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Original Article

Protective effect of moringa seed extract on kidney damage in rats fed a high-fat and high-fructose diet

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المخلص

أهداف البحث: المورينجا نبات شائع يحتوي على مستويات عالية من مضادات الأكسدة. وبالتالي، تهدف هذه الدراسة إلى تحليل التأثير الوقائي لمستخلص بذوره على الكلى لنموذج الفئران مع نظام غذائي عالي الدهون وعالي الفركتوز.

طريقة البحث: تم استخدام طريقة تصميم مجموعة التحكم قبل الاختبار وبعد الاختبار لقياس المعلمات الأيضية ووظائف الكلى ، بينما تم استخدام طريقة ما بعد الاختبار فقط في المجموعة الضابطة لتقييم الحجم الكبيبي وتعبير ديسموتازات الأكسيد الفائق. تم استخدام أخذ العينات الهادفة على 28 جرذا مقسمة إلى أربع مجموعات: مجموعة التحكم (ك1)، وثلاث مجموعات تغذت على نظام غذائي عالي الدهون وعالي الفركتوز لمدة 53 يوما (ك2 و ك3 وك4). بعد ذلك، تم إعطاء (ك3) 150 مجم / كجم من وزن الجسم / يوم؛ وتم إعطاء (ك4) 200 مجم / كجم من وزن الجسم / يوم مستخلص بذور المورينجا لمدة 28 يوما.

النتائج: أظهرت النتائج أن النظام الغذائي يزيد من مخاطر الإصابة بمتلازمة التمثيل الغذائي المتمثلة في زيادة الوزن والجلوكوز والدهون الثلاثية. أدت الجرعة المثلى من إدارة مستخلص بذور المورينجا إلى تحسن كبير في الحجم الكبيبي.

الاستنتاجات: أعطت إدارة مستخلص بذور المورينجا تأثيرا وقائيا على الكلى عن طريق خفض مستويات الكرياتينين في الدم ، وتحسين الهيكل العام ، وزيادة التعبير عن مضادات الأكسدة ديسموتازات الأكسيد الفائق.

الكلمات المفتاحية: الحجم الكبيبي؛ نظام غذائي عالي الدهون عالي الفركتوز؛ وظائف الكلى؛ مستخلص بذور المورينجا؛ ديسموتازات الأكسيدالفائق

Abstract

Objective: Moringa is a common plant that contains high levels of antioxidants. In this study, we aimed to analyze the protective effect of moringa seed extract on the kidneys of a rat model maintained on a high-fat and high-fructose (HFHF) diet.

Methods: An experiment with a pretest-posttest control group design was used to measure metabolic parameters and determine kidney function, while a posttest-only method was used for the control group to determine glomerular volume and superoxide dismutase (SOD) expression. Purposive sampling was used on 28 rats divided into four groups: a control (K1) group, and three groups fed a HFHF diet for 53 days (K2, K3, and K4). Subsequently, K3 and K4 were given 150 and 200 mg/kg BW per day moringa seed extract for 28 days. Data were analyzed using IBM® SPSS® Statistics version 22 software.

Results: Analysis showed that the diet increased the risk of metabolic syndrome, as evidenced by weight gain, glucose, and triglycerides. The optimal dose of moringa seed extract significantly improved glomerular volume ($p = 0.001$). The expression of SOD in kidney tubules and glomeruli was significantly different with each group ($p = 0.002$ and $p = 0.001$) respectively.

Conclusion: The administration of moringa seed extract provided a protective effect on the kidney by reducing

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serum creatinine levels, improving overall structure, and increasing the expression of SOD, a key antioxidant.

Keywords: Glomerular volume; High-fat high-fructose diet; Kidney function; Moringa seed extract; Superoxide dismutase

Introduction

A high-fat and high-fructose (HFHF) diet, together with sedentary behavior, can increase the risk of obesity and non-communicable diseases (NCDs). NCDs have become one of the world's leading health problems, thus resulting in the highest morbidity and mortality rates. According to data released by the World Health Organization (WHO), NCDs cause 41 million deaths every year.[1] In Indonesia, the prevalence of NCDs is increasing and this is directly proportional to an increase in risk factors. For instance, approximately 95.5% of the population consume inadequate amounts of fruits and vegetables, 33.5% engage in insufficient physical activity, 31% experience central obesity, and 21.8% become obese in adulthood.[2]

