Pharmacogn J. 2026; 18(1): 102-106
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
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Original Article

Modulation of BCL-2 Family Proteins by *Moringa oleifera* Fruit Extract in High-Fat Diet–Induced Obesity Rat Models

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History

- Submission Date: 13-11-2025;
- Review completed: 02-12-2025;
- Accepted Date: 21-01-2026.

DOI : 10.5530/pj.2026.18.122

Article Available online

http://www.phcogj.com/v18/i1

ABSTRACT

Background: Obesity induces chronic low-grade inflammation and oxidative stress, leading to mitochondrial dysfunction and dysregulation of apoptosis-related proteins, particularly the BCL-2 family (BCL-2, BAK1, BAD). *Moringa oleifera* fruits are rich in antioxidant phytochemicals, yet their effects on BCL-2 family protein expression in obesity rat models remain poorly explored. **Objective:** This study investigated the modulation of *Moringa oleifera* fruit extract on BCL-2 family protein expression in obesity rat models. **Methods:** Male Sprague Dawley rats were divided into five groups: normal control (N), obesse control (O), obese + *M. oleifera* fruit extract (500 mg/kg BW, once (OEMO1) or twice daily (OEMO2)), and obese rats + Vitamin C (OC). After 30 days, liver tissues were collected. BCL-2 family proteins (BCL-2, BAK1, and BAD) were analyzed using ELISA. **Results:** Obese rats (O) showed dysregulation of BCL-2 family protein expression, characterized by decreased pro-apoptotic and increased anti-apoptotic markers. Administration of *M. oleifera* fruit extract significantly restored BCL-2 family protein expression by increased anti-apoptotic (BCL-2) and decreased pro-apoptotic (BAK1 and BAD) signaling expression of BCL-2 family protein (OEMO2) compared to obese controls (O) p<0,001. **Conclusion:** *Moringa oleifera* fruit extract modulates apoptosis-related proteins by restoring BCL-2 family balance in obesity rat livers, suggesting its potential as a pharmacognostic candidate for obesity-related hepatic dysfunction prevention in obesity rat models.

Keywords: *Moringa oleifera* fruits; obesity rat models; BCL-2 family; pro-apoptotic; anti-apoptotic; antioxidant.

INTRODUCTION

Obesity is a complex metabolic disease characterized by excessive adipose tissue expansion, chronic low-grade inflammation, and dysregulated cellular energy homeostasis. Excessive lipid accumulation in hepatocytes elevates reactive oxygen species (ROS) production, triggering mitochondrial dysfunction and apoptosis through intrinsic pathways regulated by BCL-2 family proteins. Dysregulation of the balance between anti-apoptotic (BCL-2) and pro-apoptotic (BAK1, BAD) proteins is a crucial event in obesity-associated liver injury[1,2].

In preclinical research, high-fat diet (HFD) induced obesity rat are widely used as models to study molecular mechanisms underlying adipocyte dysfunction, apoptosis, and metabolic derangements in obesity. A **High-Fat Diet (HFD)** commonly used formulations are **45% kcal from fat, and 60% kcal from fat**. Within this context, the BCL-2 family of proteins, known regulators of mitochondrial intrinsic apoptosis pathways, have increasingly been investigated for their roles in adipose tissue remodeling, cell survival and death during obesity[3,4]. Parallelly, *Moringa oleifera*, including its fruits, has been studied for potential anti-obesity and metabolic benefits through antioxidant, anti-inflammatory, and regulatory effects on cell signaling apoptotic[5,6].

Indonesia possesses abundant medicinal plants, including *Moringa oleifera*. While leaves are extensively studied, *M. oleifera* fruits extract contain higher levels of flavonoids, quercetin, glucosinolates, and isothiocyanates with potent antioxidant and anti-inflammatory properties[7,8]. Preliminary studies indicate that *M. oleifera* fruits extract exhibit stronger antioxidant capacity than leaves[9,10], supporting their pharmacognostic relevance.

However, evidence regarding the effect of *M. oleifera* fruit extract on apoptosis-related molecular pathways, particularly BCL-2 family proteins in obesity rat models, remains limited[11,12]. Therefore, this study aimed to analyze the modulation of BCL-2 family protein expression by *M. oleifera* fruit extract in obesity rat models, contributing novel insights into its hepatoprotective mechanism through apoptotic pathways.

MATERIALS AND METHODS

Experimental Design

This laboratory experimental study used male Sprague Dawley rats (8 weeks old). Obesity was induced using a high-fat diet (HFD) in 4 weeks from RatBio (R) . Animals were randomly divided into five groups (n = 6/group): Normal control (N), Obese control (O), Obese + *M. oleifera* fruit extract 500 mg/kg BW once daily (OEMO1), Obese + *M. oleifera* fruit extract 500 mg/kg BW twice daily (OEMO2), and Obese + Vitamin C (OC).

Preparation of *Moringa oleifera* Fruit Extract

M. oleifera fruits were processed and extraction yield of the 70% ethanol extract was 12.4% (w/w) relative to the dried plant powderat the Chemistry Department

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of the Faculty of Medicine, University of Indonesia, using standardized extraction protocols. The extract was standardized to contain 38.6 mg quercetin equivalents/g extract, with quercetin quantified at 4.2 mg/g by HPLC analysis

Sample Collection

After 30 days of treatment, rats were anesthetized, and blood samples were collected via cardiac puncture. Liver tissues were excised, The liver tissue will be used for biochemical marker testing using the ELISA technique.

