Trop J Nat Prod Res, May 2025; 9(5): 2024 - 2029 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antioxidant and Anti-Obesity Potentials of *Moringa oleifera* Roots in High-Fat Diet-Induced Obesity in Rats

Marhaen Hardjo[1, 2]*, Surahmat Hamid[1], Nurfaiqa Hardjo[2], Sukaeni Ibrahim[3], Ilhamuddin Azis[1, 2], Mirna Muis[1, 4], Syahrijuita Kadir[1, 2]

[1]*Master Program of Biomedical Sciences, Graduate School Hasanuddin University, Hasanuddin University, Makassar, South Sulawesi, Indonesia*
[2]*Department of Biochemistry, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia*
[3]*Department of Public Health, Faculty of Medicine, Bosowa University, Makassar, South Sulawesi, Indonesia*
[4]*Department of Histology, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia*

ARTICLE INFO

Article history:
Received 08 February 2025
Revised 20 March 2025
Accepted 05 May 2025
Published online 01 June 2025

ABSTRACT

The prevalence of obesity is increasing globally. Obesity is associated with serious health complications, including diabetes, heart disease, and stroke. *Moringa oleifera* root (MOR) contains bioactive compounds and antioxidants that may aid in weight reduction. This study aimed to evaluate the antioxidant and anti-obesity potentials of MOR in obese rats. Preliminary phytochemical screening including total phenolic content of MOR extract was done following standard procedures. The antioxidant activity was evaluated using the 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay. The toxicity of the extract was also assessed using the brine shrimp lethality test. For the anti-obesity activity evaluation, thirty male Wistar rats were divided into five groups: normal control, negative control (distilled water), positive control (orlistat), MOR400 (400 mg/kg BW), and MOR200 (200 mg/kg BW). Obesity was induced in the rats by the administration of a high-fat diet (HFD) for seven weeks, followed by five weeks of treatment with MOR. Serum triglycerides, total cholesterol, as well as liver histopathology were also evaluated. Phytochemical analysis revealed the presence of phenolics, flavonoids, alkaloids, and terpenoids in MOR, with total phenolic content of 54.1±2.80 mg GAE/g. MOR exhibited strong antioxidant activity (IC_{50} = 37.6±0.44 µg/mL) and weak toxicity (LC_{50} = 772.6±269.9 µg/mL). MOR extract significantly ($p<0.05$) reduced body weight, Lee index, and organ (liver, lungs, heart, kidneys, and spleen) weights in obese rats. Additionally, MOR improved liver histoarchitecture in obese rats. These findings suggest that MOR has potential anti-obesity effects. Further studies are needed to explore its mechanism of action and possible clinical application.

Keywords: Moringa, Obesity, Lee Index, Liver histopathology.

Introduction

The incidence of obesity, a modern epidemic, has tripled since 1975. In 2022, 1 in 8 people in the world were living with obesity. Predisposing factors to obesity include dietary changes, increased consumption of processed food, physical inactivity, and genetics. Obesity is associated with many health complications and diseases, such as diabetes, heart disease, and stroke, among others. Obesity is a medical condition in which the body accumulates excessive fat. The body stores its fat in adipose tissue – a connective tissue containing fat.[1, 2] The state in which the amount of energy consumed and the amount of energy expended is referred to as energy balance. Energy balance is maintained through appetite suppression and increased metabolism. Long-term energy balance is regulated by the hormone leptin. Leptin levels are significantly increased in obese people as a result of the increased number of adipose cells in their bodies.

*Corresponding author. Email: Email: marhaenhardjo@gmail.com

Tel.: +62-82197222822

Citation: Hardjo M, Hamid S, Hardjo N, Ibrahim S, Azis I, Muis M, Kadir S. Antioxidant and Anti-Obesity Potentials of *Moringa oleifera* Roots in High-Fat Diet-Induced Obesity in Rats. Trop J Nat Prod Res. 2025; 9(5): 2024 – 2029 https://doi.org/10.26538/tjnpr/v9i5.21

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Nonetheless, increased leptin levels are not always followed by decreased appetite or better metabolism, a condition known as leptin resistance.[3] Obese people do not feel full even after eating plenty food due to leptin resistance. As a result, they tend to consume more food, and losing weight becomes more difficult for them.[4-6] Besides inhibiting leptin, one way to induce weight loss in obese people is to inhibit the enzyme lipase. Inhibiting lipase enzyme prevents the breakdown of fat, so the body cannot completely absorb fat from food.[7]

Polyphenols, flavonoids, saponins, and alkaloids are examples of plant phytochemicals that inhibit lipase. Studies have shown that foods rich in antioxidants can improve leptin sensitivity and prevent leptin resistance. Foods with high antioxidants contain polyphenols and flavonoids.[4-6] Research on natural ingredients in Indonesia has been focused on active ingredients with anti-obesity effects, such as polyphenols, flavonoids, and alkaloids.[8, 9]