An HFHF diet plays an important role in the progression of metabolic syndrome. This syndrome is a group of several risk factors, including insulin resistance, dyslipidemia, and hypertension, which can increase the risk of cardiovascular disease or kidney damage.[3,4] According to Yustisia et al.[5] an HFHF diet culminated in moderate-level kidney damage (45%), characterized by hydropic degeneration, hemorrhage, inflammation, and the proliferation of glomerular capillaries compared with a control group that only experienced mild kidney damage (7%). Other studies have also shown that this type of diet can damage kidney structure, increase the expression of alpha-smooth muscle actin (αSMA), as well as genes related to inflammation and glomerulosclerosis, such as tumor necrosis alpha (TNF-α), interleukin 1β (IL-1β), nuclear factor kappa B (NF-κB), poly(ADP-ribose) polymerase-1 (PARP1), and receptor-interacting protein (RIP1).[6] This damage occurs due to an increase of superoxide anions caused by endothelial dysfunction, activation of the renin-angiotensin-aldosterone system, lipotoxicity, and adipokine imbalance.[4] Excessive superoxide anion levels suppress the activity of the superoxide dismutase (SOD) enzyme.[7] A reduction in SOD activity leads to the accumulation of superoxide anions, thus resulting in oxidative stress and the exacerbation of kidney damage. Therefore, increasing SOD activity is an important means of improving kidney function due to the increased production of reactive oxygen species (ROS).[4,8]

Moringa (*Moringa oleifera* Lam.) is a plant that contains high levels of essential nutrients in its leaves, fruits, stems, and seeds.[9] The main component of seeds is oil, which is made up from unsaturated fatty acids and vitamin E; this oil is known to exert antioxidant effects and potentially increase SOD enzyme activity.[9] Moringa seed also contains phytochemicals that potentially increase SOD activity, such as flavonoids, including quercetin and kaempferol, as well as tannins and glucosinolates.[9,10] However, only a few studies have identified the effect of moringa seed extract on kidney function, histopathological changes, and SOD expression in an animal model exposed to an HFHF diet. This study aimed to investigate the protective effects of moringa seed extract on kidney damage in a rat model of metabolic syndrome induced by an HFHF diet.

Materials and Methods

Animals and material preparation

This study was conducted between 2017 and 2018 at the PSPG UGM Laboratory and the Pathological Anatomy Laboratory, Faculty of Medicine at UNS. A pretest-posttest control group design was used to measure metabolic parameters and kidney function, while SOD expression in kidney tissue was assessed with a posttest-only method in the control group. The study protocol was approved by the Health Research Ethics Committee of the Faculty of Medicine at UNS (Protocol ID: 01/18/06/117).

For sample collection, a purposive sampling technique was used and the minimum total sample in each group was determined using the Federer formula as follows:

$$(k-1)(n-1) \geq 15$$

$$(4-1)(n-1) \geq 15$$

$$3n-3 \geq 15$$

$$3n \geq 18$$

$$n \geq 6$$

where k = group totals, n = total sample per group.

Although the minimum sample size required was 6 rats in each group, a total of 7 rats were used; hence, 28 rats were sampled in this study.

The subjects were male Wistar white rats (*Rattus norvegicus*) aged 2–3 months and weighing 150–200 g. These 28 rats were randomly divided into 4 groups, with 1 group given a regular diet, consisting of BR-2 pellets and tap water (K1); the other 3 groups were given an HFHF diet for 53 days (K2, K3, K4). The HFHF diet consisted of duck egg (10 mL/kg), beef fat (10 mL/kg), oxidized oil (5 mL/kg), and fructose (1.8 g/kg). We also determined a range of metabolic parameters, including weight, and the blood concentrations of glucose and triglycerides. Kidney function was evaluated by measuring serum creatinine. Blood samples were collected through retroorbital vein puncture. K1 and K2 were given distilled water, while K3 and K4 were administered *Moringa oleifera* seed extract at doses of 150 mg/kg BW per day and 200 mg/kg BW per day, respectively, for 28 days. The extract was obtained by maceration with 70% ethanol as the solvent. The moringa seed used was obtained from the Bu Yati Kelor Bobor stall in Bantul. The extract was prepared at the PSPG Laboratory, UGM Yogyakarta.

Serum markers and histopathological analysis

After 28 days, metabolic parameters and kidney function were re-quantified; then, the rats were euthanized and kidney preparations were prepared. These preparations were stained with hematoxylin–eosin (HE) to assess the histopathological appearance of kidney, while immunohistochemical staining was carried out with anti-SOD antibodies. Glomerular volume was assessed in 30 glomeruli from each kidney using the Wiebel Gomez method, as follows.[11]

$$VG = Area^{1.5} X \frac{\beta}{K}$$

where area = glomerular surface area, β = Spheris coefficient (1,38), K = size distribution coefficient (1.01).

