diminishes, resulting in decreased hydrolysis of triglyceride levels.

Group E1 exhibited the highest triglyceride levels as this group was solely administered fructose without any additional treatment. Fructose, within the glycolysis process, undergoes a shortcut catalyzed by the enzyme fructokinase to form fructose 1-phosphate. Subsequently, the breakdown of fructose 1-phosphate by aldolase B via the enzyme glycerol dehydrogenase yields D-glyceraldehyde and dihydroxyacetone phosphate. These products are then reduced to glycerol and phosphorylated to glycerol-3-phosphate with the assistance of the enzyme glycerol kinase. Glycerol-3-phosphate is further converted into diacylglycerol 3-phosphate through esterification, utilizing free fatty acid fatty acyl-CoA as a precursor for triglyceride formation[26].

Prolonged consumption of fructose can lead to increased fat mass and heightened oxidative stress in endothelial cells, resulting in elevated blood pressure, obesity risk, and insulin resistance[27]. The absence of insulin can contribute to triglyceride elevation. Moreover, insulin resistance can enhance Very Low-Density Lipoprotein (VLDL) production, leading to the formation of atherogenic particles rich in triglycerides and LDL[28].

Group E3 exhibited the second highest mean triglyceride levels following group E1. Research by Pitoyo (2012) showed contrasting results, demonstrating a significant decrease in the group administered quercetin at a dose of 50 mg/kgBW compared to the obese control group[29]. One possible factor contributing to the ineffectiveness of quercetin in reducing triglycerides could be its poor water solubility, resulting in low bioavailability in the body[30].

The E4 group demonstrated an intermediate mean triglyceride level, indicating a suboptimal reduction in triglycerides. This could be attributed to the extraction process utilizing a water solvent, which may have been less effective in extracting bioactive compounds from Moringa leaves compared to the ethanol solvent extraction process[31].

Table 2 illustrates that the highest mean serum HDL levels were observed in group E2, followed by group E4 (administered moringa leaf intervention), while the T2DM control group (E1) exhibited the lowest HDL levels. This suggests that the administration of Moringa leaf powder can elevate serum HDL levels in rats. Group E2, with a shorter duration of fructose administration compared to groups E1, E3, and E4, demonstrated the most favorable HDL levels. The Kruskal-Wallis test revealed a p-value of 0.000 ($p<0.05$), indicating differences in serum HDL levels among treatment groups. Statistical analysis revealed a significant effect of Moringa leaf powder on the HDL levels of experimental animals, consistent with research by Saryono (2017) that Moringa leaves administered in a single dose can enhance mean HDL levels, potentially due to their vitamin C and flavonoid content[32].

The elevated mean serum HDL levels in group E4 were attributed to the administration of Moringa leaf powder over the 4-week treatment period. The Moringa leaf powder from West Nusa Tenggara variety utilized in this study contains antioxidants such as alkaloids, flavonoids, tannins, and quercetin. The alkaloid content in Moringa leaves can facilitate the reduction of blood fat levels (hypolipidemic) by enhancing the regulation of lipolytic enzyme activity or by promoting the excretion of cholesterol into bile acids through feces, thereby increasing HDL levels[17].

The flavonoid content in Moringa leaves can prevent the oxidation process of LDL and inhibit the activity of the HMG Co-A Reductase enzyme, consequently inhibiting the formation of mevalonate from HMG Co-A, which in turn can elevate HDL levels[33,34]. The tannin content functions to inhibit the HMG-CoA reductase enzyme, thereby reducing cholesterol formation. This leads to a decrease in the formation of Apo-B100 and an increase in LDL receptors, resulting in decreased LDL cholesterol and VLDL[35].

Quercetin can inhibit lipid peroxidation by binding to metal ions involved in ROS formation, thereby neutralizing free radicals. Additionally, quercetin-3-O-glucoside can enhance LDL receptor expression, contributing to improved lipid profiles by reducing LDL,

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triglycerides, and total cholesterol levels while increasing HDL[36]. The dose of quercetin administered in this study was 50 mg/kgBW, selected based on previous research indicating that this dosage has a significant effect on reducing blood glucose levels when administered daily via oral probe. However, in this study, the effectiveness of quercetin in increasing HDL was not as significant as that of Moringa leaf powder, as evidenced by the higher mean HDL levels in group E4 compared to group E3. This difference can be attributed to the presence of other antioxidants in Moringa leaf powder, such as alkaloids, tannins, and flavonoids.

The low mean serum HDL levels in group E1 indicate a reduction in HDL levels in diabetes mellitus due to alterations in fat metabolism caused by impaired insulin resistance. This leads to the activation of lipase hormone in adipose tissue, resulting in increased triglyceride lipolysis. Consequently, free fatty acids may accumulate excessively, leading to their conversion into triglycerides, which become part of VLDL in the liver. This results in VLDL rich in triglycerides, which undergoes an exchange process with cholesterol esters from HDL. As a result, HDL becomes rich in triglycerides but deficient in cholesterol esters, ultimately leading to a decrease in serum HDL due to its increased catabolism by the kidneys[37,38].

