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Effect of *Moringa* Leaves Ethanolic Extract (*Moringa oleifera, Lam*.) on Testicles Histopathological Structure in Metabolic Syndrome Rat Model

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ABSTRACT

Introduction: Metabolic syndrome was a collection of various disorders that can significantly increase the risk of experiencing insulin resistance, atherosclerotic cardiovascular disease, vascular complications, and diabetes mellitus. The detrimental effect of this condition has led to the development of various treatments options, such as Moringa leaves extract (Moringa oleifera), which was suspected to have ameliorative effects. Therefore, this study aimed to determine the effect of Moringa leaves extract on the histopathological appearance of the testes of Wistar rats induced with metabolic syndrome.

Methods: This was an experimental design study, with a post-test-only control group, involving 30 Wistar rats (Rattus norvegicus). Metabolic syndrome was induced in the samples through the administration of high-fat and fructose feeds along with streptozotocin and nicotinamide. Furthermore, the moringa leaves extract was administered to the treatment groups in different doses of 150, 250, and 350 mg/kg BW. Testicular histopathological damage scores were then assessed on each group. The Kruskal-Wallis test which followed by Mann-Whitney test was used to determine the significant differences between the groups, while the correlation was analyzed using Spearman test.

Results: There were significant differences in the histopathological scores between the treatment and control groups. Furthermore, the Wistar rats treated with Moringa extract showed better histopathological scores (2.1±0.64) compared to those without the treatment (2.7±0.54), $p<0.05$. The results also showed that higher doses of the extract significantly correlated with better outcome in testicular histopathological structure of metabolic syndrome rat model *($p=0.000$, $r=-0.396$).*

Conclusion: Moringa leaves ethanolic extract treatment improved testicular histopathological appearance in Wistar rats induced with metabolic syndrome.

Keywords: metabolic syndrome; moringa leaves; testes; white rat

INTRODUCTION

Economic development and globalization have caused significant changes in various social fields, including lifestyle and food patterns[1]. Many people who previously consumed traditional food are now switching to instant and westernized meals[2], leading to a shift in dietary habits. Several

individuals also currently prefer to eat food high in sugar, saturated fat, and salt with low fiber rather than consuming carbohydrates and fiber-rich vegetables [3]. However, high-fat diets have been linked to various health problems, such as coronary heart disease, diabetes, hypertension, and stroke, as well as other diseases, including metabolic syndrome and cancer[1,2].

Metabolic syndrome refers to a collection of various disorders that increase the risk of experiencing insulin resistance, atherosclerotic cardiovascular, vascular complications, and diabetes mellitus. They are also considered to be related to metabolic syndrome when some criteria are met, namely waist circumference of > 102 cm in men or 88 cm in women, high-density lipoprotein (HDL) cholesterol of < 40 mg/dL in men or <50 in women, triglycerides increase of >150 mg/dL, >100 mg/dL fasting glucose, and > 130 mm/Hg systolic pressure with a diastolic pressure of 85 or higher[4].

Several studies reported that metabolic syndrome is a global clinical challenge, with 1 in 3 adults in the United States currently affected by the condition[4]. Furthermore, its prevalence in Indonesia is approximately 23%, with women (26.6%) being more affected than men (18.3%). Previous reports have shown that the number of metabolic syndrome-related diseases, such as type 2 diabetes mellitus is expected to increase by five times in the next 5 to 10 years, while cardiovascular diseases are likely to increase by two times[2].

One of the consequences of metabolic syndrome is the formation of Advanced Glycation End-products (AGEs). Furthermore, AGEs are a group of lipids and proteins formed endogenously through oxidative or exogenous sources, such as food. The formation of these compounds is a precursor to the production of Reactive Oxygen Species (ROS), which can damage the antioxidant system in the body[5]. Previous studies have shown that increased levels of ROS in the body can cause damage to sperm DNA, leading to infertility in men. Metabolic syndrome is also closely related to a decrease in androgen hormone[6], which can trigger changes in the histological appearance of the testes, such as depletion of the epithelium in the seminiferous tubules, decrease in spermatozoa, degeneration of germ cells, decreased integrity of the epididymis, changes in cell plasma, and decreased Sertoli cells density[7].

