Hepatoprotector Effect of *Moringa Oleifera Lam.* Seeds Extract Through Decrease Liver Inflammation of Rats with Metabolic Syndrome

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ABSTRACT

***Background*:** *Metabolic syndrome has been associated with chronic inflammation due to the increase of lymphocyte focus on hepatic lobular tissue. Moringa oleifera Lam. is an herbal plant that potentially reduce the inflammation process. This study aims to evaluate the effect of Moringa seed extract (MSE) on hepatic lobular inflammation in rats with hepatic tissue metabolic syndrome (MetS).*

Method: *Twenty-four male Wistar rats (n = 24) were assigned into four groups: one control group (C) and three MetS groups, fed with a high-fat, high-fructose diet (HFHFD) daily for 53 days. After 53 days, respectively, MetS 2 and MetS 3 groups were given 150 and 200 mg/kg MSE. After 28 days of MSE administration, the rats were sacrificed, and then hepatic lobular inflammation measured with lobular inflammation score. The effect of MSE on hepatic lobular inflammation was analyzed with Kruskal-Wallis and Mann-Whitney test.*

Results: *There was a significant difference in hepatic lobular inflammation between four groups (p = 0.000). Mann-Whitney showed a significant difference between C vs. MetS 1, MetS 1 vs. MetS2, and MetS 1 vs. MetS 3 (p = 0.000), but not significant C vs. MetS 2 (p = 0.364), C vs. MetS 3 (p = 0.109), MetS 2 vs. MetS 3 (p = 0.533).*

Conclusion: *MSE at the dose of 150 mg/kg and 200 mg/kg significantly reduces lobular inflammation in hepatic tissue of MetS rats.*

Keywords: *metabolic syndrome, moringa oleifera Lam. seeds extract, hepatic lobular, inflammation*

ABSTRAK

Latar belakang: *Metabolik sindrom berhubungan dengan kondisi inflamasi kronis yang disebabkan karena peningkatan fokus inflamasi oleh sel limfosit pada jaringan hepar. Moringa oleifera Lam. merupakan tanaman herbal yang memiliki potensi untuk menurunkan proses inflamasi. Studi ini bertujuan untuk mengetahui pengaruh pemberian ekstrak biji kelor (MSE) terhadap kondisi inflamasi jaringan hepar tikus model sindroma metabolik (MetS).*

Metode: *Dua puluh empat tikus Wistar jantan (n = 24) terbagi menjadi empat kelompok: satu kelompok kontrol (C) dan tiga kelompok MetS, diberi diet tinggi fruktosa dan tinggi lemak (HFHFD) setiap hari selama 53 hari. Setelah 53 hari, kelompok MetS 2 dan MetS 3 diberi 150 and 200 mg/kg MSE setiap hari selama 28 hari. Setelah 28 hari pemberian MSE, tikus diterminasi dan dibuat sediaan histopatologi untuk mengamati kondisi inflamasi jaringan lobulus hepar yang diukur menggunakan skor lobular inflammation. Pengaruh pemberian MSE pada jaringan hepar dianalisis menggunakan Kruskal-Wallis dan Mann-Whitney test.*

Hasil: *Didapatkan nilai signifikan pada keempat kelompok (p = 0.000). Hasil uji Mann-Whitney menunjukan hasil signifikan pada kelompok C vs. MetS 1, MetS 1 vs. MetS 2, dan MetS 1 vs. MetS 3 (p = 0.000), tetapi tidak signifikan pada kelompok C vs. MetS 2 (p = 0.364), C vs. MetS 3 (p = 0.109), MetS 2 vs. MetS 3 (p = 0.533).*

Simpulan: *MSE pada dosis 150 mg/kg dan 200 mg/kg secara signifikan mampu menurunkan fokus inflamasi pada jaringan lobulus hepar tikus model sindroma metabolik.*

Kata kunci: *sindroma metabolik, ekstrak biji moringa oleifera Lam., lobulus hepar, inflamasi*