SOD expression was assessed semi-quantitatively using the Intensity Distribution Score (IDS) formula: (percentage of strongly positive stained cells × 3) + (percentage of moderately positive stained cells × 2) + (percentage of weakly positive stained cells × 1) + (percentage of negatively stained cells × 0). The cells evaluated in this study were obtained from the tubules and glomerulus. SOD expression was considered positive when there was a color reaction in the cell membrane and cytoplasm after staining. The intensity of cell staining was considered strong, medium, weak, or negative if it was solid dark brown, light brown, golden yellow, or bluish-purple, respectively.

Statistical analysis

The serum creatinine data obtained were analyzed with the Shapiro Wilks normality test, while serum creatinine level data were analyzed with the Wilcoxon test. We applied the Kolmogorov–Smirnov normality test for glomerular volume and SOD expression data. If data were normally distributed, we performed one-way analysis of variance (ANOVA) and post hoc Tukey tests. In contrast, Kruskal–Wallis and Mann–Whitney post hoc tests were carried out when the data were not normally distributed. All statistical analyses were conducted using IBM® SPSS® Statistical software version 22.

Results

Metabolic parameters

Analysis of body weight, glucose, and triglycerides was performed to assess the comparison of metabolic status before and after rats were fed an HFHF diet. Blood samples were taken by puncturing the retroorbital vein and the results obtained are shown in Table 1.

Kidney function parameters

Analysis of kidney function was performed by measuring the serum level of creatinine with a kitDiaSys (Diagnostic System Holzheim Germany). Serum creatinine levels were measured before and after being the rats were fed an HFHF diet, as well as moringa seed ethanolic extract. Table 2 shows the mean serum creatinine levels in experimental and control rats.

The serum creatinine data were not normally distributed ($p < 0.05$) and the Wilcoxon test showed that the administration of moringa seed extract significantly improved serum creatinine levels ($p = 0.028$ and $p = 0.018$, respectively).

Kidney histopathology

Histopathological analysis of the kidney involved measuring the glomerular volume, as calculated using the Wiebel-Gomez formula. The mean glomerular volume results are listed in Table 3; Figure 3 shows the histopathology of the kidneys from rats taken from all four groups.

The Kolmogorov–Smirnov test showed that the glomerular volume data were normally distributed ($p > 0.05$). Thus, this data was analyzed by one-wat ANOVA. There was a significant difference in glomerular volume between groups

Table 2: Serum Creatinine Levels Before and After Rats were Fed a high-fat and high-fructose diet, and After being Given Moringa Seed Extract.

Group	Serum creatinine (mean ± SD) (mg/dl)		
	Before	After	After giving moringa seed extract
K1*	0.64 ± 0.06	0.64 ± 0.04	0.66 ± 0.01
K2	0.64 ± 0.03	3.57 ± 0.24	3.58 ± 0.18
K3	0.65 ± 0.05	3.83 ± 0.11	2.20 ± 0.19
K4	0.65 ± 0.03	3.52 ± 0.18	0.92 ± 0.09

SD: standard deviation.
*K1 is the control group.

Table 1: Mean Body Weight and the Blood Levels of Glucose and Triglycerides of Rats Before and After being fed a high-fat and high-fructose diet.

Group	Weight (mean ± SD) (gram)		Glucose (mean ± SD) (mg/dl)		Triglycerides (mean ± SD) (mg/dl)	
	Before	After	Before	After	Before	After
K1*	158.29 ± 3.86	204.57 ± 3.78	70.47 ± 2.13	74.29 ± 1.97	25.63 ± 2.70	26.40 ± 2.66
K2	160.86 ± 5.76	227.00 ± 5.23	70.37 ± 3.00	159.55 ± 3.06	25.00 ± 2.48	69.20 ± 1.13
K3	156.29 ± 2.21	223.57 ± 3.05	68.51 ± 1.70	160.05 ± 2.15	25.46 ± 2.00	70.00 ± 1.81
K4	161.00 ± 3.92	229.00 ± 4.40	69.87 ± 2.37	160.75 ± 2.44	25.53 ± 1.32	68.12 ± 1.91

SD, standard deviation.
*K1 is the control group.

Table 3: Mean Glomerular Volume of Kidney Tissue.

Group	Glomerular volume (mean ± SD) (m^3)	Comparison	*p value*
K1	4.961 ± 1.662	–	N/A
K2	6.919 ± 2.502	K1	0.001*
K3	6.558 ± 2.818	K2	0.069
K4	6.2935 ± 2.487	K2	0.001*

Table 4: Mean SOD Expression IDS Scores in Rat Kidney Tubules and Glomeruli.

