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Effect of Etanolic Extract of Moringa (*Moringa oleifera, Lam.*) Leaf on Seminiferous Tubules of Wistar Rats (*Rattus norvegicus*) Model of Metabolic Syndrome

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ABSTRACT

Introduction: Metabolic syndrome define as symptoms of body abnormalities that increased reactive oxygen species and cause disturbances of the germ cell layer thickness in seminiferous tubules. The content of Moringa oleifera has anti-diabetic, anti-dyslipidemia, and hypotensive effects. This study aimed to find out theeffect of ethanolic extract of moringa leaves (*Moringa oleifera, Lam.*) on the histopathology of seminiferous tubules of Wistar rats (*Rattus norvegicus*) induced metabolic syndrome with Streptozotocin-Nicotinamide and high-fat diet.

Methods: This research was experimental post-only control group design. The subjects were 30 male *Rattus norvegicus* grouped into normal group (K1), metabolic syndrome group (K2), and 3 metabolic syndrome groups with ethanolicextract of moringa leaf at doses of 150 mg/kg (K3), 250 mg/kg (K4) and 350 mg/kg of body weight (K5).

Results: Thickness of tubules (μm) of group K1 (107.48±10.74), K2 (53.87±25.09), K3 (80.52±3.50), K4 (81.94±9.40), K5 (94.04±8.66). Average number of cell layers of group K1 (8.00±1.09), K2 (3.65±1.35), K3 (5.99±0.36), K4 (6.57±0.36), K5 (6.72±0.20). There was a significant difference in tubule wall thickness and the number of cell layers between the five groups ($p<0.05$). The K3, K4, and K5 differed significantly from the K2 ($p<0.05$). K5 has value closest to K1.

Conclusion: Ethanolic extract of moringa (*Moringa oleifera, Lam.*) leaf increased the wall thickness and number of cell layers of seminiferous tubules of Wistar rats (*Rattus norvegicus*) model of metabolic syndrome with ethanolicextract of moringa leaf at doses of 350 mg/kg ofbody weight most effectively improved the histopathological of the seminiferous tubules.

Keywords: Metabolic syndrome; *Moringa oleifera*; *Rattus norvegicus*; seminiferous tubules.

INTRODUCTION

Changes in people's lifestyles resulted in 25% of the world's population suffering from metabolic syndrome, according to The International Diabetes Federation (IDF) in 2015. This number continues to increase every year. The increase affects the prevalence of metabolic syndrome in developed and developing countries. Indonesia is one of the countries that has a high metabolic syndrome prevalence rate, which is 21.66% [1].

Metabolic syndrome is defined as symptoms of body's metabolic abnormalities such as hypertension, insulin resistance, dyslipidemia, and obesity[2]. This symptoms increase reactive oxygen species (ROS) which causes disturbances in the thickness of the germ cell layer in the seminiferous tubules, germinal atrophy, and spermatogenesis arrest[3]. Metabolic syndrome also causes impaired spermatogenesis, decreased sperm quality, and decreased maturation of sperm cells[4,5].

Currently, the use of natural herbal medicines or phytopharmaceuticals is more widely chosen by the public than conventional medicine[6]. One of the widely used plants that is *Moringa oleifera* or moringa. Moringa is known as "The Miracle Tree" because it has many benefits. This plant can be use from leaves to roots[6–9]. Indonesians often use the leaves as medicines, food sources, cultural rituals, and cosmetic ingredients[7,8].

Moringa oleifera has benefits in insulin regulation and exhibits anti-hyperglycemia, anti-diabetic, anti-dyslipidemia, hypotensive, cholesterol-lowering effects, Low-Density Lipoprotein-lowering effects, and Very Low-Density Lipoprotein-lowering effects[7,10].

There have been no previous studies on the effect of moringa leaf on the testicles of metabolic syndrome-induced rats. This study aimed to find out the effect of ethanolic extract of moringa (*Moringa oleifera, Lam.*) leaf on the histopathology of seminiferous tubules of the testicles of Wistar rats (*Rattus norvegicus*) induced metabolic syndrome.

METHODS

This research is experimental with a posttest-only control group design. The research was conducted at the Laboratory of the Center for Food and Nutrition Studies of Universitas Gadjah Mada and the Laboratory of Anatomical Pathology of Universitas Sebelas Maret.