Moringa oleifera root extract (MOR) has been found to contain bioactive polyphenols and flavonoids, such as niazirin, caffeoylquinic acid, and quercetin.[10,11] Preliminary phytochemical tests on moringa root extract has also shown that the plant part contain alkaloids, tannins, phenolics, saponins, triterpenoids, and steroids. Morphin and moriginin are alkaloids present in moringa.[12] Moringa root contains vitamin B3, also known as niacin, and vitamin C.[13] In addition, moringa root also contains unique compounds, such as glucosinolates and isothiocyanates. Studies have shown that the isothiocyanate content of moringa contribute to weight reduction, improve lipid profile, reduce leptin level, and inhibit lipase enzyme.[14,15]

This study aimed to investigate the anti-obesity potential of *Moringa oleifera* root (MOR). Unlike previous studies which primarily focused

on *Moringa oleifera* leaves, this study explores the effects of its root extract, providing new insights into its bioactive components, antioxidant capacity, and efficacy in an *in vivo* obesity model.

Materials and Methods

Plant collection and identification

Fresh *Moringa oleifera* root (MOR) samples were collected on March 20, 2024, from Kabupaten Sidrap, South Sulawesi, Indonesia. The plant material was taxonomically identified at the Herbarium of the Botany Laboratory, Universitas Hasanuddin, Indonesia. Herbarium specimen with voucher number UH-MO-1120 was deposited at the Laboratory of Biochemistry, Universitas Hasanuddin, Indonesia. The samples were thoroughly cleaned with distilled water, cut into small pieces, and air-dried at room temperature (approximately 27 – 32°C). The dried MOR was pulverized into a fine powder using a mechanical grinder.

Extraction

The powdered MOR was extracted by maceration in 96% ethanol (Merck, Germany) containing 1% glacial acetic acid (≥99.7% purity – Sigma-Aldrich, USA) at room temperature for 72 h with periodic stirring. The extract was decanted and filtered. The filterate was concentrated using a rotary evaporator at approximately 40°C under a pressure of 100 mbar until a thick extract was obtained.[16]

Phytochemical screening

The presence or absence of phytoconstituents in MOR extract was determined by phytochemical screening methods using several reagents. Ferric chloride ($FeCl_3$) reagent (Merck, Germany) was used to test for phenolic or flavonoid groups. Wagner reagent (Sigma-Aldrich, USA) was used to identify alkaloids. Additionally, the Liebermann-Burchard reagent (Merck, Germany) was used to test for steroid or terpenoid compounds.[17]

Determination of total phenolic content (TPC)

The TPC of MOR extract was determined using the method previously described by Sinay *et al*. (2022).[18] Stock solution MOR extract (1 mg/mL) was made by dissolving 10 mg of moringa root ethanol extract in 10 mL ethanol. To this solution was added 0.4 mL of Folin Ciocalteau reagent (Merck, Germany), shaken, and left to stand for 4 - 8 minutes. To the mixture was added 4.0 mL of Na_2CO_3 solution (Merck, Germany), and shake until homogeneous. Thereafter, the reaction mixture was made up to 10 mL with the addition of distilled water. The mixture was kept at room temperature for 2 h. The absorbance was measured at 750 nm with a UV-Vis spectrophotometer (GENESYS 150, Thermo Scientific, USA). Calibration curve of gallic acid (≥99%) (Sigma-Aldrich, USA) at various concentrations (10, 20, 30, 40, and 50 µg/mL) was prepared. The TPC of the MOR extract was then calculated from the equation of the gallic acid calibration curve, and the result was expressed as milligram gallic acid equivalent per gram of extract (mgGAE/g extract)

Determination of antioxidants activity

The antioxidant activity of MOR extract was determined using the 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay according to the method previously described by Okafor *et al*. (2024).[19] A stock solution of DPPH (0.2 mM) was prepared in ethanol. The DPPH solution (1 mL) was added to 1 mL of the following concentrations of MOR extract; 20, 40, 60, 80, and 100 µg/mL, and made up to 10 mL with ethanol. The mixture was mixed thoroughly, and allowed to stand at room temperature for 30 minutes, then the absorbance was measured at 517 nm using a UV-Vis spectrophotometer (GENESYS 150, Thermo Scientific, USA). The percentage DPPH radical scavenging activity of MOR extract was calculated using the formula below.

$$\text{DPPH scavenged [\%]} = \frac{\text{Control Absorbance} - \text{Sample Absrobance}}{\text{Control Absorbance}} \times 100$$

A linear regression curve of the sample concentration versus percentage inhibition was plotted. The equation of the linear regression plot was used to determine the IC_{50} (50% Inhibitory Concentration) value of MOR extract.