Group	Mean IDS tubules SOD expression	Mean IDS Glomerulus SOD expression
K 1	122.00	13.00
K 2	25.52	1.65
K 3	118.00	47.24
K 4	135.65	55.43

IDS, Intensity Distribution Score; SOD, superoxide dismutase (see Fig. 1).

(p = 0.001). Post hoc Tukey analysis identified a significant difference in glomerular volume between the control group and the group fed an HFHF diet (p = 0.001). Administration of the optimal dose of moringa seed extract led to a significant improvement in glomerular volume (p = 0.001).

Immunohistochemistry

The mean SOD expression IDS scores in the tubules and glomeruli of kidney tissue are shown in Table 4.

SOD expression was detected by immunohistochemical staining; Figures 2 and 3 show the expression of SOD in kidney tubules and glomeruli, respectively.

Data relating to the expression of SOD in tubules and glomeruli in kidney tissue were then analyzed by the Kruskal–Wallis test (p = 0.002 and p = 0.001, respectively). These data indicated that the four groups showed significant differences in terms of SOD expression. To specify these significant differences between the groups, we next performed a Mann–Whitney post hoc test (Table 5).

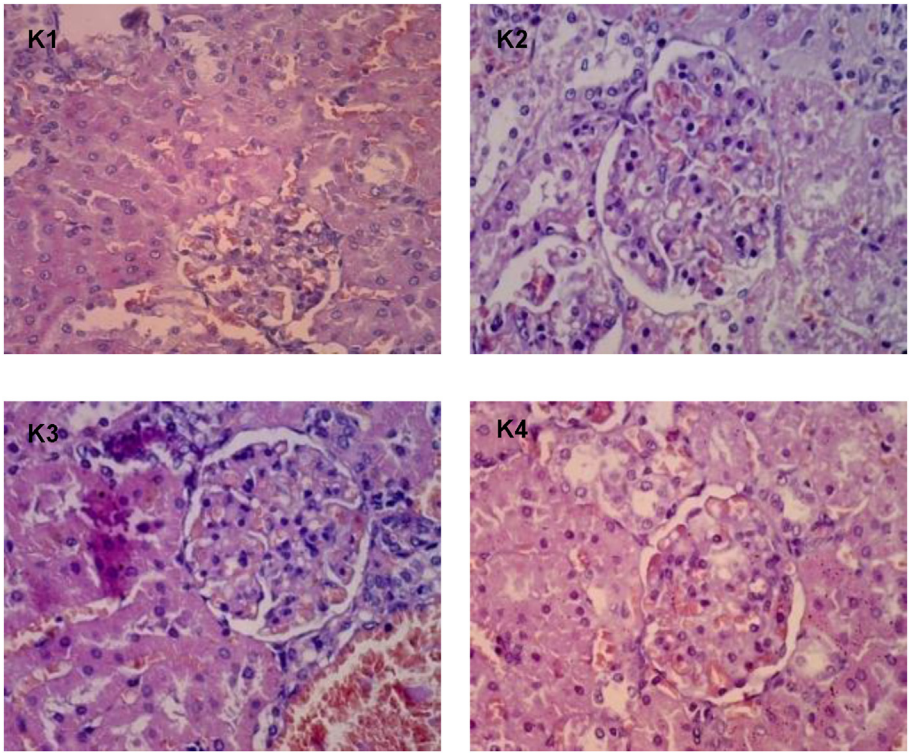

Figure 1: Overview of kidney glomerular volume in Wistar white rats stained with HE at 400 × magnification. K1 = L = 5.214,66 μm^2, K2 = 6.964,21 μm^2, K3 = 6.272,5 μm^2, K4 = 6.094,49 μm^2.