The subjects of this study were 30 male white rats (*Rattus norvegicus*) of Wistar strain, aged 2-3 months, and had a body weight of 150-200 grams. Meanwhile, the excluded criteria were rat that pale, often sneezed, hair loss and dullness, weight loss after adaptation, and died before the research end. The sampling used is purposive sampling, which is sampling by selecting subjects based on criteria. Randomization was done on the sample to divide the subjects into 5 groups. The sample size was calculated using the Federer's formula, as follow:

$$(k-1)(n-1) \geq 15$$
$$(5-1)(n-1) \geq 15$$
$$4n-4 \geq 15$$
$$4n \geq 19$$
$$n \geq 4{,}75$$

k: number of groups
n: number of samples per groups

The minimum number of samples per group was 5 mice, plus 1 rat per group so the number of mice used was 30 mice.

The sample is divided into 5 groups, named K1: control group; K2: metabolic syndrome group; K3: metabolic syndrome induced group with ethanolicextract of moringa leaf at doses of 150 mg/kg of body weight; K4: metabolic syndrome induced group with ethanolic extract of moringa leaf at doses of 250 mg/kg of body weight; K5: metabolic syndrome induced group with ethanolicextract of moringa leaf at doses of 350 mg/kg of body weight.

The metabolic syndrome condition is performed by induction of oxidized oil, duck egg yolk, and cow fat for 28 days and high induction of fructose by injection of Streptozotocin-Nicotinamide on day 25. On the day 57, the animal is dissected and a testicular organ is taken. Organs cut and inserted into cassette tissue. Staining the object using Hematoxylin-Eosin (HE). The object then observed using a microscope with a magnification of 100x in 9 viewing fields. Tubule wall thickness and number of cell layers measured using image raster.

Normality test using the Shapiro-Wilk test. Normal distributed data, it is continued using one-way anova and continued post hoc analysis using the Fisher LSD (Least Significant Difference) test. If the distribution is abnormal, the Kruskal-Wallis test is used and the post hoc analysis is continued with the Mann-Whitney test.

This study's ethical clearance was approved by The Health Research Ethics Committee Dr. Moewardi, with reference number 1.297/X/HREC/2022.

RESULTS

Measurement Results Data

The observed histopathological picture of seminiferous tubules is the thickness of the walls of the seminiferous tubules and the number of layers of germ cells in the tubules. The histopathological picture from the five groups is shown in the following figure:

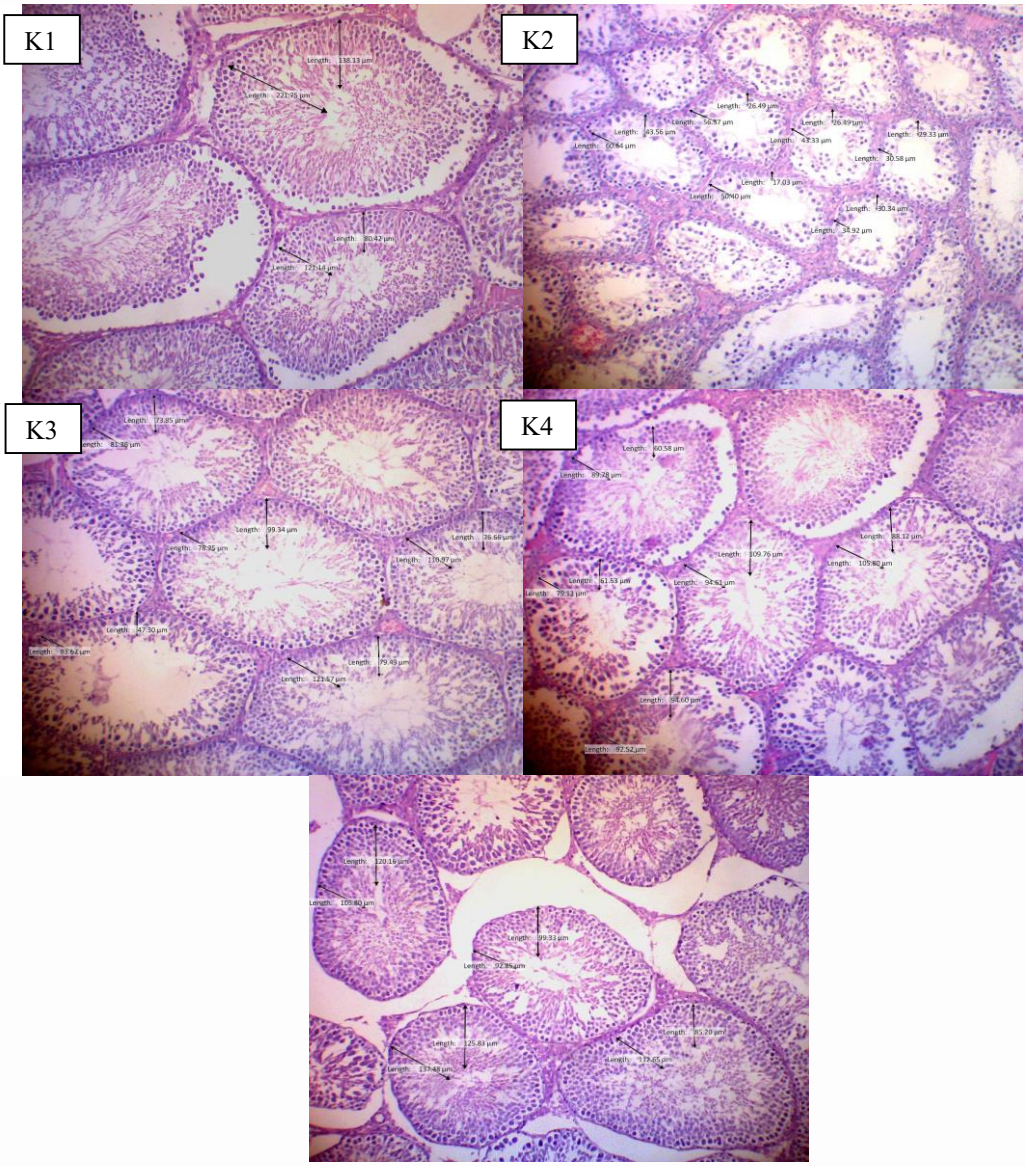

Figure 1. Histopathology of seminiferous tubules

The measurement results of each group will be averaged to get a score for each group. Measurement results are shown in table 1.

Table 1. Average wall thickness of the tubules and number of cell layers

Group	Mean±SD	
	Thickness of the tubules (µm)	Number of cell layers
K1	107.48±10.74	8.00±1.09
K2	53.87±25.09	3.65±1.35
K3	80.52±3.50	5.99±0.36
K4	81.94±9.40	6.57±0.36
K5	94.04±8.66	6.72±0.20

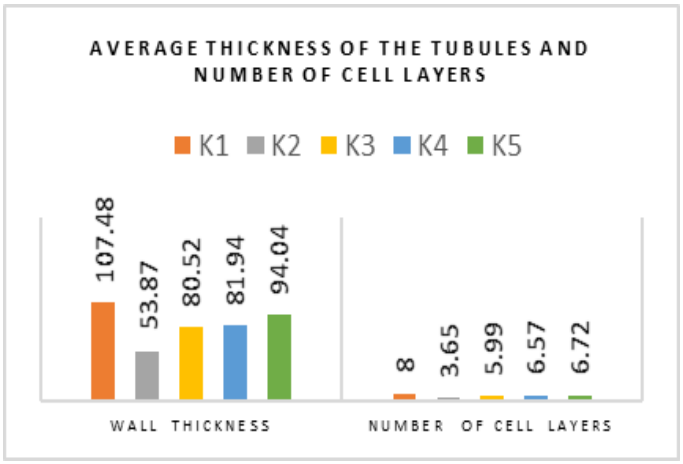

Figure 2. Diagram of the average wall thickness of the tubules and number of cell layers