Brine shrimp lethality test

A vessel of seawater was seeded with 50 to 100 mg of brine shrimp (*Artemia salina*) eggs to be hatched and then divided into two parts: the dark zone and the light zone. The aerator and eggs were in the dark zone, while the lamp was in the light zone. After 48 hours, mature nauplii emerged, and they were exposed to the test sample (MOR) at various concentrations (10, 100, and 1000 µg/mL). Two drops of DMSO (Sigma-Aldrich, USA) were added to ensure complete dissolution of the sample in the medium. After 24 hours incubation, the number of live and dead larvae from each vial was counted, and the 50% lethal concentration (LD_{50}) was determine using probit analysis.[20]

Determination of anti-obesity potential

Animals

Thirty (30) adult male rats 2-3 months old with a weight of 150 - 200 g were obtained from the Laboratory Animal Facility, Faculty of Medicine, Universitas Hasanuddin, Indonesia. The rats were kept in well-ventilated cages, and acclimatized to the laboratory condition for one week. The rats were fed with standard rodent pellets, and allowed access to drinking water *ad libitum*.

Ethical approval

Ethical approval was granted by the research ethics committee of the State University Hospital, Hasanuddin University (RSPTN UH) – Dr. Wahidin Sudirohusodo Hospital, Makassar with approval reference number: 940/UN4.6.4.5.31/PP36/2023 and protocol number UH23110863. All experimental procedures were conducted in accordance with ethical guidelines.

Animal grouping and induction of obesity

The rats were divided into five groups of 6 rats per group. All the rats except those in Group 1 were fed with a high-fat diet (HFD), and given a high fructose drink for 7 weeks to induce obesity. The rats were thereafter treated as follows: Group 1 represents the normal control group, they were not induced and no extract treatment, Group 2 represents the negative control group (obese rats administered distilled water only), Group 3 represents the positive control group (obese rats administered orlistat at a dose of **20 mg/kg body weight**), Groups 4 and 5 represent the extract treatment groups (obese rats administered *Moringa oleifera* root extract at doses of 200 mg/kg body weight (MOR200) and 400 mg/kg body weight (MOR400), respectively). All the treatments were given orally once daily for 5 weeks.

Evaluation of serum triglyceride and total cholesterol level

At the end of the treatment period, blood samples were collected from the rats. The blood sample was allowed to clot, then centrifuged to obtain serum. The serum samples were used for the triglycerides and cholesterol tests. For the triglycerides test, 10 µL of serum was mixed with 1 mL of triglyceride reagent, the mixture was incubated at 25°C for 15 minutes. The absorbance of the mixture was measured at 500 nm using a UV-Vis spectrophotometer (GENESYS 150, Thermo Scientific, USA). The serum triglycerides concentration was estimated from a standard triglyceride calibration curve. For the serum cholesterol test, the same procedure for triglycerides was followed except that cholesterol reagent was used in place of triglyceride reagent. Serum cholesterol level was estimated from cholesterol calibration curve.

Histopathological analysis of the liver

The rats were anesthetized, and their livers were surgically removed and immediately placed in a fixation liquid (formalin) to preserve the cellular structure and prevent unwanted changes before further processing. After fixation, the liver tissue samples were dehydrated by immersing the tissue in a series of graded alcohol solutions. After dehydration, the liver tissues were impregnated in liquid paraffin, which allows the tissue to harden and become easier to cut with a microtome. After paraffin impregnation, thin sections were cut from the tissue using a microtome. The tissue sections were placed on glass slides and stained with hematoxylin and eosin (H&E) dye. H&E staining provides good

contrast between different tissue structures and allows for better observation under the microscope. After staining, the glass slide was covered with another coverslip using a mounting medium. The slides were then observed under the microscope for histopathological analysis. Micrographs (at X100 combined magnification) were taken using an Amscope 14MP digital microscope camera fitted on a NOVEX compound microscope with Hi-PLAN objectives.[21]

Statistical analysis

Data were reported as mean ± standard deviation (SD). Differences between means were analyzed using one-way analysis of variance (ANOVA). A P-value of < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS software version 22.0 (IBM Corp., Armonk, N.Y., USA).