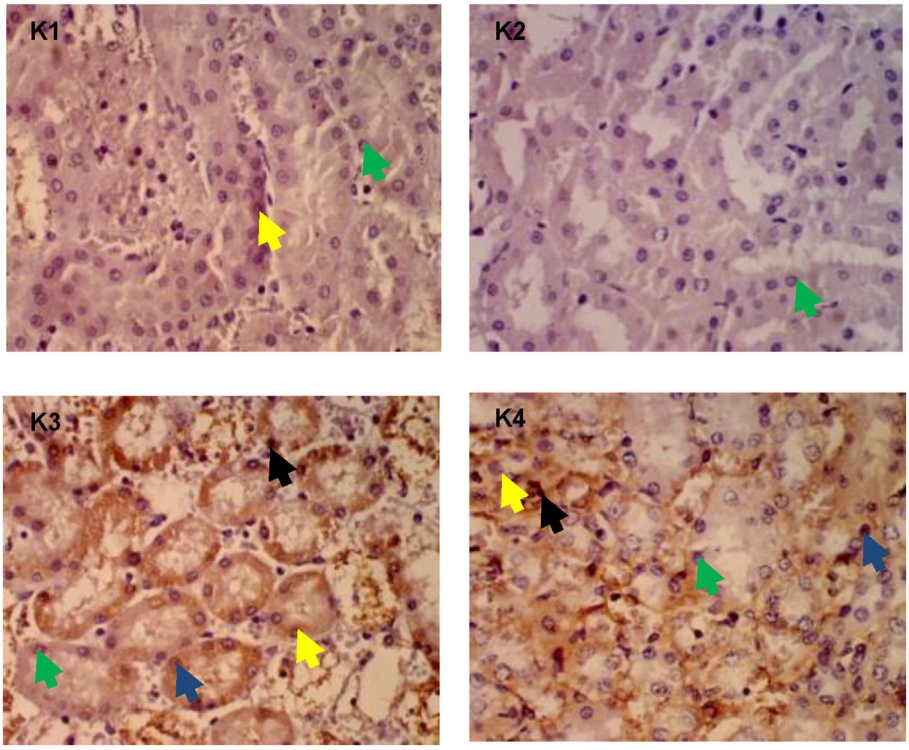

Figure 2: Cytoplasmic SOD expression in the kidney tubules of white Wistar rats with IHC at 400× magnification. Green arrow, negative; yellow arrow, weak positive; blue arrow, medium positive; black arrow, strong positive.

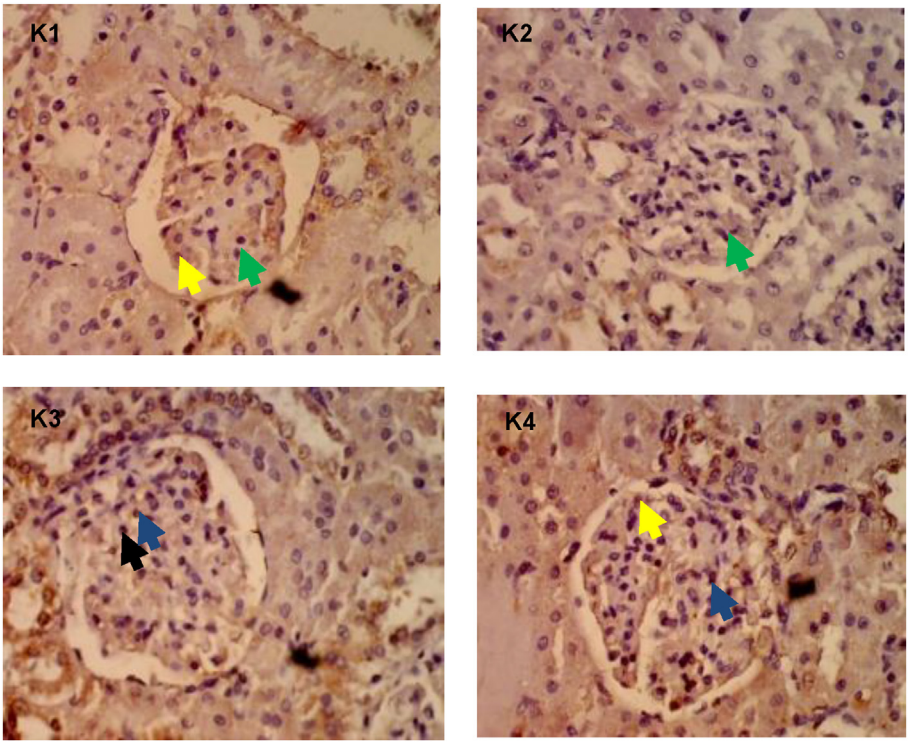

Figure 3: Cytoplasmic SOD expression in the glomeruli of white Wistar rats with IHC at 400× magnification staining. Green arrow, negative; yellow arrow, weak positive; blue arrow, medium positive; black arrow, strong positive.

Table 5: Mann–Whitney Post Hoc Test for IDS SOD Expression in Kidney Tubules and Glomeruli from experimental and control rats.