INTRODUCTION

Metabolic syndrome is an accumulation of several disorders, which together raise the risk of an individual developing atherosclerotic cardiovascular disease, insulin resistance, and diabetes mellitus, and vascular and neurological complications such as a cerebrovascular accident. The diagnosis of metabolic syndrome can be determined if 3 of the 5 criteria are found. The criteria are obesity, hyperglycemia, hypertension, low high density lipoprotein (HDL) cholesterol and high triglycerides. The prevalence of metabolic syndrome increases almost in every country. Most countries show 20-30% of the adult population suffering from metabolic syndrome. While in Indonesia, the prevalence of metabolic syndrome in the elderly population is around 14.9%. The high prevalence of metabolic syndrome is influenced by factors such as body mass index and total serum cholesterol.[1-3] Dyslipidemia is the imbalance of lipids such as cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides, and high-density lipoprotein (HDL). This condition can result from diet, tobacco exposure, or genetic and can lead to cardiovascular disease with severe complications. Hypertriglycerides in dysplidemia is related to increase systemic oxidative stress and decrease levels of HDL as an antioxidant in people with metabolic syndrome. The presence of systemic oxidative stress affects the occurrence of fat accumulation in the visceral organs. The main organ that plays a role in the metabolism of fat and cholesterol is the liver.[3,4]

Liver disorders mechanism is mediated by the accumulation of triglycerides in the liver and the inflammatory response due to the increase of pro-inflammatory cytokines, adipocytokines, mitochondrial dysfunction, oxidative stress, and lipid peroxidation. According to the NASH clinical research network scoring system, liver disorder consists of steatosis, lobular and portal inflammation, and fibrosis. The inflammation is induced by the high FFA that triggers the release of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IFN γ) that are channeled through the portal vein in excess, thus attract inflammatory cells to arrive. Insulin resistance also causes an increase in lipolysis and de novo lipogenesis which further aggravates inflammation in liver.[5]

Moringa Oleifera (MO), a plant from the family Moringacea is a major crop in Asia and Africa. MO contains bioactive components and nutrients that have activities as antifibrosis, antihyperglycemic, anticancer, anti-inflammatory, and antioxidant. Moringa seeds contain bioactive components, such as vitamin C, β-carotene, α-tocopherol, γ-tocopherol, β-sitosterol, and vitamin A. Moreover, Moringa seeds are also rich in polyphenol content consisting of quercetin, kaempferol, flavonoids, anthocyanins, and can also be found alkaloids, glucosinolates, and isothiocyanates. Moringa seed has antioxidant effects that can reduce cell damage due to oxidative processes.[6,7] This study aimed to evaluate the effect of Moringa seed extract (MSE) on hepatic lobular inflammation in rats with hepatic tissue metabolic syndrome (MetS).

METHOD

This experimental laboratory study was conducted at the Center for Food and Nutrition Study Laboratory of Universitas Gadjah Mada and Anatomic Pathology Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Indonesia, from August 2017 to March 2018. A post-test-only control group design was used in this study. The sampling technique employed was purposive sampling with a sample size of 24 rats (n = 24), divided randomly into four groups: one control diet group (C) and three MetS groups that would be fed with high-fat, high-fructose diet (HFHFD).

Ethanolic extract of *Moringa oleifera Lam.* seeds (MSE) was used as the material of this study. The seeds were obtained from Warung Bobot Kelor Bu Yati, Indonesia, and extracted at the UGM GMG Laboratory, Indonesia. Seeds were dried and converted into simplicial form, then extracted by maceration method using a solution of 70% ethanol.

The subjects of this study were twenty-four Wistar (*Rattus norvegicus*) male rats (n = 24), which met

the following conditions: (1) aged 2-3 months; (2) weight of 150-200 g; (3) active and not physically disabled. The subject criteria for MetS rats consisted of three following conditions: (1) obesity; (2) hypercholesterolemia; (3) hyperglycemia. The subjects (n = 24) then randomly assigned into four groups: control (C), MetS 1, MetS 2, and MetS 3 groups. HFHFD was given to the MetS groups. Meanwhile, the C group was given BR-2 pellets and tap water. The given HFHFD consisted of duck egg 10 mL/kg, beef fat 10 mL/kg, oxidized oil 5 mL/kg, and fructose 1.8 gram/kg. The condition of MetS was obtained after 53 days. In this study, MSE was given orally with the gastric tube for MetS 2 and MetS 3 groups, with the dosage of 150 and 200 mg/kg/day, respectively, for 28 consecutive days.

After induction, subjects were assessed whether they met the MetS criteria discussed in the review. Established MetS model rats were characterized as having the following traits: hyperglycemia, obesity, and hypercholesterolemia. Obesity was determined by assessing body weight before and after HFHFD induction. Serum blood glucose level and serum cholesterol level were assessed to determine hyperglycemia and hypercholesterolemia, respectively. After the laboratory assessment, each subject would be sacrificed and assessed microscopically altogether to determine their mean of focal inflammation by hematoxylin eosin (HE) staining. The test carried out was a post-test-only group design.