Moringa oleifera is a plant that is commonly found in Asia and Africa and can be used as an alternative treatment for metabolic syndrome to prevent progression into degenerative diseases[2,8]. The plant contains several bioactive components that act as natural antioxidants, including ascorbic, phenolic, tannins, saponins, flavonoids, and carotenoids. Furthermore, its leaves have been found to be beneficial in reducing hypercholesterolemia, diabetes, hypertension, insulin resistance, and cancer. [8,9].

Previous studies have also shown that *Moringa oleifera* leaves extract can significantly reduce ROS production in sperm[10]. Other studies reported that it is beneficial in improving the histological appearance of the testicle of Wistar rats with diabetes mellitus by reducing fat degeneration and necrosis[11]. The administration of *Moringa oleifera* leaves ethanolic extract can also decrease the expression value of TNF-α in metabolic syndrome models[12]. Therefore, this study aims to determine the effect of *Moringa oleifera* leaves on the histopathological appearance of the testes of Wistar rats induced with metabolic syndrome.

METHOD

This study employed an experimental design, with a post-test-only control group, involving 30 Wistar rats (Rattus norvegicus). All the procedures were carried out at the Food and Nutrition Laboratory of the Center for Food and Nutrition Studies (PSPG) UGM and the Anatomical Pathology Laboratory of the Faculty of Medicine UNS. The sample population consisted of white rats obtained from the PSPG UGM Yogyakarta Laboratory. The inclusion criteria included white male rats of the Wistar strain, aged 2-3 months, and weighing 150-200 grams. Meanwhile, samples that drastically lost weight, died before the study and had signs of disease, such as smelly, flaBWy, and watery feces were excluded. Purposive sampling was used to select the rats used, while the sample size was

determined using Federer's formula. The independent variable was the dose of ethanolic extract of Moringa leaves, while the dependent variable was the histopathological appearance score of Wistar rat testicle[13].

The testicular histopathology score implemented in this study was based on the method developed by Hari and Priya with few modifications. Furthermore, score 1 indicated normal testicles, score 2 showed atrophy with reduced spermatozoa in seminiferous tubules or dilated distance, and score 3 indicated atrophy with reduced spermatozoa in the seminiferous tubules accompanied by the dilated distance or loss of epithelial integrity.

This study involved 30 Wistar rats, which were divided into 5 groups, namely K1 (control without treatment), as well as K2-K5 induced with metabolic syndrome by feeding them with a high-fat and fructose diet (1 ml/100 g/kg BW of oxidized oil, 1 ml/100 g BW of beef fat, and 1 ml/100 g BW of duck egg yolk) for 28 days. Furthermore, they were injected with 45 mg/kg BW Streptozotocin and 110 mg/kg BW Nicotinamide on days 25, 26, 27, and 28. *Moringa* leaves ethanolic extract doses of 150, 250, and 350 mg/kg BW were then given on days 29-56 in groups K3, K4, and K5, respectively. Measurements were taken on days 0 and 28 including body weight, fasting blood sugar, triglycerides, and HDL, to determine changes in metabolic syndrome. On day 56, the testicles of the rats were taken and processed using a histopathological procedure and then observed under a light microscope with a magnification of 100x with nine fields of view on each rat.

The study data were processed using SPSS, and differences between treatment groups were analyzed using the Kruskall-Wallis test, followed by Mann-Whitney posthoc test. The correlation between increased doses of *Moringa* leaves ethanolic extract administration and the histopathological appearance of the testicles was analyzed using *the Spearman test.*

This study was approved by the Health Research Ethics Committee of RSUD Dr. Moewardi Surakarta with reference number 711/V/HREC/2022.