Table 2 illustrates the mean serum LDL levels from highest to lowest as follows: groups E1, E4, E3, E2, and K0, respectively. The One-Way ANOVA test resulted a p-value of 0.153 ($p>0.05$), indicating no significant difference in mean serum LDL levels between groups. Previous research administering Moringa leaf extract at doses of 300, 500, and 700 mg/kgBW/day to male white rats induced by a high-fat diet found no effect on the ratio of blood plasma LDL cholesterol levels[39]. This lack of effect could be attributed to stress induced by the probe usage. Groups E3 and E4 received probe twice, namely fructose and quercetin or moringa. According to Budiyono and Candra (2013), stress can elevate triglyceride synthesis and VLDL secretion, leading to increased LDL and decreased HDL levels[40].

The highest serum LDL levels were observed in group E1. Hyperglycemia in diabetes increases LDL levels due to impaired fat metabolism. This disturbance can activate lipase hormone and escalate triglyceride lipolysis in adipose tissue, resulting in excess free fatty acids. These elevated free fatty acid levels contribute to LDL oxidation, which is atherogenic[41].

Comparatively, LDL levels in groups E3 and E4 were lower than those in group E1. The reduction in serum LDL levels in rats receiving the Moringa leaf intervention was attributed to the antioxidant compounds present in Moringa leaf powder. Flavonoids inhibit the non-competitive absorption of fructose in GLUT 2 in the intestinal mucosa[42]; while tannins are thought to act as LDL oxidation inhibitors[43]. Antioxidants also inhibit lipoprotein lipase in fat metabolism, reducing triglyceride levels and lipolysis in the liver[44].

The decrease in serum LDL levels in the group of rats given quercetin was attributed to the aromatic hydroxyl group, which scavenge ROS and free radicals, along with its capacity to enhance the activity of endogenous enzymes like SOD, CAT, and GSH-Px. Quercetin and polyphenols in Moringa leaves improve dyslipidemia conditions by increasing PPAR-γ expression and decreasing SREBP-1c in rat liver, thus reducing triglyceride synthesis. Additionally, quercetin may act as a ligand binding to receptors, influencing insulin resistance through transcriptional regulation of resistin or other adipose cell genes[15].

Table 2 shows the mean total cholesterol levels, with the highest observed in the K0 group and the lowest in the E1 group. The results of the One-Way ANOVA test indicate significant differences in mean total serum cholesterol levels between treatment groups, with a p-value of 0.004 ($p<0.05$). Post hoc analysis further reveals a significant difference between the K0 treatment group and the other treatments, as indicated by the distinct notations for each treatment. Based on the results of statistical tests, total serum cholesterol levels in the control group (K0) were higher than those in the DMT2 group (E1). This contrasts with research by Susanti et al., that a 60% high fructose diet for 8 weeks significantly increased total cholesterol, LDL, and triglyceride levels in mice.

The lipid profile in experimental animals fed a high fructose diet can lead to elevated levels of total cholesterol, LDL, triglycerides, and total protein[46]. Serum total cholesterol levels rise due to fructose metabolism, wherein fructose is phosphorylated by the fructokinase enzyme into fructose 1-phosphate. This compound is then converted into dihydroxy acetone phosphate and glyceraldehyde 3-phosphate, eventually forming glycerol 3-phosphate and acetyl-CoA. The acyl-CoA derived from acetyl-CoA combines with glycerol 3-phosphate to produce triglycerides[23]. Elevated triglyceride levels can increase HDL catabolism, leading to low HDL levels and increased total cholesterol in the blood, as cholesterol fails to return to the liver[4].

The high serum total cholesterol levels in the K0 group may be attributed to the absence of a pre-test, resulting in unknown serum total cholesterol levels prior to the study. It is likely that the total serum cholesterol levels in the K0 group, which were higher than those in the DMT2(E1) group, were already elevated before the study commenced. In contrast, serum total cholesterol levels in the E4 group, which received the Moringa leaf powder intervention, were lower (49.50 ± 12.26 mg/dl) compared to those in the E2 group (50.50 ± 8.12 mg/dl), indicating a decrease in total serum cholesterol despite no significant difference between E2 and E4 groups.

Several factors may have contributed to the decrease in total serum cholesterol levels. The experimental animals were subjected to a high fructose diet for 28 days, concurrently with the intervention of Moringa leaf powder. This combination likely resulted in fructose intake imbalance which has the potential to increase total serum cholesterol with antioxidant intake, preventing the anticipated increase in total serum cholesterol levels[47]. Additionally, factors such as the duration and dose of Moringa leaf powder administration could have influenced the insignificant decrease in cholesterol levels. Research suggests that experimental animals administered with Moringa leaf extract water at 600 mg/kgBW exhibited a reduction in total serum cholesterol, although not statistically significant[47].