A series of analysis tests were carried out to test the effect of giving Moringa seed ethanolic extract on the focal lobular inflammation in liver tissue of sample rats. First, a normality test was carried out to see the data distribution. Because the data was less than 50, the data analysis used was the Shapiro-Wilk test. The data of weight, serum blood glucose, and cholesterol were then analyzed by the Friedman test and followed by Wilcoxon test. Then the data of focal lobular inflammation were analyzed by the Kruskal-Wallis test followed by the Mann-Whitney posthoc test.

RESULTS

Serum Markers

The Shapiro-Wilk test for the sample showed that the data were not normally distributed ($p < 0.05$) and fulfilled of Friedman test for data of weight, serum glucose and cholesterol. The summary of the data is shown in Table 1.

The MetS groups successfully developed the experimental MetS after the HFHFD induction. The experimental MetS criterion previously mentioned had been met by the MetS groups: obesity, hypercholesterolemia, and hyperglycemia.

Measurement of Focal Lobular Inflammation

Hepatic lobular inflammation was observed by counting the number of focal inflammation that were further categorized into scores. Observation used 5 fields with a 100x magnification. The mean of each group's focal lobular inflammation is shown in Tables 2. It shows the differences in focal lobular inflammation between four groups.

Table 1. Mean weight of glucose and cholesterol levels in each group

Group	Weight				Glucose				Cholesterol			
	Pre (mean± SD)	Post (day 53) (mean± SD)	Post (day 82) (mean± SD)	p	Pre (mean± SD)	Post (day 53) (mean± SD)	Post (day 82) (mean± SD)	p	Pre (mean± SD)	Post (day 53) (mean± SD)	Post (day 82) (mean± SD)	p
C	158.29 ± 3.86	204.57 ± 3.78	223,83 ± 3,71	< 0.001	70.47 ± 2.12	74.30 ± 2.00	78,50 1,90	0.009	67.75 ± 2.27	69.06 ± 2.15	69.73 ± 2.17	0.328
MetS1	160.86 ± 5.75	227.00 ± 5.23	251,57 2,44	< 0.001	70.37 ± 2.99	159.55 ± 3.05	160,67 ± 2,86	< 0.001	69.05 ± 3.67	170.39 ± 3.52	171.32 ± 3.54	< 0.001
MetS2	156.29 ± 2.21	223.57 ± 3.05	264,67 4,18	< 0.001	68.51 ± 1.70	161.48 ± 4.19	130,42 ± 1,62	< 0.001	69.70 ± 2.35	167.93 ± 2.88	127.08 ± 3.61	< 0.001
MetS3	161.00 ± 3.91	229.00 ± 4.39	249,57 4,86	< 0.001	69.87 ± 2.37	160.75 ± 2.44	112,21 ± 3,26	< 0.001	70.56 ± 2.75	168.59 ± 2.88	99.62 ± 1.66	< 0.001

Note: Between-group differences were analyzed using Friedman-test

Table 2. Total of lobular inflammation score in each group

Group	Field	Lobular Inflammation score	n (%)
C	30	0	14 (46.7)
		1	16 (53.3)
		2	0 (0)
		3	0 (0)
MetS1	30	0	0 (0)
		1	27 (90.0)
		2	3 (10.0)
		3	0 (0)
MetS2	30	0	11 (36.7)
		1	18 (60)
		2	1 (3.3)
		3	0 (0)
MetS3	30	0	8 (26.6)
		1	21 (70.0)
		2	1 (3.3)
		3	0 (0)