Group	Kidney Tubules	Kidney Glomerulus
	p value	p value
K1 and K2	0.006*	0.004*
K1 and K3	0.109	0.004*
K1 and K4	0.078	0.004*
K2 and K3	0.013*	0.014*
K2 and K4	0.006*	0.004*
K3 and K4	0.065	0.016*

Discussion

This experimental study aimed to investigate the effects of moringa seed extract on kidney function in rats given a high-fat and high-fructose supplement. After the treatment, we identified an increase in several metabolic parameters, including a 2.5-fold elevation in body weight and triglyceride levels, as well as a mean glucose level of 100 mg/dL. The diet became an independent predictor of the occurrence of metabolic syndrome. This was due to high-fructose content that could stimulate visceral fat deposition, thus leading to an increase in fatty acids and fat accumulation in insulin-sensitive tissues. Furthermore, high-fructose also led to an increase in *de novo* lipogenesis.[5,12]

Moringa seeds have recently been characterized with regards oil content. The mature seeds of the moringa plant contain 38–54% of edible oil. The oil of moringa seeds were found to contain high levels of unsaturated fatty acids. The dominant saturated fatty acids were palmitic acid, lauric acid, stearic acid, linoleic acid and linolenic acid. The major sterol components of the *M. oleifera* seed oil were β-sitosterol, campesterol, stigmasterol and Δ5, avenasterol. The major sterol components of the moringa oil were α-,ɣ- and δ-tocopherols.[13]

In this study, serum creatinine levels increased 5-fold after rats were given an HFHF diet treatment. Increased serum creatinine could represent a marker of reduced kidney function. This was due to the obesity and insulin resistance associated with metabolic syndrome which could trigger activation of the renin-angiotensin-aldosterone system (RAAS).[14,15] The activation of this system can cause an increase in the levels of angiotensin II which then increase the levels of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, an enzyme known to play a role in the formation of ROS. An increase in ROS can potentially trigger kidney tissue damage due to the mechanism of oxidative stress, thereby reducing the glomerular filtration rate and increasing the levels of serum creatinine.[12]

In the group treated with moringa seed extract, there was a significant decrease in serum creatinine. This finding was consistent with a study conducted by Margret et al.,[16] in which moringa seed extract was shown to improve kidney function by improving glycemic markers and their antioxidant effects, as indicated by a reduction in urea and serum creatinine in a streptozotocin-induced rat model of diabetes. Another study showed that the administration of moringa seed extract significantly improved serum creatinine levels in a male white rat model of diabetic nephropathy.[17]

In this study, kidney structural damage was identified as one of the long-term complications of consuming an HFHF diet. There was an expansion of glomerular volume; this was caused by increased oxidative stress. Optimal dosages of moringa seed extract can improve kidney structure. A study by Gabr et al.[17] reported improvements in kidney structure in rat a model of diabetic nephropathy given moringa seed extract at a dose of 500 mg/kg BW per day for 10 weeks. Another experimental study showed that this extract improved the appearance of tubular necrosis in the kidney glomeruli.[18]

An HFHF diet can reduce the expression of SOD as an antioxidant. This diet triggers the dysregulation of adipokine secretion, thus leading to the increased production of NADPH oxidase, pro-inflammatory cytokines, and ROS that can suppress the activity of endogenous antioxidant enzymes, specifically SOD.[19] Reduced levels of adiponectin and an increased level of in leptin in the kidney can reduce the activity of AMP-protein kinase (AMPK), an enzyme that can regulate metabolism; this can cause an upsurge in the production of monocyte chemoattractant protein-1 (MCP-1) in tubules and mesangial cells in the kidney. Subsequently, MCP-1 will recruit more macrophages, culminating in the production of cytokines (TNF-α, IL-6, IL-1), tumor growth factor-β (TGF-β) as a profibrotic factor, increased NF-κB activity, and NADPH oxidase (6,19,20). With a high-fat diet, the process of fatty acid β-oxidation in the mitochondria also increases, thus causing electron transport leakage at complexes I and III, thus leading to the formation of more superoxide anions.[20]

After rats were given the moringa seed extract, there was an increase in SOD expression in both tubules and glomeruli in the kidney. This occurred presumably because moringa seed contains an abundance of antioxidants. In plant seeds, Kaempferol can reduce the production of superoxide anions by preventing the oxidation process of LDL by activating peroxidase in hemoglobin, thereby preventing the accumulation of H_2O_2.[9] Another flavonoid, quercetin, is known to increase the activity of endothelial nitric oxide synthase (eNOS). Therefore, quercetin can prevent the formation of peroxynitrite and reduce the production of superoxide anions in a manner that is mediated by NADPH.[8,9,21] Glucomoringin and tocopherol are considered to be capable of increasing the activity of antioxidant enzymes such as SOD and catalase by reducing lipid oxidation and forming non-radical products by transferring H atoms.[22] Moringa seed extract also increases the activity of peroxisome proliferator activated receptor alpha (PPARα), which has been reported to play a key role in modulating redox balance. PPARα acts as a transcription factor for a diverse array of target genes possessing a PPAR response element (PPRE) in their promoter region. The PPRE, the binding site for PPARα, has been identified in the promoter regions of genes encoding SOD. Therefore, the PPRE could increase the expression of SOD.[23]

Our results are consistent with previous studies that evaluated the effect of moringa extract on SOD. According to a systematic review conducted by Akter et al.,[18] moringa extract potentially exerts a protective effect on the kidneys by increasing the activity of antioxidant enzymes such as SOD, catalase (CAT), and glutathione (GSH), as well as reducing the activity of proinflammatory cytokines.