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Increasing the dosage has the potential to further reduce total serum cholesterol due to the increased levels of antioxidants, which can inhibit cholesterol formation[47]. Additionally, the insignificant decrease in cholesterol levels is also influenced by the activity of antioxidant compounds present in Moringa leaf powder.

Antioxidant compounds in Moringa leaves, including flavonoids, polyphenols, tannins, and quercetin, play a vital role in regulating the metabolic system. They inhibit lipogenesis through the Nf-kb pathway, enhance insulin secretion sensitivity, and inhibit the formation of cholesterol, such as LDL, VLDL, and TG[48]. Flavonoid compounds specifically inhibit HMG-CoA reductase, an enzyme crucial for cholesterol synthesis in the liver[49], while tannins increase bile acid excretion, contributing to decreased total serum cholesterol levels[50].

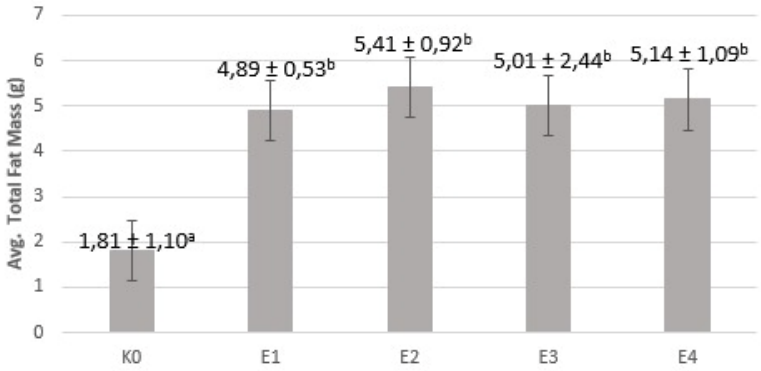

Figure 1. Average White Fat Tissue Mass

The average mass of white fat tissue in rats, as illustrated in Figure 1, reveals that the lowest mass was observed in the control group (K0), while the highest was in the prediabetes control group (E2). The results of the difference test yielded $p = 0.010$ ($p < 0.05$), indicating a significant difference in the average mass of white fat tissue among treatment groups. Further analysis indicated that the normal control group (K0) significantly differed from the other groups.

The differences in fat tissue mass between the control group (K0) and the intervention groups (E1, E2, E3, and E4) are likely attributed to variations in fructose consumption. While the normal control group received no fructose, the intervention groups were administered a 66% fructose solution at a dose of 9 mg/kgBW. High fructose intake has been associated with increased accumulation of white fat tissue in both humans and experimental animals. These findings align with the research conducted by Bursac et al. (2014), that reported increased accumulation of visceral white adipose tissue in rats subjected to a 60% fructose diet for 9 weeks compared to those on a normal diet[51].

High fructose consumption may impact systemic factors contributing to the increase of white adipose tissue. These factors include increased fructose energy intake mediated by leptin resistance, the action of glucagon-like peptide-1 receptor (GLP-1R) antagonists in the brain, and inflammation of visceral white adipose tissue[52]. The results indicate that the administration of 66% fructose (9 mg/kgBW) increases the mass of white fat tissue in mice.

White adipose tissue, predominant in humans and associated with diabetes and cardiovascular disease, makes up the majority of body fat and serves as the primary storage site for excess dietary triglycerides, which may lead to cellular hypertrophy during obesity[53]. Enlargement of fat tissue cells (hypertrophy) triggers local hypoxia in the endoplasmic reticulum, adipocyte death, and macrophage infiltration, subsequently increasing the secretion of proinflammatory cytokines and causing local and systemic inflammation, thereby disrupting insulin signaling and promoting insulin resistance[54].

Administration of quercetin and Moringa leaf powder did not affect the mass of white fat tissue in rats fed a high fructose diet, as evidenced by the absence of significant differences in white fat tissue mass among groups E1, E2, E3, and E4. This lack of effect could be attributed to the antioxidant activity of the Moringa leaves utilized, which is classified as weak (> 500 ppm), with an IC_{50} value of 1312.99 ± 10.04 ppm. A lower IC_{50} value indicates stronger antioxidant activity. The IC_{50} value of Moringa leaf powder obtained in this study was higher compared to the research conducted by Ardiansyah (2021), where Moringa leaves from Cianjur Regency exhibited an IC_{50} value of 81.35 ± 0.57 ppm, indicating a potent antioxidant profile[55]. The relatively weak antioxidant activity of Moringa leaves in this study may account for the variance in fat tissue mass between the control and treatment groups.