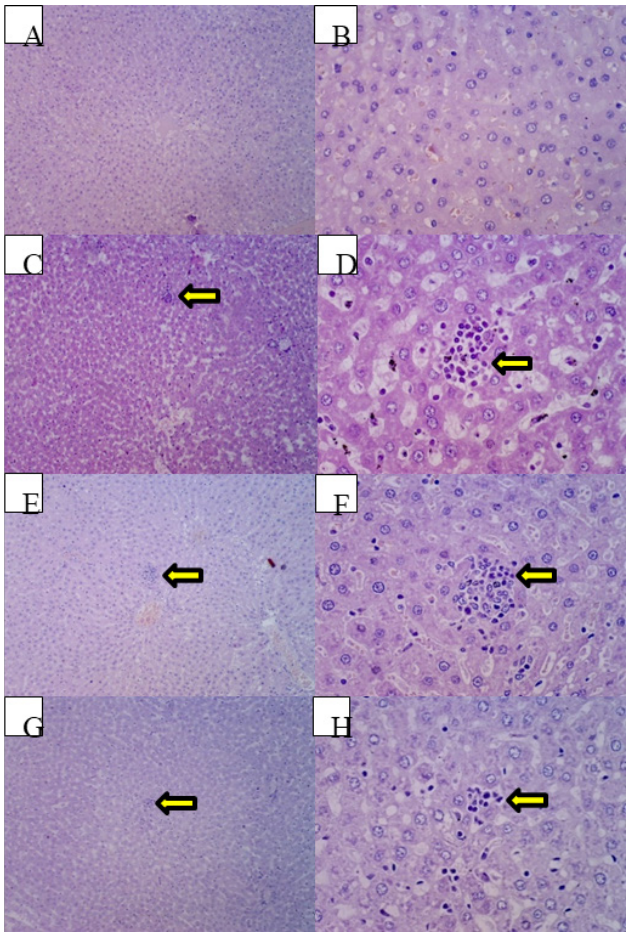

Figure 1. (A;B) C group with HE (100x); (400x) ; (C;D) MetS 1 group with (100x); (400x) ; (E;F) MetS 2 group with (100x); (400x) ; (G;H) MetS 3 group with (100x); (400x); yellow arrow show the inflammation focus

The hematoxylin eosin (HE) staining of focal lobular inflammation on hepatic tissue of metabolic syndrome rats result displayed in Figure 1.

The Shapiro-Wilk test for the sample showed that the data were not normally distributed ($p < 0.05$) and require of Kruskal-Wallis test for focal lobular inflammation. Kruskal-Wallis revealed a significant difference between four groups ($p = 0.000$; $p < 0.05$). Between the sample groups, the Mann-Whitney test was conducted and summarized in Table 3. The test uncovered significant differences between C and MetS 1 groups ($p = 0.000$), and between the MetS groups (MetS 1 vs. 2 ($p = 0.000$) and MetS 1 vs. 3 ($p = 0.001$)) but not significant between C vs. MetS 2 ($p = 0.364$), C vs. MetS 3 ($p = 0.109$), and MetS 2 vs. 3 ($p = 0.533$).

Table 3. Mann-Whitney Test of focal lobular inflammation

Group	p	Sig.
C – MetS1	0.000	Sig.
C – MetS2	0.364	Not Sig.
C – MetS3	0.109	Not Sig.
MetS1 – MetS2	0.000	Sig.
MetS1 – MetS3	0.000	Sig.
MetS2 – MetS3	0.533	Not Sig.

DISCUSSION

In this study, we sought to examine the effect of *Moringa oleifera Lam.* seeds extract and liver focal inflammation of MetS rats. Our findings revealed that *Moringa oleifera Lam.* seeds extract was significantly associated with decreased focal lobular liver inflammation. After the high-fat and high-fructose diets (HFHFD), the mean body weight of subjects in MetS 1, MetS 2, and MetS 3 groups showed an increment of at least 8%. It achieved an increase of glucose levels (> 126 mg/dL), cholesterol level (> 110 mg/dL), but not in triglyceride level (> 150 mg/dL). It might be due to triglycerides in very-low-density lipoprotein (VLDL) already converted by the lipoprotein lipase enzyme in blood vessels into low density lipoprotein (LDL), containing much cholesterol and a little triglyceride. Based on these results, the criterion for high-fat and high-fructose-fed rats conditions had been fulfilled so that the discussion could be carried out on the effect of MSE on focal lobular inflammation on liver tissue.