RESULT

Achievement of Metabolic Syndrome

The results showed that all the groups experienced a weight increase of more than 8%, as shown in Table 1. The fasting glucose in K1 was shown to be <200 ml/dL, compared to K2, K3, K4, and K5 with higher levels. Furthermore, its triglyceride levels were < 150 mg/dL, while others had values greater than this threshold. The HDL in K1 was more than 80 mg/dL compared to other groups with < 35 mg/dL. Based on the criteria, metabolic syndrome was absent in K1, while K2, K3, K4, and K5 had achieved this condition by fulfilling 4 of the 5 criteria[14].

Histopathological Structure

The testicular organs were taken and processed to create preparations with a thickness of 4-5 µm. The samples were then marked with a pen to ensure objective observation. The preparatory stage was then photographed using a light microscope with a magnification of 100x in 9 fields of view. Furthermore, the observations were photographed and assessed using an assessment of the histopathological damage score[13].

The frequency of histopathological scores of each group is presented in Table 1. The results showed that K1, K3, K4, and K5 were dominated by score 2 with an average of 1.54 ± 0.539, 2.20 ± 0.683, and 2.17 ± 0.666, and 1.98±0.532, respectively. Meanwhile, K2 was dominated by score 3 with an average of 2.70 ± 0.537.

Table 1. Parameters of Wistar Rat Metabolic Syndrome

		K1	K2	K3	K4	K5
N		6	6	6	6	6
Body weight (g)	Day 0	173±5,0	173±4,9	172±4,7	174±4,1	175±3,3
	Day 28	193±4,0	201±5,4	200±5,0	202±4,7	202±3,4
	%	11,0%	16,2%	16,0%	16,2%	15,3%
Fasting blood sugar (mg/dL)	Day 0	68±1,7	67±1.2	68±1,6	67±1,3	68±2,0
	Day 28	75±0,8	273±3,8	271±4,3	272±4,5	270±1,7
	%	10,6%	304,3%	295,7%	304,9%	297,7%
Triglycerides (mg/dL)	Day 0	64±4,0	64±3,8	64±4,6	62±3,0	64±1,7
	Day 28	80±3,3	151±6,4	150±7,6	155±1,9	150±3,3
	%	26,0%	134,4%	131,8%	149,6%	134,3%
HDL (mg/dL)	Day 0	79±3,3	78±2,6	77±3,7	75±4,6	80±2,5
	Day 28	82±3,1	25±2,3	24±3,1	25±2,6	26±2,0
	%	3,6%	-67,0%	-68,1%	-65,6%	-67,1%

Table 2. Frequency of Histopathological Scores of Each Group

Group	N	Field of view	Score			Average
			1	2	3	
K1	6	54	26	27	1	1.6±0.54
K2	6	54	2	12	40	2.7±0.54
K3	6	54	8	27	19	2.2±0.68
K4	6	54	8	29	17	2.2±0.67
K5	6	54	8	39	7	2.0±0.53

The study data were analyzed to determine whether it was normally distributed using the Shapiro-Wilk normality test. Based on the results, each group had a value of p= 0.00, indicating that the data was not normally distributed. Furthermore, the Kruskal-Wallis nonparametric test was used to determine the significant difference.

The Kruskal-Wallis results showed a value of p= 0.000 ($p < 0.05$), indicating that there was a significant difference in the histopathological structure of the testicles of Wistar rats induced with metabolic syndrome. The *Mann-Whitney* posthoc test revealed a significant difference between K1 and K2, with a p-value of 0.000 ($p<0.05$). Based on the results, the induced metabolic syndrome caused damage to the histopathology of the testes of Wistar rats. The results also showed significant differences between K2 and K3, K4, and K5, with p-value = 0.000 ($P<0.05$). This indicated that the administration of ethanol extract of *Moringa* leaves improved the histopathology of the samples, but there were no differences between K3, K4, and K5 ($p>0.05$).