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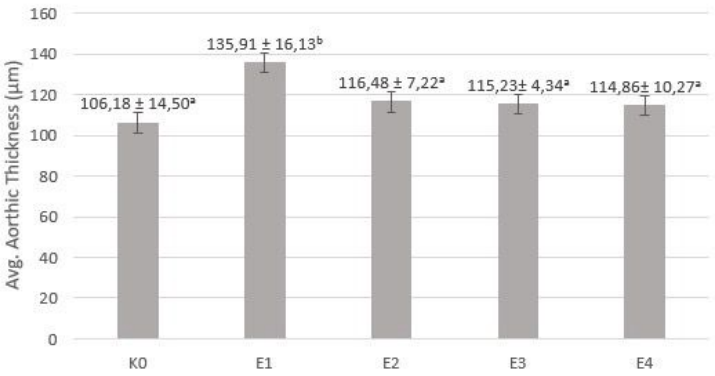

Figure 2. Average Aortic Wall Thickness

Figure 2 illustrates that the highest mean aortic thickness in rats was observed in the diabetes control group (E1), whereas the control group (K0) exhibited the lowest value. The One-Way ANOVA test yielded a p-value of 0.002 ($p < 0.05$), indicating a significant difference in mean aortic thickness among the treatment groups. Futher tests revealed that the diabetes control group (E1) displayed significant differences from the other groups.

The aortic wall thickness in group E1 tended to exceed that of group E2, where fructose intervention in group E1 extended over 19 weeks compared to 15 weeks in E2. Research by Yoo et al. (2017) demonstrated that rats subjected to a 30% fructose solution for 8 weeks experienced a notable increase in total fat weight and thickening of the aortic tunica media, potentially leading to endothelial dysfunction[56].

Aortic wall thickening represents an advanced stage following foam cell formation, which originates from intracellular lipid accumulation in artery walls during atherosclerosis. Oxidative stress triggered by NADH or NADPH oxidation induces endothelial expression of adhesion molecules such as vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, leading to monocyte infiltration. Macrophages are formed after monocytes enter the arterial intimal walls through the influence of macrophage-colony stimulating factor (MCSF). MCSF also stimulates the expression of scavenger receptors, leading to the phagocytosis of macrophages, which in turn convert oxidized LDL into foam cells[57,58].

The inflammation intensifies as MCSF, a key player in foam cell formation, triggers the release of pro-inflammatory cytokines, including TNF-α, IFN-ɤ, and lymphotoxin. Additionally, fibrous mediators and platelet-derived growth factors are generated upon monocyte infiltration into the artery wall, leading to the migration and proliferation of smooth muscle cells from the tunica media to the tunica intima. This process results in the formation of lesions characterized by fatty streaks or fat spots, accompanied by the migration and proliferation of smooth muscle cells stimulated by growth factors like platelet-derived growth factor. The accumulation of extracellular matrix components such as collagen, fibroblast connective tissue, and elastin fibers in the intima contributes to the thickening of the aortic wall[59,60]. Despite the absence of significant differences in fructose intake between treatment groups, it is evident that internal metabolic damage occurs in rats subjected to a 66% high fructose diet. This aligns with research by Febrianingsih (2019), which suggests that not all mice become obese, but the consumption of fructose as the primary energy source disrupts internal metabolism, leading to foam cell formation and thickening of the aortic walls in rats[20].

Group E3, which received quercetin intervention for 4 weeks, exhibited lower aortic wall thickening compared to group E4, which was given Moringa leaf intervention for 4 weeks. This demonstrates that quercetin intervention tends to reduce aortic thickness in diabetic rats more effectively than Moringa leaf intervention. Alkaloids and flavonoids, such as quercetin, are among the antioxidants known to prevent atherosclerosis. Rats subjected to quercetin intervention presented fewer atherosclerotic plaques, indicating the ability of quercetin to inhibit the formation of atherosclerotic areas in the aorta of mice on a high fructose diet. Quercetin has been shown to inhibit the proliferation of various growth factor receptors, the ROS pathway, and gene transcription that induces inflammation in endothelial cells, thus mitigating aortic wall thickening[61].

The decrease in aortic thickness observed in rats receiving Moringa leaf intervention is supported by previous research demonstrating the antioxidant effects of Moringa Oleifera leaves as modulators of inflammatory cells. Research by Randriamboavonjy et al. (2019) showed that hypertensive rats receiving 750 mg/kgBW of Moringa leaves for 4 weeks experienced a significant reduction in aortic wall thickness due to increased bioavailability of NO[62]. The treatment group receiving quercetin and Moringa leaves experienced decreased aortic thickness because the antioxidants in Moringa Oleifera leaves can reduce cholesterol absorption and inflammatory reactions, thereby inhibiting atherosclerosis and reducing the thickness of the aortic blood vessel walls[63].

CONCLUSIONS

This study concludes that administering Moringa Oleifera leaf powder at a dose of 500 mg/kgBW to male Wistar rats did not significantly affect feed intake, changes in body weight, triglyceride levels, LDL, and white fat tissue mass. However, it demonstrated an effect on increasing HDL levels and reducing total cholesterol and aortic thickness. Further research is needed to determine the optimal dosage of Moringa leaf powder

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and the duration of treatment necessary to influence triglyceride, LDL, and white fat tissue levels. Moreover, using ethanol as a solvent for dissolving Moringa leaf powder is recommended over water, as ethanol can extract more active substances from plants, potentially enhancing the efficacy of intervention in comparison to groups not receiving Moringa leaf powder.