A high-fat high-fructose diet in metabolic syndrome may increase free fatty acid (FFA) uptake in liver. FFA is synthesized into VLDL which is hydrolyzed into LDL. LDL is released and accumulated in plasma, thereby increasing the amount of total cholesterol in the blood plasma. Lipid accumulation in metabolic syndrome can cause adipocyte cells hypertrophy which are more prone to adipocyte rupture. It will

release free fatty acids in the blood circulation and trigger inflammatory cells through the production of reactive oxygen species (ROS) and pro-inflammatory cytokines. ROS causes oxidative stress that can induce hepatotoxin to activate macrophages and release more chemokines and pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), Interleukin (IL-6, IL-1β, IL-8), Interferon γ (IFN-γ). These cytokines engage inflammatory cells, consisting of granulocytes, macrophages, T lymphocyte cells, B lymphocyte cells, and plasma cells to move towards liver.[8,9]

In this study, there were difference between focal lobular liver inflammation score in C-MetS1 ($p = 0.000$). C as a control group compared to MetS1 as a metabolic syndrome-induced group showed a difference with a higher MetS1 lobular inflammation score than C group. This showed high-fat high-fructose diet affects hepartic lobular inflammation. After giving ethanolic extract of Moringa seeds, there was a significant decrease in total cholesterol levels in MetS2 group and Mets3 group. The decrease in the average total cholesterol level occurs due to the effects of secondary metabolites contained in the ethanolic extract of Moringa seeds. These metabolites are β-sitosterol and anthocyanin. β-sitosterol is a phytosterol that occupies cholesterol receptors in the intestinal micelles because of its structure similar to cholesterol, thus causing a decrease in total cholesterol in the blood because the cholesterol receptors are replaced by β-sitosterol.[10]

Anthocyanin is secondary antioxidant that inhibits the absorption of cholesterol in the intestines and the synthesis of cholesterol in the liver. According to Manurung et al (2015), anthocyanin reduces LDL levels by inhibiting the work of 3-Hydroxy-3-methylglutaril coenzyme A reductase (HMG Co-A reductase). The enzyme catalyzes the change of HMG Co-A into mevalonic acid which is the initial stage of cholesterol synthesis. Because cholesterol synthesis decreases, it will also decrease total cholesterol levels in the blood.[11]

In addition, the administration of Moringa seed extract can reduce hepatic lobular inflammation which is seen based on liver histopathology. In this study, it showed MetS1 vs. MetS2 ($p = 0.000$) and MetS1 vs. MetS3 ($p = 0.000$) which means there was a significant difference in the group of mice-made metabolic syndrome (MetS1) compared to mice-made metabolic syndrome and given ethanolic extract of Moringa seeds at a dose of 150 mg/KgBB (MetS2) and 200 mg/kgBB (MetS3).

Phytochemicals contents such as flavonoids and phenolic acids are antioxidants which play a role in anti-inflammatory and anti-bacterial activities. Antioxidant in moringa seed extract has anti-inflammatory activity by inhibiting the production of nitrites-oxide (NO) which is a pro-inflammatory mediator. NO plays a role in increasing vascular permeability and activation of T lymphocyte cells. Araujo et al (2013) proved that moringa seed-aquades extract inhibits the production of TNF-α and IL-1β which are strong pro-inflammatory mediators. Another content that reduces inflammation is β-sitosterol. Mahajan and Mehta (2011) stated that β-sitosterol also plays a role in anti-inflammatory activity through a decrease in the production of TNF-α which has been proven in airway inflammation. The active compounds such as β-carotene, α-tocopherol and γ-tocopherol is hepatoprotective. The antioxidant compounds of β-carotene, α-tocopherol and γ-tocopherol are able to reduce the amount of free radicals in body and inhibit lipid peroxidation reactions, thereby reduce liver damage and inhibit inflammatory reactions in liver.[2,12]

In this study, MetS2 vs. MetS3 ($p = 0.533$) showed that the dose variation of ethanolic extract of Moringa seeds did not have a different effect on the lobular inflammation score. Meanwhile, C vs. MetS2 ($p = 0.364$) and C vs. MetS3 ($p = 0.109$) showed that the decrease in lobular inflammation scores in the group that was given a dose of 150 mg/KgBB and 200 mg/kgBB did not have significant difference, which means by giving ethanolic extract of Moringa seeds in those dosages can reduce lobular liver inflammation equivalent to the control group.

Nevertheless, this study has some limitations, which should be acknowledged. Our study dosage had limited variation, consisting of only two variations (150 mg/kg and 200 mg/kg). Furthermore, more dosage variations will be needed for the next experiment to know the effective dosage. Another limitation is that our study was an animal experiment; additional research is needed on how it will affect humans and how much dosage is needed.

CONCLUSION

MSE at the dose of 150 mg/kg and 200 mg/kg significantly reduces lobular inflammation in hepatic tissue of MetS rats.

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