Results and Discussion

Phytochemical constituents of MOR extract

Preliminary phytochemical analysis of MOR extract revealed the presence of phenolics, flavonoids, alkaloids, and terpenoids, while steroids were absent (Table 1). This observation is in line with several other studies, which have shown the presence of phenolics and flavonoids such as niazirin, caffeoylquinic acid, and quercetin in *Moringa oleifera* root.[10,11] MOR have also been shown to contain alkaloids, tannins, saponins, and triterpenoids. Morphine and moringin are some of the alkaloids found in MOR.[12]

Phenolic compounds are secondary metabolites of plants that are mostly present as hydroxycinnamic acid derivatives (free phenolics) and hydroxybenzoic acid (bound phenolics). These compounds have one or more hydroxy groups connected directly to the aromatic ring and can be found in plant material as esters or glycosides. These hydroxyl groups are responsible for the high free radical scavenging activity of phenolic compounds.[22]

Total phenolic content and antioxidant activity of MOR extract

Moringa oleifera plant has been found to contain several phenolic compounds, and their bioactivity have been confirmed by *in vitro* and *in vivo* analyses. For this reason, MOR extract was evaluated for its total phenolic content, and the result showed a high total phenolic content of 76.80 ± 2.80 mg GAE/g extract (Table 2).

Table 1: Phytochemical constituents of *Moringa oleifera* root extract

Phytoconstituent	Result
Phenolic/Flavanoid	+
Alkaloid	+
Steroids	-
Terpenoids	+

Note: '+' indicates the presence of phytoconstituent, while '- 'indicates absence of phytoconstituent

In the present investigation, the antioxidant activity of MOR extract was evaluated through DPPH radical scavenging assay. The result showed that MOR extract has a very strong antioxidant activity with IC_{50} value of 37.60 ± 0.44 µg/mL (Table 2). The antioxidant activity of extract can be categorized based on the IC_{50} value as follows: very strong (< 50 µg/mL), strong (50 - 100 µg/mL), moderate (101 - 150 µg/mL), weak (151 - 200 µg/mL), and very weak (> 200 µg/mL).[18] The very strong antioxidant activity of MOR extract could be linked to its high phenolic content. Phenolic compounds are known to possess antioxidant activity through their ability to counteract free radicals.

Cytotoxic effect of MOR extract

The cytotoxic effect of MOR extract was assessed using the brine shrimp lethality test (BSLT). Plant toxicity can be assessed using the LC_{50} (50% Lethal Concentration) in various *in vitro* toxicity tests, one of which is the Brine Shrimp Lethality Test (BSLT). The nature of toxicity can be categorized according to Clarkson classification of toxicity as follows; highly toxic ($LC_{50} < 100$ µg/mL), moderate ($LC_{50} =$ 101 - 500 µg/mL), weak ($LC_{50} =$ 501 - 1000 µg/mL), non-toxic (LC_{50} >1000 µg/mL). Based on this classification, and from the result obtained (Table 3), MOR extract is said to be weakly toxic with LC_{50} of 772.6 ± 269.9 µg/mL.[20]

Table 2: Total phenolic content and antioxidant activity of *Moringa oleifera* root extract

Sample	Total Phenolic content (mg/GAE/g)	DPPH RSA (IC_{50} value in µg/mL)	(T-Test) p-value
MOR	76.80 ± 2.80	37.60 ± 0.44	<0.001
Ascorbic Acid	-	6.10 ± 0.50	

Note: GAE = Gallic acid equivalent, RSA = Radical scavenging activity

Table 3: Cytotoxic activity of *Moringa oleifera* root extract

Extract Conc. (µg/mL)	Log C	Percent Mortality (%)	Probit Value	LC_{50} (µg/mL)	Toxicity Properties
1000	3	55	5.13	772.6 ± 269.9	Weak
100	2	29	4.45		
10	1	13	3.87		
Control	0	0	0.00		

Anti-obesity activity of MOR extract

Anti-obesity test of MOR extract was evaluated following a high fat diet (HFD) induced obesity in rats. Several parameters of obesity including body weight, Lee index (ratio of body weight and length), organ weight

(liver, lungs, kidneys, heart, and spleen), serum triglyceride (TG) and total cholesterol (TC) levels were measured, as well as liver histopathological investigation.

As shown in Figure 1 and Table 4, there was a significant increase in the body weight and Lee index of rats after induction with HFD. Increased body weight and high Lee index (> 0.3) are characteristic of obesity.[23,24] Administration of MOR extract and positive control (orlistat) for 5 weeks exhibited anti-obesity effect by significantly reducing body weight and Lee index in the obese rats.

Table 4: Lee Index changes before and after treatment

Group	Lee Index			p-value
	acclimation	HFD	treatment	
Negative Control	0.291 ± 0.010	0.340 ± 0.007	0.317 ± 0.016	0.090
Positive Control	0.290 ± 0.006	0.345 ± 0.010	0.271 ± 0.007	0.001
MOR400	0.289 ± 0.012	0.342 ± 0.010	0.284 ± 0.003	0.001
MOR200	0.292 ± 0.007	0.338 ± 0.008	0.289 ± 0.005	0.001
Normal	0.287 ± 0.010	0.293 ± 0.010	0.263 ± 0.011	0.059

Note: Significant if $p < 0.05$.