One advantage of this study is its use of an induced animal model with phenotypic similarities to human disease, along with easily controllable variables that do not require extensive space for maintenance. Furthermore, this research has the potential to generate new insights, contribute to scientific knowledge, address problems, inform policy development, and enhance understanding of relevant phenomena. Additionally, conducting this research can help improve the analytical and methodological skills of researchers. However, this study has some limitations. It requires a relatively long duration and a high dose of fructose, which may increase the risk of diarrhea and mortality in experimental animals (rats).

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Conflict of Interest and Funding Disclosure

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Author Contributions

ADA, ARC, IK, DH: shaping the research concept, determining technical research methods, and executing the study; AC, SS, DA, UA, PS, HM, RAW: caring for the experimental animals, monitoring their condition throughout the study, and collecting and analyzing data on research parameters.

REFERENCES

1. Setyawati, V. A. V. & Rimawati, E. Pola Konsumsi Fast Food dan Serat sebagai Faktor Gizi Lebih pada Remaja. *Unnes Journal of Public Health* **5**, 275-284 (2016).
2. Kemenkes. Laporan Riskesdas 2018 Nasional. (2018).
3. Lumbuun, N. & Kodim, N. Pengaruh Konsumsi Fruktosa pada Minuman Kemasan terhadap Toleransi Glukosa Terganggu pada Kelompok Usia Dewasa Muda di Perkotaan Indonesia. *Jurnal Epidemiologi Kesehatan Indonesia* **1**, 19-23 (2017).
4. Kuswandari, L. S. Pengaruh Pemberian Diet High Fat High Fructose Modifikasi Ain-93M Terhadap Jumlah Sel Beta dan Histopatologi Pankreas Tikus Sprague Dawley Jantan Model Obesitas. (Universitas Brawijaya, 2019).
5. Refdanita, Musnelina, L., Teodhora & Aprianis, H. H. U. Gambaran Terapi Diabetes dengan Penyakit Penyerta Hiperlipidemia di Rumah Sakit. *Jurnal Endurance* **6**, 103–113 (2021).
6. Association, A. D. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2020. *Diabetes Care* **43**, (2020).
7. Pham, N. M., Eggleston K. Prevalence and Determinants of Diabetes and Prediabetes among Vietnamese Adults. *Diabetes Res Clin Pract*. 2-9 (2016)
8. Yudkin, J. S., Montori, V. M. The Epidemic of Pre-diabetes: The Medicine and The Politics. *BMJ*. 1-6 (2014).
9. World Health Organisation. Diabetes mellitus: Report of a WHO Study Group. *World Health Organisation. GenevaSwitzerland*. 25-36 (2006).
10. Pekerti, A. C., Kurniasari, F. N. & Kusumastuty, I. Jus Jambu Merah dan Jeruk Siam Menurunkan Trigliserida pada Wanita Dislipidemia. *Indonesian Journal of Human Nutrition* **6**, 1-9 (2019).
11. Wari, A. T., Muhlishoh, A., Nurzihan, N. C. Hubungan Indeks Glikemik dan Beban Glikemik Makanan Terhadap Kadar LDL Dan RLPP Pasien Diabetes Mellitus Tipe-2. (Universitas Kusuma Husada Surakarta, 2022).
12. Rantung, A. A., Umboh, A. & Mantik, M. F. J. Hubungan Hiperkolestrolemia dengan Obesitas pada Siswa SMP Eben Haezar Manado. *e-CliniC* **2**, (2014).

How to cite: *Ariestiningsih, A. D., Cempaka, A. R., Kusumastuty, I., Cahyaningrum, A., Setiawati, S., Arintya, D., Azmi, U., Salwa, P., Malahayati, H., Wibowo, R. A., & Handayani, D. (2024) Moringa Leaf Powder Improves Lipid Profiles and Aortic Thickness in Wistar Rats Model of Prediabetes Mellitus: Pemberian Serbuk Daun Kelor terhadap Perbaikan Profil Lipid dan Ketebalan Aorta pada Tikus Model Prediabetes Melitus. Amerta Nutrition, 8(2), 278–289.*