Table 1 and figure 2 shows the mean thickness of the tubules (µm) and the number of cell layers in all five groups. In the control group (K1), the average tubule thickness was 107.48±10.74, the metabolic syndrome group (K2) was 53.87±25.09, the metabolic syndrome induced group with ethanolic extract of moringa leaf at doses of 150 mg/kg of body weight group (K3) was 80.52±3.50, the metabolic syndrome induced group with ethanolic extract of moringa leaf at doses of 250 mg/kg of body weight group (K4) was 81.94±9.40, and the metabolic syndrome induced group with ethanolic extract of moringa leaf at doses of 350 mg/kg of body weight group (K5) was 94.04±8.66. Table 1 and figure 2 shows the average number of cells layers in the control group (K1) was 8.00±1.09, the metabolic syndrome group (K2) was 3.65±1.35, the metabolic syndrome induced group with ethanolic extract of moringa leaf at doses of 150 mg/kg of body weight group (K3) was 5.99±0.36, the metabolic syndrome induced group with ethanolic extract of moringa leaf at doses of 250 mg/kg of body weight group (K4) was 6.57±0.36, and the metabolic syndrome induced group with ethanolic extract of moringa leaf at doses of 350 mg/kg of body weight group (K5) was 6.72±0.20. Based on table 1 and figure 2, there is an increase in the average thickness of the tubules and the number of cell layers in the group with ethanolic extract of moringa leaf at doses of 150 mg/kg of body weight (K3), 250 mg/kg of body weight (K4), and 350 mg/kg of body weight (K5). The increase was in line with an increase in dose. The group with ethanolic extract of moringa leaf at doses of 350 mg/kg of body weight (K5) showed the highest mean increase in tubule thickness and number of cell layers and it had an average closest to the control group (K1).

Analysis of Tubule Thickness

Table 2. Kruskal-Wallis test on tubule thickness

Group	Tubule Thickness			*p-value*
	Mean (µm)	95% CI for Mean		
		Lower Bound	Upper Bound	
K1	107.48	96.20	118.75	
K2	53.87	27.53	80.20	
K3	80.52	76.85	84.19	<0.001
K4	81.94	70.26	93.62	
K5	94.04	84.95	103.13	

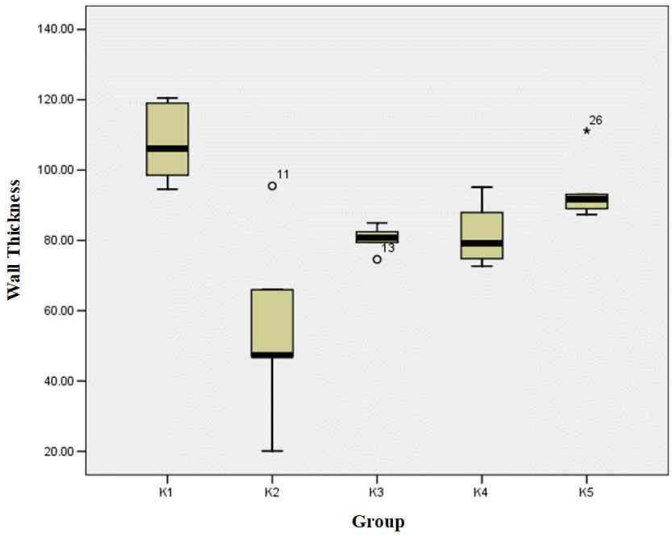

Figure 3. Boxplots diagram of the average wall thickness of the tubules

Based on table 2, the results of the *Kruskal-Wallis* statistical test obtained a value of p=0.001 (<0.05), which means that there are significant differences between the five treatment groups.

Table 3. Post hoc Mann-Whitney test on tubule thickness

Group	Against Groups	*p-value*
K1 (107.48)	K2 (53.87)	0.006
	K3 (80.52)	0.004
	K4 (81.94)	0.011
	K5 (94.04)	0.025
K2 (53.87)	K3 (80.52)	0.055
	K4 (81.94)	0.068
	K5 (94.04)	0.037
K3 (80.52)	K4 (81.94)	0.855
	K5 (94.04)	0.004
K4 (81.94)	K5 (94.04)	0.100

Based on table 3, it is known that the control group (K1) differs significantly from the metabolic syndrome group (K2) with a value of p<0.05, which means that the metabolic syndrome induced of the K2 group was successful and had an impact on reducing the thickness of the tubule walls.