Furthermore, Aju et al.[24] showed that supplementation with moringa leaf extract at a dose of 300 mg/kg BW per day for 60 days increased SOD activity in a rat model of diabetes. *In vitro* studies have also shown that moringa leaf extract induces antioxidant effects by increasing the activity of antioxidant enzymes, including SOD.[25] Another *in vitro* study reported that supplementation with this extract increased SOD activity by 97.8% in goat liver.[26]

Nevertheless, this study has some limitations that need to be considered. Our study dosage has limited variation, consisting of only two doses (150 mg/kg and 200 mg/kg). Furthermore, the antioxidant parameters only relate to SOD due to limitations in antibody availability. Further studies are needed to identify, isolate, and purify compounds contained in *Moringa oleifera* Lam. seed extract, use more varied doses, as well as evaluate the expression of other antioxidant enzymes.

Conclusion

Moringa oleifera seed extract has a potential protective effect on the kidneys by reducing serum creatinine levels, improving overall structure, and increasing the expression of the antioxidant enzyme SOD in rats maintained on an HFHF diet.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

The study protocol was approved by the Health Research Ethics Committee of the Faculty of Medicine at UNS (Protocol ID: 01/18/06/117).

Authors' contributions

Conceptualization: ISP, GNS, DRB. Data curation: ISP, GNS, DRB. Formal analysis: ISP. Funding acquisition: ISP. Investigation: ISP, GNS, DRB, BER. Methodology: ISP, GNS, DRB. Project administration: ISP. Resources: ISP, GNS, DRB, BER. Software: ISP. Supervision: DRB. Validation: ISP, GNS, DRB, BES Visualization: ISP, GNS, DRB. Writing – original draft: ISP, GNS. Writing, reviewing and editing: ISP, GNS, DRB, BER

All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

References

1. World Health Organization. Noncommunicable disease 2022. https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases (accessed December 13, 2022).
2. Kementrian Kesehatan Republik Indonesia. *Riset Kesehatan Dasar Nasional*. Jakarta: Badan Penelitian dan Pengembangan Kesehatan; 2018.
3. International Diabetes Federation. *The IDF consensus worldwide definition of the metabolic syndrome*; 2006. Belgium.
4. Zhang X, Lerman LO. The metabolic syndrome and chronic kidney disease. **Transl Res 2017**; 183: 14–25. https://doi.org/10.1016/j.trsl.2016.12.004.
5. Yustisia I, Tandiari D, Cangara MH, Hamid F, Daud NA. A high-fat, high-fructose diet induced hepatic steatosis, renal lesions, dyslipidemia, and hyperuricemia in non-obese rats. **Heliyon 2022**; 8. https://doi.org/10.1016/j.heliyon.2022.e10896.
6. Elsisy RA, El-Magd MA, Abdelkarim MA. High-fructose diet induces earlier and more severe kidney damage than high-fat diet on rats. **Egyptian J Histol 2021**; 44: 535–544. https://doi.org/10.21608/ejh.2020.31508.1304.
7. Kesh SB, Sarkar D, Manna K. High-fat diet-induced oxidative stress and its impact on metabolic syndrome: a review. **Asian J Pharmaceut Clin Res 2016**; 9: 47–52.
8. Salvamani S, Gunasekaran B, Shaharuddin NA, Ahmad SA, Shukor MY. Antiartherosclerotic effects of plant flavonoids. **BioMed Res Int 2014**; 2014. https://doi.org/10.1155/2014/480258.
9. Kong L, Luo C, Li X, Zhou Y, He H. The anti-inflammatory effect of kaempferol on early atherosclerosis in high cholesterol fed rabbits. **Lipids Health Dis 2013**; 12. https://doi.org/10.1186/1476-511X-12-115.
10. Gopalakrishnan L, Doriya K, Kumar DS. Moringa oleifera: a review on nutritive importance and its medicinal application. **Food Sci Hum Wellness 2016**; 5: 49–56. https://doi.org/10.1016/j.fshw.2016.04.001.
11. Weibel ER, Gomez DM. A principle for counting tissue structures on random sections1 A principle for counting tissue structures on random sections. **Appl Phys J 1962**. https://doi.org/10.1152/jappl.1962.17.2.343.
12. Moughaizel M, Dagher E, Jablaoui A, Thorin C, Rhimi M, Desfontis JC, et al. Long-term high-fructose high-fat diet feeding elicits insulin resistance, exacerbates dyslipidemia and induces gut microbiota dysbiosis in WHHL rabbits. **PLoS One 2022**; 17. https://doi.org/10.1371/journal.pone.0264215.
13. Özcan MM. Moringa spp: composition and bioactive properties. **South Afr J Bot 2020**; 129: 25–31. https://doi.org/10.1016/j.sajb.2018.11.017.
14. Underwood PC, Adler GK. The renin angiotensin aldosterone system and insulin resistance in humans. **Curr Hypertens Rep 2013**; 15: 59–70. https://doi.org/10.1007/s11906-012-0323-2.
15. Thethi T, Kamiyama M, Kobori H. The link between the renin-angiotensin-aldosterone system and renal injury in obesity and the metabolic syndrome. **Curr Hypertens Rep 2012**; 14: 160–169. https://doi.org/10.1007/s11906-012-0245-z.
16. Margret N, Nadro MS, Audu AS, Glen E. Anti-diabetic effects of aqueous extract and oil of moringa oleifera seed on liver and kidney functions in streptozotocin-induced diabetes in rats hypoglycaemic effects of fractions and crude methanolic leaf extract of Phyllanthus fraternus in streptozotocin induced diabetic rats. View project anti-diabetic effects of aqueous extract and oil of moringa oleifera seed on liver and kidney functions in streptozotocin-induced diabetes in rats. **Am J Biochem 2018**; 8: 69–74. https://doi.org/10.5923/j.ajb.20180804.01.
17. Gabr NM, Fouda ABA. Effects of moringa oleifera seeds aqueous extract on type-II diabetic nephropathy in adult male albino rat. **Med J Cario Univ 2021**; 89: 1129–1139. https://doi.org/10.21608/MJCU.2021.185000.
18. Akter T, Rahman MA, Moni A, Apu MAI, Fariha A, Hannan MA, et al. Prospects for protective potential of moringa oleifera against kidney diseases. **Plants 2021**; 10. https://doi.org/10.3390/plants10122818.