13. Alhakmani, F., Kumar, S. & Khan, S. A. Estimation of Total Phenolic Content, In-Vitro Antioxidant and Anti-Inflammatory Activity of Flowers of Moringa Oleifera. *Asian Pac J Trop Biomed* **3**, 623-627 (2013).
14. Adawiyah, R., Sartika, F. & Arfianto, F. Potensi Ekstrak Akar Kalakai (Stenochlaena palutris Bedd) sebagai Antihiperlipidemia yang Diuji Secara In Vivo. *Jurnal Pharmascience* **7**, 62-71 (2020).
15. Monika, A. & Lestariana, W. Pengaruh Pembeian Kombinasi Kuersetin dan Glibenklamid terhadap Kadar Kolesterl LDL pada Tikus Diabetes Melitus Tipe 2. *JKKI* **6**, 27–36 (2014).
16. Dwika, W., Putra, P., Agung, A., Oka Dharmayudha, G. & Sudimartini, L. M. Identifikasi Senyawa Kimia Ekstrak Etanol Daun Kelor. *Indonesia Medicus Veterinus Oktober* **5**, 464-473 (2016).
17. Aborhyem, S., Ismail, H., Agamy, N. & Tayel, D. Effect of Moringa Oleifera on Lipid Profile in Rats. *Journal of High Institute of Public Health* **46**, 8–14 (2016).
18. Romero-Nava, R., Zhou, D., Garcia, N., Ruiz-Hernandez, A., Si, Y., Sanchez-Munoz, F., *et al.* Evidence of Alterations in The Expression of Orphan Receptors GPR26 and GPR39 Due to The Etiology of The Metabolic Syndrome. *Journal of Receptors and Signal Transduction* **37**, 1-8 (2017).
19. Villarruel-López, A., Lopez-de la Mora, D. A., Vazquez-Paulino, O. D., Puebla-Mora, A.G., Torres-Vitela, M. R., Guerrero-Quiroz, L. A., *et al.* Effect of Moringa Oleifera Consumption on Diabetic Rats. *BMC Complement Altern Med* **18**, (2018).
20. Febrianingsih, E. Pengaruh Pemberian Diet High Fat High Fructose Aorta Tikus Sprague Dawley Jantan Model Obesitas. (Universitas Brawijaya, 2019).
21. Catena, C., Giacchetti, G., Novello, M., Colussi, G., Cavarape, A., Sechi, L. A. Cellular Mechanisms of Insulin Resistance in Rats with Fructose-induced Hypertension. *Am J Hypertens* **16**, 973-978 (2003).
22. Siregar NS. Karbohidrat. *Jurnal Ilmu Keolahragaan* **13**, 38–44 (2014).
23. Rahmawati, Y. W., Ulfa, E. U. & Rachmawati, E. Pengaruh Ekstrak Metanol Daun Kayu Kuning (Arcangelisia flava (L.) Merr) terhadap Histopatologi Aorta Tikus Wistar Hiperlipidemia. *e-Jurnal Pustaka Kesehatan* **4**, 241–248 (2016).
24. Astuti, I. L. P. Pengaruh Pemberian Diet High Fat High Fructose Modifikasi AIN-93M terhadap Kadar Serum Trigliserida dan Low Density Lipoprotein pada Tikus (Rattus Norvegicus) Galur Sprague Dawley Jantan. (Universitas Brawijaya, 2019).
25. Masnunah. Efek Ekstrak Daun Kelor (Moringa Oleifera) Terhadap Kadar Trigliserida Tikus Jantan Galur Sprague Dawley Diabetes Melitus yang Diinduksi Streptozotocin. (Universitas Islam Negeri Syarif Hidayatullah Jakarta, 2019).
26. Horton, J. D., Goldstein, J. L. & Brown, M. S. SREBPs: Activators of The Complete Program of Cholesterol and Fatty Acid Synthesis in The Liver. *Journal of Clinical Investigation* **109**, 1125–1131 (2002).
27. Haris, S. & Tambunan, T. Hipertensi pada Sindrom Metabolik. *Sari Pediatri* **11**, 257-263 (2016).
28. Hassing, H. C., Surendran, R. P., Mooij, H. L., Stroes, E. S., Nieuwdorp, M., Dallinga-Thie, G. M. Pathophysiology of hypertriglyceridemia. *Biochim Biophys Acta Mol Cell Biol Lipids* **1821**, 826–832 (2012).
29. Pitoyo, F. L. H. & Fatmawati, H. The Effect of Quercetine to Reduced Trigliceride and Blood Glucose Level in Animal Model Diet-Induced Obesity. *Jurnal Medika Planta* **1**, 36–46 (2012).
30. Cahyani, M. Formulasi dan Uji Pelepasan Kuersetin Ekstrak Daun Jambu Biji (Psidium Guajava L.) pada Mikroemulsi dalam Basis Gel Menggunakan Virgin Coconut Oil (VCO) sebagai Fase Minyak. (Universitas Islam Negeri Maulana Malik Ibrahim, 2017).
31. Wardani, E., Sunaryo, H., Sopiani, M. Z. &

How to cite: *Ariestiningsih, A. D., Cempaka, A. R., Kusumastuty, I., Cahyaningrum, A., Setiawati, S., Arintya, D., Azmi, U., Salwa, P., Malahayati, H., Wibowo, R. A., & Handayani, D. (2024) Moringa Leaf Powder Improves Lipid Profiles and Aortic Thickness in Wistar Rats Model of Prediabetes Mellitus: Pemberian Serbuk Daun Kelor terhadap Perbaikan Profil Lipid dan Ketebalan Aorta pada Tikus Model Prediabetes Melitus. Amerta Nutrition, 8(2), 278–289.*

Fatahillah, M. Aktivitas Antihipertrigliserida dan Antihiperglikemik Ekstrak Daun Kelor (Moringa Oleifera Lam.) pada Tikus Hipertrigliserida Diabetes. *Media Farmasi* **12**, 199–212 (2015).