The Spearman test showed a significant correlation (p=0.000), with a coefficient of -0.396. This indicated that there was a fair correlation between the increase in the dose of ethanolic extract of *Moringa* leaves and the appearance of testicular histopathological damage. The higher the dose of the extract given, the lower the histopathological damage score.

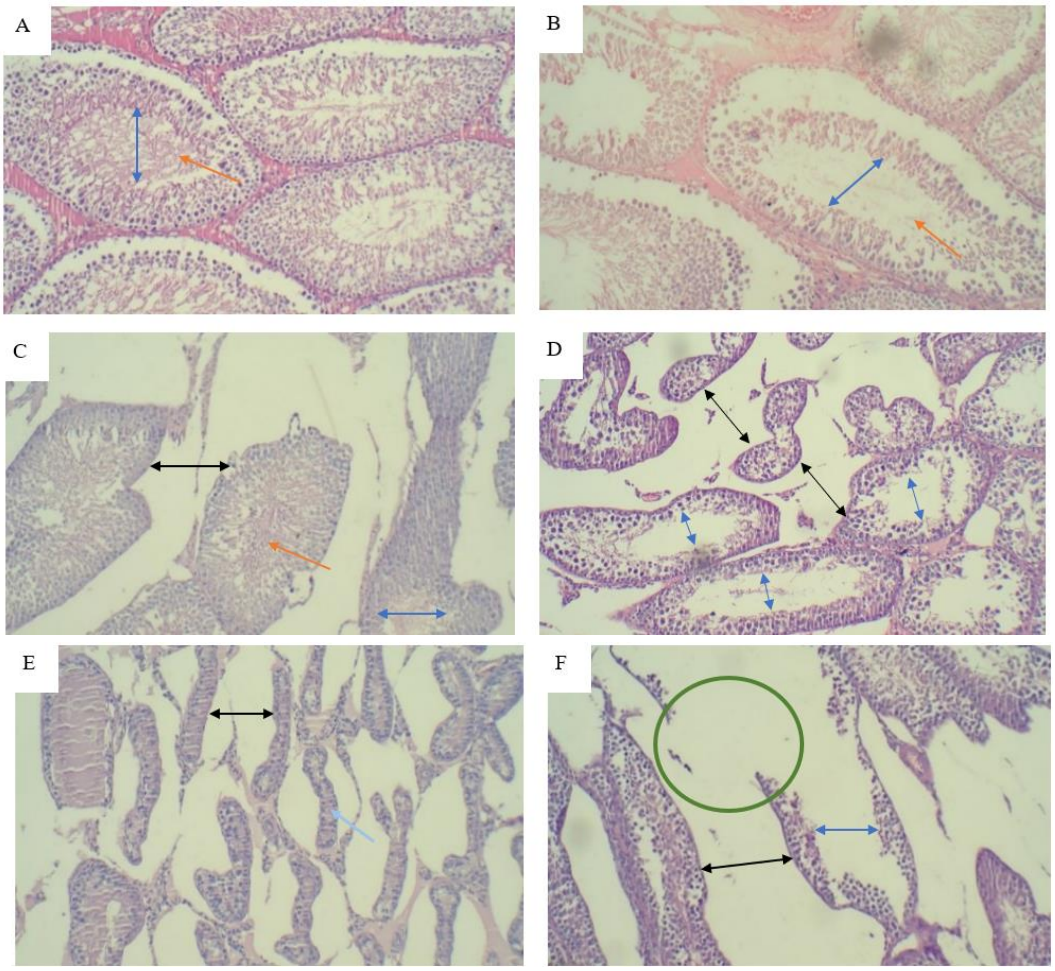

Figure 1. Testicular histopathological preparations with an enlargement of 100x. The lumen of seminiferous tubule(↔), spermatozoa(→), the distance between seminiferous tubules(↔), atrophy of seminiferous tubules(→), loss of epithelial integrity of seminiferous tubules(O). (A) Score 1, normal testicles. (B) Score 2, atrophy of seminiferous tubules with the dilated lumen of seminiferous tubules and reduced spermatozoa. (C) Score 2, the distance between the dilated seminiferous tubules. (D) Score 3, atrophy of seminiferous tubules with the dilated lumen of seminiferous tubules and reduced spermatozoa accompanied by the distance between dilated seminiferous tubules. (E) Score 3, atrophy of seminiferous tubules accompanied by a distance between the dilated seminiferous tubules. (F) Score 3, loss of epithelial integrity of the seminiferous tubules

DISCUSSION

Animal Model of Metabolic Syndrome

Metabolic syndrome is a metabolic disorder with symptoms of central obesity, insulin resistance, hypertension, high triglycerides, and low HDL. Based on NCEP-ATP criteria, it was characterized by 3 or more signs[4,15], including an 8% increase in BW, fasting glucose >200 mg/dL, triglycerides >150 mg/dL, cholesterol >110 mg/dL, and HDL <35 mg/dL[14].

This study showed that K1 did not meet the metabolic syndrome criterion due to an increase in only BW. However, K2, K3, K4, and K5 met 4 out of the five criteria, namely an 8% increase, fasting glucose >200 mg/dL, triglycerides >150 mg/dL, and HDL <35 mg/dL. Based on these findings, metabolic syndrome was absent in K1, but was evident in K2, K3, K4, and K5.

Effect of *Moringa* Leaves Ethanolic Extract on Testicular Histopathological Structure Induced by Metabolic Syndrome