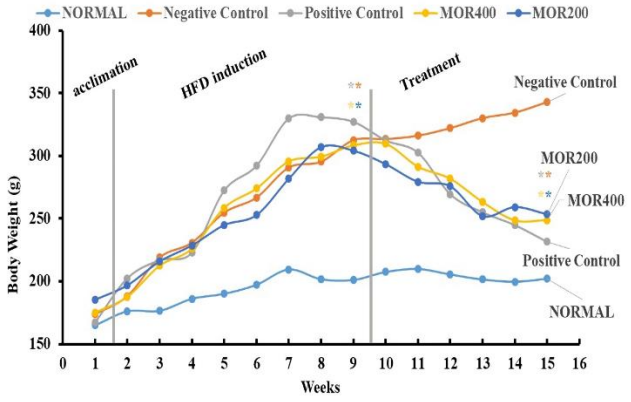

Figure 1: Body weight changes of rats following high fat diet induced obesity and treatment with MOR extract
Note: '*' indicates significant difference compared to normal control ($p < 0.05$).

The weight loss effect of the 400 mg dose of MOR was similar to that of the positive control orlistat, although, these treatments were unable to reverse the body weight and lee index to that of the normal control. Similarly, MOR extract treatment was also able to reduce the weight of the liver, lungs, kidneys, heart, and spleen (Figure 2). Although, only the weight of the lungs in the extract groups reduced to the same extent as seen with that of the positive control group. Comparatively, the effect of the 400 mg/kg dose of MOR was better than that of the 200 mg/kg dose in terms of body weight reduction, reduction of lee index, and organ weight reduction. The positive control drug orlistat exerts its anti-obesity effect by inhibiting the lipase enzyme.

However, the long-term use of orlistat is limited due to its relatively expensive price, and side effects on the gastrointestinal tract, kidney, and liver. Therefore, the search for alternative anti-obesity drugs with pancreatic lipase inhibitory activity is very important.[25, 26] pancreatic lipase hydrolyzes triglycerides found in food into free fatty acids (FFA) and monoglycerides, which are then absorbed by the small intestine. By inhibiting lipase activity, this process is disrupted so that triglycerides

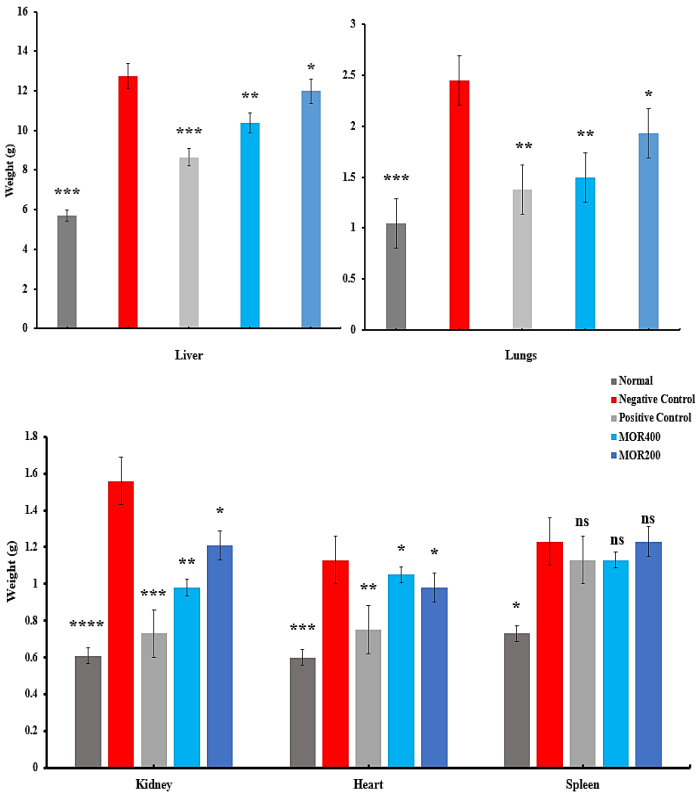

Figure 2: Comparison of organ weights of rats after treatment with MOR extract
Note: '*' indicates significant difference ($p < 0.05$) compared to negative control, ns = non-significant.

are not broken down and cannot be absorbed by the body. This causes the triglycerides to be excreted through the faeces, ultimately reducing calorie intake and supporting weight loss.[4, 5, 26]

MOR as herbal medicine remains safe despite long-term consumption. Based on the findings from the present study, MOR extract may be thought to exert its anti-obesity effect in a similar fashion as orlistat, that is by inhibiting lipase enzyme activity.