In the group with ethanolic extract of moringa leaf at doses of 350 mg/kg of body weight (K5) increased the thickness of the tubules and showed significantly different results from the K2 group with a value of p=0.037 (p<0.05). Thus, the K5 group was the most effective at increasing the thickness of

the tubules compared to the moringa leaf treatment group at doses of 150 mg/kg of body weight (K3) and 250 mg/kg of body weight (K4).

Analysis of Number of Cell Layers

Table 4. Anova test on number of cell layers

Group	Number of Cell Layers			*p-value*
	Mean	95% CI for Mean		
		Lower Bound	Upper Bound	
K1	8.00	6.85	9.14	
K2	3.65	2.23	5.07	
K3	5.99	5.61	6.37	<0.001
K4	6.57	6.13	7.02	
K5	6.72	6.51	6.93	

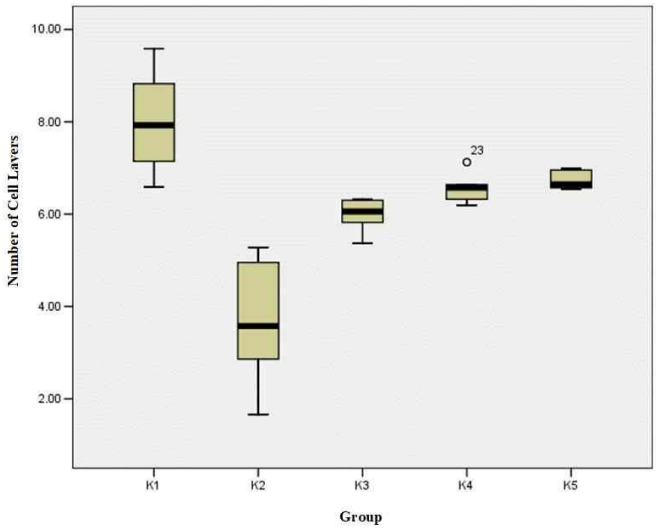

Figure 4. Boxplots diagram of the average number of cell layers

Based on table 4, anova's statistical test results obtained a value of p=0.001 (<0.05) which means that there is a significant difference between the five groups.

Table 5. Post hoc LSD test on number of cell layers

Group	Against Groups	*p-value*
K1 (8.00)	K2 (3.65)	<0.001
	K3 (5.99)	<0.001
	K4 (6.57)	<0.001
	K5 (6.72)	0.014
K2 (3.65)	K3 (5.99)	<0.001
	K4 (6.57)	<0.001
	K5 (6.72)	<0.001
K3 (5.99)	K4 (6.57)	0.257
	K5 (6.72)	0.137
K4 (6.57)	K5 (6.72)	0.764

Based on table 5, it is known that the group with ethanolic extract of moringa leaf moringa leaf extract treatment groups (K3, K4, K5) showed significantly different results from the control group (K1) with a p<0.05 value, which means that there was a significant difference in the number of cell layers between the moringa leaf extract treatment groups and the control group.

The moringa leaf extract treatment groups (K3, K4, K5) differ significantly from the metabolic syndrome group (K2) with a value of $p<0.05$, which means that the treatment of moringa leaf extract effectively increases the number of cell layers. The moringa leaf extract treatment group at a dose of 350 mg/kg of body weight (K5) increased the number of cell layers compared to moringa leaf extract doses of 150 mg/kg of body weight (K3) and 250 mg/kg of body weight (K4).

DISCUSSION

The results of the measurement of seminiferous tubules showed a decrease in thickness and number of cell layers in the metabolic syndrome-induced group. These results are in line with the Alkandurur dan Kum (2021) research which mentions a decrease in the thickness of the seminiferous tubules and the number of spermatogenic cells which has an effect on the decrease in the number of cell layers in metabolic syndrome conditions. This occurs as a result of cell degeneration due to increased necrosis and cell apoptosis events due to lipid peroxidation. Lipid peroxidation is triggered by oxidative stress conditions, where there is an imbalance between ROS and the body's antioxidants. In addition to spermatogenic cells, ROS also causes degeneration in Leydig and Sertoli cells so that it will lower testosterone levels and affect the function of testicular spermatogenesis[11–14].