19. Lozano I, Van Der Werf R, Bietiger W, Seyfritz E, Peronet C, Pinget M, et al. High-fructose and high-fat diet-induced disorders in rats: impact on diabetes risk, hepatic and vascular complications. **Nutr Metab (Lond) 2016**; 13. https://doi.org/10.1186/s12986-016-0074-1.
20. Jarukamjorn K, Jearapong N, Pimson C, Chatuphonprasert W. A high-fat, high-fructose diet induces antioxidant imbalance and increases the risk and progression of nonalcoholic fatty liver disease in mice. **Scientifica (Cairo) 2016**; 2016. https://doi.org/10.1155/2016/5029414.
21. Alkhalidy H, Moore W, Zhang Y, McMillan R, Wang A, Ali M, et al. Small molecule kaempferol promotes insulin sensitivity and preserved pancreatic β -cell mass in middle-aged obese diabetic mice. **J Diabetes Res 2015**; 2015. https://doi.org/10.1155/2015/532984.
22. Ahn M, Kim J, Bang H, Moon J, Kim GO, Shin T. Hepatoprotective effects of allyl isothiocyanate against carbon tetrachloride-induced hepatotoxicity in rat. **Chem Biol Interact 2016**; 254: 102–108. https://doi.org/10.1016/j.cbi.2016.05.037.
23. Qi L, Zhou Y, Li W, Zheng M, Zhong R, Jin X, et al. Effect of Moringa oleifera stem extract on hydrogen peroxide-induced opacity of cultured mouse lens. **BMC Compl Alternative Med 2019**; 19. https://doi.org/10.1186/s12906-019-2555-z.
24. Aju BY, Rajalakshmi R, Mini S. Protective role of Moringa oleifera leaf extract on cardiac antioxidant status and lipid peroxidation in streptozotocin induced diabetic rats. **Heliyon 2019**; 5. https://doi.org/10.1016/j.heliyon.2019.e02935.
25. Ceci R, Maldini M, Olson ME, Crognale D, Horner K, Dimauro I, et al. Moringa oleifera leaf extract protects C2C12 myotubes against H2O2-induced oxidative stress. **Antioxidants 2022**; 11. https://doi.org/10.3390/antiox11081435.
26. Moyo B, Oyedemi S, Masika PJ, Muchenje V. Polyphenolic content and antioxidant properties of Moringa oleifera leaf extracts and enzymatic activity of liver from goats supplemented with Moringa oleifera leaves/sunflower seed cake. **Meat Sci 2012**; 91: 441–447. https://doi.org/10.1016/j.meatsci.2012.02.029.

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