In addition, one of the hormones responsible for maintaining energy balance is leptin. Leptin signals appetite suppression and increase metabolism when one is full after eating. However, leptin resistance results in the loss of this function so that appetite is not suppressed and there is always the feeling of hunger even after consuming a huge meal, this results in weight gain and obesity. Studies have shown that foods rich in antioxidants such as polyphenols and flavonoids can improve leptin sensitivity and prevent leptin resistance. Therefore, the high phenolic content and very strong antioxidant activity of MOR extract may significantly contribute to increase leptin sensitivity and prevent leptin resistance so that energy balance can be regulated, leading to weight loss in obesity.[4-6]

Effect of MOR extract on serum triglycerides and total cholesterol levels

The serum triglycerides and total cholesterol levels in rats in the different groups are presented in Figure 3. The results showed that MOR extract reduced serum triglyceride level significantly and to similar level as that of the normal control. In the same vein, MOR extract reduced serum total cholesterol level, although not statistically significant compared to the negative control. There was no significant difference in the serum triglycerides and total cholesterol reduction effects between the MOR extract doses (200 and 400 mg/kg). This is in line with several previous studies which showed that moringa plants can reduce and improve serum triglyceride and total cholesterol levels.[27-29]

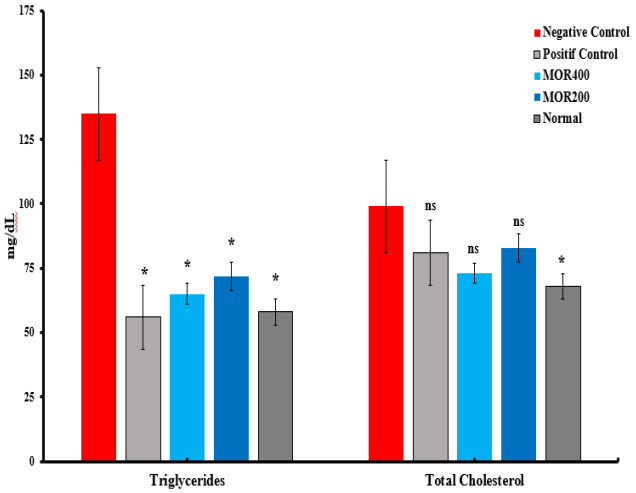

Figure 3: Effect of MOR extract on serum triglycerides and total cholesterol levels

Note: '*' indicates significant difference ($p < 0.05$) compared to negative control, ns = non-significant.

Histopathological effect of MOR extract on rat liver

As shown in Figure 4, the liver histopathology in the negative control group showed several liver abnormalities such as congestion, fibrosis, necrosis, and inflammation due to HFD induction and obesity in rats. However, the administration of MOR extract, especially at 400 mg/kg was able to improve the abnormalities in the liver, even though some abnormalities were still present. This is in line with study of Zaheer *et al*.[30] where *Moringa oleifera* leaf extract was found to significantly improve bisphenol-A-induced histological changes of hepatocytes in rats. On the other hand, orlistat, which was used as the positive control, also improved liver histoarchitecture in HFD-induced obesity in rats, although some inflammation and hydrophilic degeneration were still present.

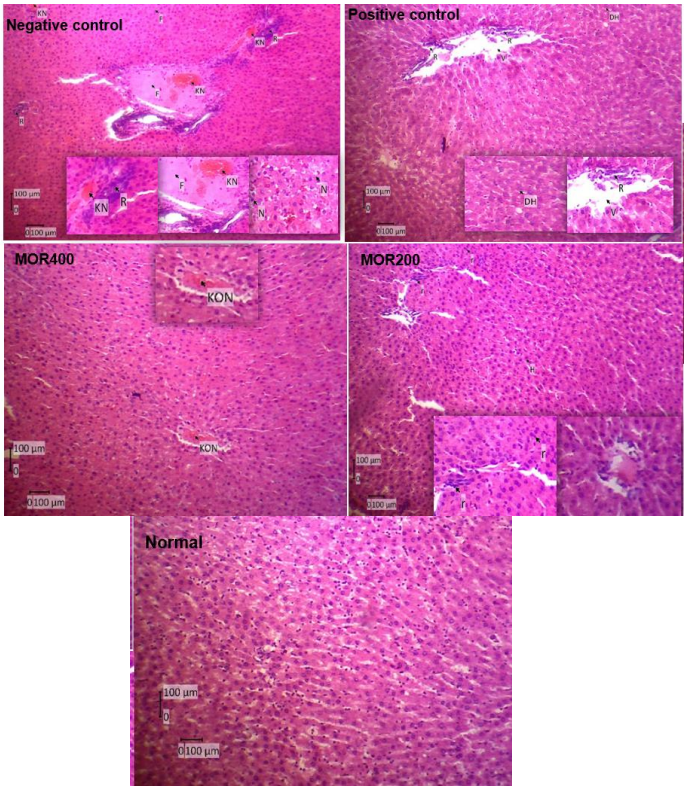

Figure 4: Photomicrograph of liver tissue of rats in the various groups

Note: Liver abnormalities; congestion (KN/KON), fibrosis (F), necrosis (N), inflammation (R/r), and hydropic degeneration (DH).