In the moringa leaf extract groups (K3, K4, K5), the average value of tubule thickness and the number of cell layers approached the control group (K1) in line with the increase in dose. The metabolic syndrome group (K2) showed the lowest values on the mean thickness of the tubules and the number of layers of the seminiferous tubule cells. These results showed that the administration of ethanolic extract of moringa leaves was able to improve the thickness of the tubules and the number of layers of seminiferous tubule cells in metabolic syndrome conditions. This happens because moringa leaves contain phytochemical compounds that act as antioxidants, such as tannins, steroids, flavonoids, saponins, phenolic acids, and anthraquinones[15,16]. Antioxidants will inhibit the production of ROS thus preventing oxidative stress and protecting cell membranes from lipid peroxidation and DNA damage[17,19]. In addition, vitamins C and E in Moringa leaves also play a role in inhibiting the increase in ROS. Antioxidants and vitamins can improve the thickness of the epithelium of the seminiferous tubules and increase the number of spermatogenic cells[19].

Moringa leaves can also increase testosterone levels which serve for the process of spermatogenesis[17]. The results of this study are also supported by Ogunsola (2017) research which proves that the effect of *Moringa oleifera* on the reproductive organs shows improvements in the histological function and physiology of the seminiferous tubules of the testes.

The post hoc test results for the thickness of the seminiferous tubules, it showed that the moringa leaf extract treatment group with a dose of 350 mg/kg of body weight (K5) was the highest improving the thickness of the tubules compared to the ethanolic extract treatment group of moringa leaves at doses of 150 mg/kg of body weight (K3) and 250 mg/kg of body weight (K4). The K5 group also showed significantly different results from the metabolic syndrome group (K2). Thus, the group of moringa leaves at doses of 350 mg/kg of body weight (K5) treatment is most effective in fixing the thickness of the tubules.

In the post hoc test of the number of cell layers, the group with moringa leaf extract with dose of 150 mg/kg of body weight (K3), 250 mg/kg of body weight (K4), and 350 mg/kg of body weight (K5) improved the number of cell layers with significantly different results from the metabolic syndrome group (K2). The moringa leaf extract groups (K3, K4, K5) are effective in increasing the number of cell layers. Moringa leaf extract dose of 350 mg/kg of body weight group (K5) showed the highest improvement compared to moringa leaf extract dose of 250 mg/kg of body weight group (K4) and 350 mg/kg of body weight group (K5).

These results are in line with the Jannah *et al* (2018) study that the most effective dose of improving testicular picture after diabetes mellitus (DM) induced was 400 mg/kg of body weight, which means that administering moringa leaf extract at a dose of 400 mg/kg of body weight is sufficient to lower fat degeneration and necrosis in cells in the mouse testicles of Wistar model DM. Gunawati *et al* (2019) research also proved that the dose of moringa leaf extract of 300 mg/kg of body weight is most effective in increasing spermatogenic cells after intervention of excessive physical activity compared to doses of 100 and 200 mg/kg of body weight. Meanwhile, Adedapo *et al* (2009) research mentioned that the effective dose that can prevent damage from rat testicles is 500 mg/kg of body weight. Stohs dan Hartman (2015) research proved the extract dose of Moringa leaves is safe to be administered peroral to rats up to a dose of 2000 mg/kg of body weight, but at a dose of 800 mg/kg of body weight showed an increase in some liver enzymes which means there began to be damage to the organs.

This study only used a narrow dose of moringa leaf extract, so research on the effect of moringa leaves on the histopathology of the seminiferous tubules of the testicles of Wistar rats with wider dose intervals is needed to determine the optimal dose and toxic dose. Preliminary research on testosterone levels is also needed to determine the relationship of testosterone levels with the histopathological picture of the seminiferous tubules of the testicles of Wistar rat model of metabolic syndrome induced.

CONCLUSION

Ethanolic extract of moringa leaves (*Moringa oleifera, Lam*.) has the effect of increasing the wall thickness and the number of layers of seminiferous tubule tubules seminiferous testicles of Wistar rats (*Rattus norvegicus*) model of metabolic syndrome- induced. A dose of 350 mg/kg of body weight most effectively improved the histopathology of the seminiferous tubules of the testicles of Wistar rats (*Rattus norvegicus*) model of metabolic syndrome.

CONFLICT OF INTEREST

The authors reported no competing interests.

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