Induction of metabolic syndrome in Wistar rats for 28 days caused severe damage to the reproductive organs. The results showed that there was a significant difference between the control and the group induced without ethanolic extract from *Moringa* leaves (p=0.000). This indicated that the induction of metabolic syndrome in Wistar rats for 28 days caused histopathological damage to the testicles of the samples. Histopathological damage was evident through the presence of edema and vast distances between seminiferous tubules, degenerative changes in seminiferous tubules characterized by incomplete constituent cells of the epithelium, increasing lumen width, and a decrease in sperm cells in the lumen.

Metabolic syndrome has been reported to cause hormonal disorders in the form of LH and FSH inhibition. The inhibited LH can lead to the inhibition of Leydig cell stimulation, leading to the suppression of testosterone formation as well as disruption of the spermiogenesis process in seminiferous tubule germ cells. Meanwhile, the inhibited FSH often affected spermatogenesis, causing a decrease or stoppage of sperm production. The inhibition of LH and FSH can cause degenerative changes, including atrophy of the seminiferous tubules, loss of germinal cell constituents, increase in the lumen width of the seminiferous tubules, and decrease in the volume of sperm cells[16–18]. Hari Priya and Reddy[13] reported the occurrence of oxidative stress conditions and chronic testicular damage, which are characterized by germinal cell death, widening of the distance between the tubules, and loss of testicular structure integrity.

The results showed that the administration of Moringa oleifera extract at a dose of 150 mg/kg repaired testicular damage caused by metabolic syndrome. This finding was supported by the statistically significant difference in histopathological score between K2 and K3. A previous study showed that the intake of *Moringa oleifera* ethanolic extract at a dosage of 100 mg/kg began to provide an improvement in the testicle histopathological structure of Wistar rats who were previously affected by side effects from HIV treatment. Furthermore, the testicular morphology began to show recovery, which was indicated by the typical appearance of the germ cells of the seminiferous tubules and an improvement in spermatogenic cells[19]. This was due to the antioxidant content of the extract with the ability to suppress testosterone, which caused a reduction in spermatogenic cells[20].