Conclusion

The findings from this study have shown that *Moringa oleifera* root (MOR) has potential anti-obesity effects coupled with very strong antioxidant activity and weak toxicity. MOR was able to reduce body weight, Lee index, and organ weights of liver, lungs, heart, kidneys, and spleen, as well as reduced serum triglyceride and total cholesterol levels in HFD-induced obesity in rats. In addition, MOR was also able to reverse to a great extent the liver histological damage due to obesity. The present study serves as a foundation upon which further evaluation of MOR as potential anti-obesity agent would be done.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The author gratefully acknowledges the Faculty of Medicine and the Postgraduate Program at Hasanuddin University for their financial support through the research grant with reference number: 00309/UN4.22/PT.01.03/2024.

Funding

The funding for this research was obtained from a grant from the Postgraduate Program, Hasanuddin University in 2024 with grant number: 00309/UN4.22/PT.01.03/2024.

References

1. Kozlov AI. Carbohydrate-Related Nutritional and Genetic Risks of Obesity for Indigenous Northerners. Vopr Pitan. 2019; 88(1):5-16. doi: 10.24411/0042-8833-2019-10001.
2. World Health Organization. Obesity and overweight. 2024. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.
3. Sharma S and Tailor A. Relationship Between Leptin Levels and Obesity. Int J Obes. 2019; 43(4):699-707.
4. Borges V, Monteiro JA, Teixeira ML. Anti-Inflammatory and Antioxidant Properties of Food: Impact on Obesity. J Funct Foods. 2021; 75:104291.
5. Chuang L, Shen Y, Li X, Wang H. Effect of Antioxidants on Leptin Signaling in Obesity. Nutrients. 2022; 14(11):3462.
6. Jiang W, Wang X, Zhang H. The Role of Antioxidants in Leptin Resistance. Nutrients. 2022; 14(11):3459.
7. Patil S, Patil M, Maheshwari VL, Patil RH. Pancreatic Lipase (PL) Inhibitors from Medicinal Plants and their Potential Applications in the Management of Obesity. In: Maheshwari VL, Patil RH. (eds) Nat Prod Enzyme Inhib. Springer Singapore 2022; 93-112p. https://doi.org/10.1007/978-981-19-0932-0_7.
8. Gupta A, Kumar R, Singh R. Effect of *Moringa oleifera* Root Extract on Lipid Profile and Lipase Activity in High-Fat Diet-Induced Obese Rats. J Pharm Pharmacol. 2021; 73(1):1-12.
9. Kumar A, Kumar S, Pandey S. Effect of *Moringa oleifera* Leaf Extract on Lipid Profile and Lipase Activity in High-Fat Diet-Induced Obese Rats. J Pharm Pharmacol. 2022; 74(2):242-250.
10. Bharali MR, Jong AN, Kawabata J. Antidiabetic Potential of Nepalese Herbal and Food Plants. Funct Foods Chronic Dis. 2003; 3(1):123-132.
11. Krisnadi AD. Kelor Super Nutrisi. Kunduran: Media Peduli Lingkungan; 2015.
12. Gopalakrishnan P, Sundaresan K, Rajagopalan S. Phytochemical Analysis of *Moringa oleifera* Leaves. J Pharm Res. 2016; 9(1):161-164.
13. Igwilo U, Nwokedi CO, Ogunsanwo OO. Nutritional and Phytochemical Composition of *Moringa oleifera* Leaves, Pods and Roots. J Agric Food Chem. 2017; 65(34):6366-6373.
14. Lopez-Rodriguez N, Gaytán-Martínez M, Reyes-Vega ML, Loarca-Piña G. Glucosinolates and Isothiocyanates from *Moringa oleifera*: Chemical and Biological Approaches. Plant Foods Hum Nutr. 2020; 75:447–457. https://doi.org/10.1007/s11130-020-00851-x.
15. Redha A, Perna S, Riva A, Petrangolini G, Peroni G, Nichetti M, Allegrini P, Alalwan TA, Rondanelli M. Novel Insights on the Anti-Obesity Potential of the Miracle Tree, *Moringa oleifera*: A Systematic Review. J Funct Foods. 2021; 84:104600. https://doi.org/10.1016/j.jff.2021.104600.