32. Handayani, S., Saryono & Hernayanti. Effek Daun Alpukat (Persea Americana M.) dan Daun Kelor (Moringa Oleifera L.) terhadap Peningkatan Kadar HDL Pada Model Tikus Putih Hiperlipidemia. *Jurnal Keperawatan Soedirman* **12**, 47-55 (2017).
33. Utami, T. B. Aktivitas Penghambat Enzim HMG-CoA Reduktase dan Peroksidasi Lipid dari Sepuluh Tanaman Indonesia Secara Ex Vivo. (Sekolah Tinggi Farmasi Bandung, 2017).
34. Satrianawaty, L. D., Sumarno, T. M. & Prabowo, S. Pengaruh Pemberian Ekstrak Daun Kelor (Moringa Oleifera) Terhadap Kadar Kolesterol Hdl Tikus Putih (Rattus Norvegicus) Jantan Galur Wistar Hiperglikemia dengan Induksi Aloksan. *Jurnal Medical Hang Tuah* **17**, 30-43 (2019)
35. Agustina, D. & Murwani R, H. Pengaruh Pemberian Jus Biji Pepaya (Carica Papaya L.) Terhadap Rasio Kolesterol LDL:HDL Tikus Sprague Dawley Dislipidemia. *Journal of Nutrition College* **2**, 302–311 (2013).
36. Lin M, Zhang J, & Chen X. Bioactive flavonoids in Moringa Oleifera and Their Health-promoting Properties. *J Funct Foods* **47**, 469-479 (2018).
37. Malau, S. R. Hubungan Kadar Glukosa Darah Puasa dengan Profil Lipid pada Diabetes Melitus Tipe 2. (Universitas HKBP Nommensen Medan, 2014).
38. Pratiwi, W. R., Hediningsih, Y. & Isworo, J. T. Hubungan Kadar Glukosa Darah Dengan Kadar HDL (High Density Lipoprotein) Pada Pasien Diabetes Melitus Tipe 2. *Jurnal Labora Medika* **5**, 29–34 (2021).
39. Maulana, A. R., Seto, P. & Rahmat, B. Pengaruh Pemberian Ekstrak Daun Kelor (Moringa Oleifera) terhadap Rasio Kadar LDL/HDL Kolesterol pada Tikus Putih (Rattus norvegicus) Dislipidemia. (Universitas Mataram, 2017).
40. Wahyu, B. & Aryu, C. Perbedaan Kadar Kolesterol Total dan Trigiserida sebelum dan setelah Pemberian Sari Daun Cincau Hijau (Premna Oblongifolia Merr) pada Tikus Dislipidemia. *Journal of Nutrition College* **2**, 118–125 (2013).
41. Putri, S. R. & A, D. I. Obesitas sebagai Faktor Resiko Peningkatan Kadar Trigliserida. *Jurnal Majority* **4**, 78–82 (2015).
42. Wulandari, L., Nugraha, A. S. & Azhari, N. P. Penentuan Aktivitas Antioksidan dan Antidiabetes Ekstrak Daun Kepundung (Baccaurea racemosa Muell.Arg.) secara In Vitro. *Jurnal Sains Farmasi & Klinis* **7**, 60-68 (2020).
43. Umarudin, Susanti, R. & Yuniastuti, A. Efektifitas Ekstrak Tanin Seledri Terhadap Profil Lipid Tikus Putih Hiperkolesterolemi. *Unnes Journal of Life Science* **1**, 78–85 (2012).
44. Zulviyati. Uji Aktivitas Antioksidan dan Antihiperlipidemia Ekstrak Daun Kepuh (Sterculia foetida): Metode DPPH dan Hambatan Lipase In Vitro. (Universitas Jember, 2015).
45. Susanti, N., Rahmawati, E. & Kristanti, R. A. Efek Diet Tinggi Fruktosa terhadap Profil Lipid Tikus Rattus Rattus norvegicus Strain Wistar. *Journal of Islamic Medicine* **3**, 26–35 (2019).
46. Rajesh, R. & Venugopal, S. High Fructose Diet-induced Metabolic Syndrome and The Functional Abnormalities in The Liver and Kidney oOf Wistar Albino Rats. *Natl J Physiol Pharm Pharmacol* **11**, 156–159 (2020).
47. Zuhdi, F. Pengaruh Pemberian Ekstrak Air (Moringa Oleifera Lam.) terhadap Kadar Kolesterol Total dan Trigliserida Serum Tikus Putih (Rattus norvegicus) Strain Wistar yang Diberi Diet Aterogenik. (Universitas Brawijaya, 2016).
48. Wardhani, T. M. Pemanfaatan Tanaman Kelor (Moringa Oleifera Lam.) sebagai Sumber Terapi Preventif dan Kuratif pada Pasien Perlemakan Hepar dengan Sindrom Metabolik. *SCRIPTA SCORE Scientific Medical Journal* **1**, 1-12 (2020).
49. Maryani, P. E., Ulfa, E. U. & Rachmawati, E. Pengaruh Ekstrak Metanol Daun Kayu Kuning