At a dose of 250 mg/kg, the treatment improved the testicular histopathological structure caused by metabolic syndrome. This was supported by the statistically significant difference in histopathological score between K2 and K4. The administration of a dose of 250 mg/kg showed a more noticeable improvement compared to 150 mg/kg. These results were in line with the Spearman Test (r:-0.396), that the increase in the dose of ethanolic extract of *Moringa* leaves from 150 mg/kg to 250 mg/kg correlated with the histopathological structure enhancement of Wistar rats testicles. Several studies have shown that the ethanolic extract of *Moringa* leaves at 200 mg/kg can provide better improvement compared to 100 mg / kg[11]. Furthermore, the ameliorative effect of the treatment was characterized by decreased fat degeneration and necrosis. These findings were consistent with Prabsattroo et al that ethanolic extract of *Moringa* leaves at 250 mg/kg increased the testosterone hormone levels and promoted spermatogenesis in the testicles of Wistar rats subjected to stress immobilization[21].

The administration of the extract at a dosage of 350 mg/kg had the best effect on testicular damage. This improvement was supported by the Spearman Test (r:-0.396) in groups K2, K3, K4, and K5. Compared to other studies, the dose of 350 mg/kg was included as one of the dosages with good histopathological structure enhancement. These results were supported by Ogunlade et al, that 300 mg/kg ethanolic extract of *Moringa oleifera* provided protection and improvement in the testicle histopathological structure of Wistar rats who were previously affected by the adverse effects of HIV

treatment. The treatment effect was indicated by the normal appearance of seminiferous tubule germ cells and spermatogenic cells in seminiferou[19]. This finding was in line with Jannah et al, that the best dose was 400 mg/kg. Furthermore, the dosage used had a significant effect on decreasing fat degeneration and necrosis in diabetic-induced Wistar rats[11].

In this study, *Moringa* leaves ethanolic extract (Moringa oleifera) was proven to repair and protect against testicular damage due to metabolic syndrome induction. These effects can be attributed to the high antioxidant content of the leaves, such as flavonoids, tannins, and saponins[8,9,22]. Flavonoids functioned as antioxidants by inhibiting the enzymes XO and NO, and reducing ROS, thereby accelerating the repair of cell damage. The derivatives of these compounds, such as quercetin, can also lower triglycerides and cholesterol levels. Quercetin has been reported to have the ability to decrease lipid peroxidation, inhibit intestinal glucose absorption, and protect β-pancreatic cells due to its potent antioxidant effect[10,21–24]. Furthermore, saponins have been found to possess antihyperglycemic properties and can stimulate insulin production in the pancreas. These compounds can also inhibit cholesterol formation, lower triglycerides, and suppress ROS formation due to metabolic syndrome[25,26]. Tannins and their derivatives have been reported to have antioxidant effects by inhibiting the production of NO. They also served as an anti-inflammatory agent and can reduce the production of ROS[9,10,27,28]. Alkaloids played a role in reducing oxidative stress parameters, which helped to lower lipids, cholesterol, and triglycerides while increasing antioxidant levels[9,10,29].

Alkafafy et al showed that ethanolic extract of *Moringa* leaves at 300 mg/kg improved mouse testicular dysfunction with a high-fat diet-induced obesity model, which was indicated by enhancement in testosterone hormone, LH, and FSH levels. The results showed that there was a decrease in degenerative changes in spermatogenic cells along with an increase in cell count and sperm motility[30]. Jannah et al reported that rats who had diabetes mellitus experienced histopathological improvement after the administration of ethanolic extract of *Moringa* leaves at 200 mg/kg, 300 mg/kg, 400 mg/kg, and 500 mg/kg doses, with the best histopathological structure observed at 400 mg/kg. The testicular improvement in this study was characterized by a decrease in fatty degenerations and decreased necrosis in the histopathological structure of rat testicles[11].

CONCLUSION

Moringa leaves ethanolic extract *(Moringa oleifera)* improved the testicular histopathological structure of the metabolic syndrome rat model in a dose-dependent manner.

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CONFLICT OF INTEREST

The authors reported no potential competing interests

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