16. Zhu Y, Wang X, Zhang Y, Li X, Zhang X. Optimization of the Extraction of Bioactive Compounds from *Moringa oleifera* Roots by Microwave-Assisted Extraction. Food Sci Technol. 2022; 60(2):1073-1082.
17. Ankad GM, Pai SR, Upadhya V, Hurkadal PJ, Hegde HV. Pharmacognostic Evaluation of *Achyranthes coynei* Leaf. Egypt J Basic Appl Sci. 2015; 2:25-31.
18. Sinay H, Mahulette F, Yáñez JA. Determination of Free Radical Scavenging Activity, Phenolic and Flavonoid Content of Seven Corn Cultivars from the Southwest Maluku District, Indonesia. Biodiversitas. 2022; 23(11):5974-5981. https://doi.org/10.13057/biodiv/d231151.
19. Okafor CE, Ijoma IK, Igboamalu CA, Ezebalu CE, Eze CF, Osita-Chikeze JC, Uzor CE, Ekwuekwe AL. Secondary Metabolites, Spectra Characterization, and Antioxidant Correlation Analysis of the Polar and Nonpolar Extracts of *Bryophyllum pinnatum* (Lam) Oken. J Biotechnol Comput Biol Bionanotechnol. 2024; 105(2):121–136.
20. Braguini WL, Pires NV, Alves BB. Phytochemical Analysis, Antioxidant Properties, and Brine Shrimp Lethality of Unripe Fruits of *Solanum viarum*. J Young Pharm. 2018; 10(2):159-163. https://doi.org/10.5530/jyp.2018.10.36.
21. Aguwa US, Ogwo R, Evaristus C, Onyejike DN, Abazuh UD. Delayed Tissue Fixation for as Short as Five Minutes Can Distort Tissue Architecture and Falsify Research Results. Trop J Nat Prod Res. 2025; 9(1):112-118. https://doi.org/10.26538/tjnpr/v9i1.16
22. Kashyap P, Kumar S, Riar CS, Jindal N, Baniwal P, Guiné RPF, Correia PMR, Mehra R, Kumar H. Recent Advances in Drumstick (*Moringa oleifera*) Leaves Bioactive Compounds: Composition, Health Benefits, Bioaccessibility, and Dietary Applications. Antioxidants. 2022; 11(2):402. doi:10.3390/antiox11020402.
23. Lozano I, Van Der Werf R, Bietiger W, Seyfritz E, Peronet C, Pinget M, Jeandidier N, Maillard E, Marchioni E, Sigrist S, Dal S. High-Fructose and High-Fat Diet-Induced Disorders in Rats: Impact on Diabetes Risk, Hepatic and Vascular Complications. Nutr Metab (Lond). 2016; 13:15. doi:10.1186/s12986-016-0074-1.
24. Lee S-II, Kim JW, Lee YK, Yang Y, Kim SD. Anti-Obesity Effect of *Monascus pilosus* Mycelial Extract in High-Fat Diet-Induced Obese Rats. J Appl Biomol Chem. 2011; 54:197-205.
25. Alkhudhayri DA, Osman M, Ghedeir M, Alshammari A, Al-Johani NS, Alsagaby SA, Aldakheel FM, Alharbi NS, Khan MI, Al-Hamoud GA, Ghaith MM, Aljohani AS, Baothman O, Syed R. *Moringa peregrina* Leaf Extracts Produce Anti-Obesity, Hypoglycemic, Anti-Hyperlipidemic, and Hepatoprotective Effects on High-Fat Diet-Fed Rats. Saudi J Biol Sci. 2021; 28(6):3333-3342. doi:10.1016/j.sjbs.2021.02.078.
26. Pirahanchi Y and Sharma S. Biochemistry, lipase. [Updated 2023 Jun 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537346/.
27. Saleem H, Al-Dujaily ANG, Al-Murshidi MHH. Effect of Methanolic Leaf Extract of *Moringa oleifera* on Biochemical Markers in Obesity-Induced Rats. Res J Pharm Biol Chem Sci. 2016; 7(3):2222-2232.
28. Kim YJ and Kim HS. Screening *Moringa* Species Focused on the Development of Locally Available Sustainable Nutritional Supplements. Nutr Res Pract. 2019; 13(6):529-534. https://doi.org/10.4162/nrp.2019.13.6.529.
29. Alhabeeb MK and Fawzy GH. Comparing the Effect of Moringa Aqueous Extract and Selenium Nanoparticles Against Complications of Type 2 Diabetes Mellitus. Pak J Biol Sci. 2023; 26(5):249-265. https://doi.org/10.3923/pjbs.2023.249.265.
30. Zaheer A, Nazir S, Sidiqque N, Qamar F, Hussain A, Ahmed M. Effect of *Moringa oleifera* Leaves on Bisphenol-A Induced Histological Changes of Hepatocytes in Albino Rats. Pak Postgrad Med J. 2020; 30(1):17-21. https://doi.org/10.51642/ppmj.v30i01.255.