How to cite: *Ariestiningsih, A. D., Cempaka, A. R., Kusumastuty, I., Cahyaningrum, A., Setiawati, S., Arintya, D., Azmi, U., Salwa, P., Malahayati, H., Wibowo, R. A., & Handayani, D. (2024) Moringa Leaf Powder Improves Lipid Profiles and Aortic Thickness in Wistar Rats Model of Prediabetes Mellitus: Pemberian Serbuk Daun Kelor terhadap Perbaikan Profil Lipid dan Ketebalan Aorta pada Tikus Model Prediabetes Melitus. Amerta Nutrition, 8(2), 278–289.*

(Arcangelisia flava (L.) Merr.) terhadap Kadar Kolesterol Total dan Trigliserida Tikus Hiperlipidemia. *e-Jurnal Pustaka Kesehatan* **4**, 241–248 (2016).

50. Zulviana, E., Rahman, N. & Supriadi, S. Pengaruh Pemberian Ekstrak Buah Kelor (Moringa Oleifera) Terhadap Penurunan Kadar Kolestrol pada Darah Hewan Mencit (Mus musculus). *Jurnal Akademika Kimia* **6**, 15-20 (2017).
51. Bursac, B. N., Vasiljevic, A. D., Nestorovic, N. M., Velickovic, N. A., Milutinovic, D. D. V., Matic, G. M., *et al.* High-fructose Diet Leads to Visceral Adiposity and Hypothalamic Leptin Resistance in Male Rats - Do Glucocorticoids Play A Role? *Journal of Nutritional Biochemistry* **25**, 1-9 (2014).
52. Burmeister, M. A., Ayala, J., Drucker, D. J. & Ayala, J. E. Central Glucagon-like Peptide 1 Receptor-induced Anorexia Requires Glucose Metabolism-mediated Suppression of AMPK and Is Impaired by Central Fructose. *Am J Physiol Endocrinol Metab* **304**, 677-685 (2013).
53. Zwick, R. K., Guerrero-Juarez, C. F., Horsley, V. & Plikus, M. V. Anatomical, Physiological, and Functional Diversity of Adipose Tissue. *Cell Metabolism* **27**, 68-83 (2018).
54. Item, F. & Konrad, D. Visceral Fat and Metabolic Inflammation: The Portal Theory Revisited. *Obesity Reviews* **13**, 30-39 (2012).
55. Ardiansyah, A. Aktivitas Antioksidan Daun Kelor (Moringa Oleifera Lam) Segar dan Kering dengan Metode DPPH. (Universitas Bhakti Kencana, 2021).
56. Yoo, S. Y., Ahn, H. & Park, Y. K. High Dietary Fructose Intake on Cardiovascular Disease Related Parameters in Growing Rats. *Nutrients* **9**, 1-12 (2017).
57. Rocha, V. Z. & Libby, P. Obesity, Inflammation, and Atherosclerosis. *Nat Rev Cardiol* **6**, 399–409 (2009).
58. Bobryshev, Y. V., Ivanova, E. A., Chistiakov, D. A., Nikiforov, N. G. & Orekhov, A. N. Macrophages and Their Role in Atherosclerosis: Pathophysiology and Transcriptome Analysis. *Biomed Res Int* **2016**, 1-12 (2016).
59. Sakakura, K., Nakano, M., Otsuka, F., Ladich, E., Kolodgie, F. D., Virmani, R. Pathophysiology of Atherosclerosis Plaque Progression. *Heart Lung and Circulation* **22**, 399–411 (2013).
60. Karakas, M. & Koenig, W. The Role of Inflammation in Atherosclerosis - An Update. *Der Klinikarzt* **39**, 140–146 (2010).
61. Halkos, M. E., Nicholas, J., Li, S. K., Burke, J. R. & Milner, R. Endovascular Management of Blunt Abdominal Aortic Injury. *Vascular* **14**, 223–226 (2006).
62. Randriamboavonjy, J. I., Heurtebise, S., Pacaud, P., Loirand, G. & Tesse, A. Moringa Oleifera Seeds Improve Aging-related Endothelial Dysfunction in Wistar Rats. *Oxid Med Cell Longev* **2019**, 1-8 (2019).
63. Chumark, P., Khunawat, P., Sanvarinda, Y., Phornchirasilp, S., Morales, N. P., Phivthong-Ngam, L., *et al.* The In Vitro and Ex Vivo Antioxidant Properties, Hypolipidaemic and Antiatherosclerotic Activities of Water Extract of Moringa Oleifera Lam. leaves. *J Ethnopharmacol* **116**, 439–446 (2008).

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