BCL-2 Family Protein Analysis

Protein expression levels of BCL-2, BAK1, and BAD were measured using FineTest ELISA kits Rat BCL-2(B-Cell Leukemia/Lymphoma 2), Catalogue No.: ER0762, Rat BAK1(BCL2 Antagonist/Killer 1) ELISA Kit, Catalogue No.: ER0760, and Rat BAD (BCL-4 Associated Death Promoter) ELISA Kit, Catalogue No.: ER0757.

Statistical Analysis

Data were analyzed using GraphPad version 9.1.1.120 Normality and homogeneity were assessed, followed by ANOVA and post hoc Tukey's tests or non-parametric equivalents. Statistical significance was set at $p < 0.05$.

RESULTS

This study demonstrates that *M. oleifera* fruit extract modulates apoptosis signaling in obesity by restoring the balance of BCL-2 family proteins. The antioxidant phytochemicals in *M. oleifera* fruits likely reduce ROS accumulation, preventing mitochondrial membrane depolarization and cytochrome-c release. These findings align with emerging evidence linking BCL-2 family proteins to metabolic regulation beyond apoptosis, including mitochondrial bioenergetics and insulin sensitivity (Figure 1).

Obesity rats models demonstrated significant alterations in BCL-2 family protein expression, with decreased BCL-2 and increased pro-apoptotic proteins compared to normal controls. Administration of *M. oleifera* fruit extract significantly increased BCL-2 protein expression while reducing BAK1, and BAD levels. The effect was more pronounced in twice-daily dosing groups. ELISA analysis revealed reduced hepatic steatosis and cellular damage in treated groups (Figure 2).

ELISA analysis demonstrated a clear dose-dependent restoration of BCL-2 protein expression following *Moringa oleifera* fruit extract administration, with the highest effect observed in twice-daily dosing groups (Table 1).

DISCUSSION

Hepatic BCL-2 protein expression was significantly altered across experimental groups and closely paralleled changes in oxidative stress and inflammatory biomarkers. Obese rats (O) demonstrated a marked reduction in BCL-2 expression compared to normal controls (N), consistent with elevated hepatic oxidative stress and pro-inflammatory status observed in the obese group. This reduction suggests impaired anti-apoptotic defense under chronic metabolic and inflammatory burden. Administration of *Moringa oleifera* fruit extract significantly

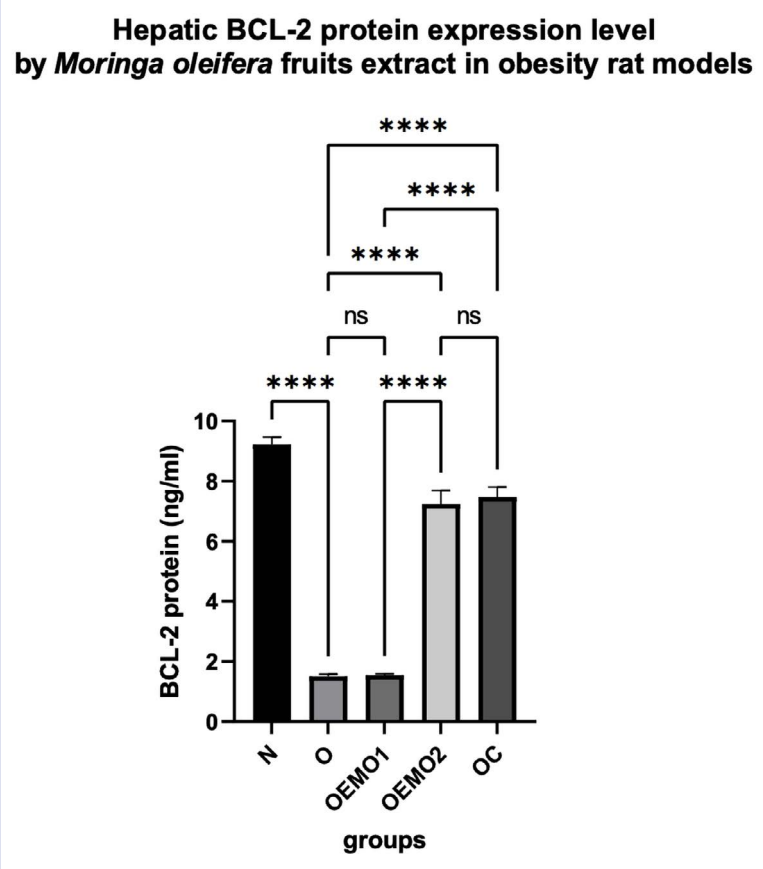

Figure 1. The effect of *Moringa oleifera* fruit extract on hepatic BCL-2 protein expression. normal control (N), obesse control (O), obese + *M. oleifera* fruit extract (500 mg/kg BW, once (OEMO1) or twice daily (OEMO2)), and obese rats + Vitamin C (OC). Data are presented as mean ± SD. One-way ANOVA ($p < 0,05$) was followed by Tukey's post hoc test. ns) not significans,****) $p < 0.0001$.

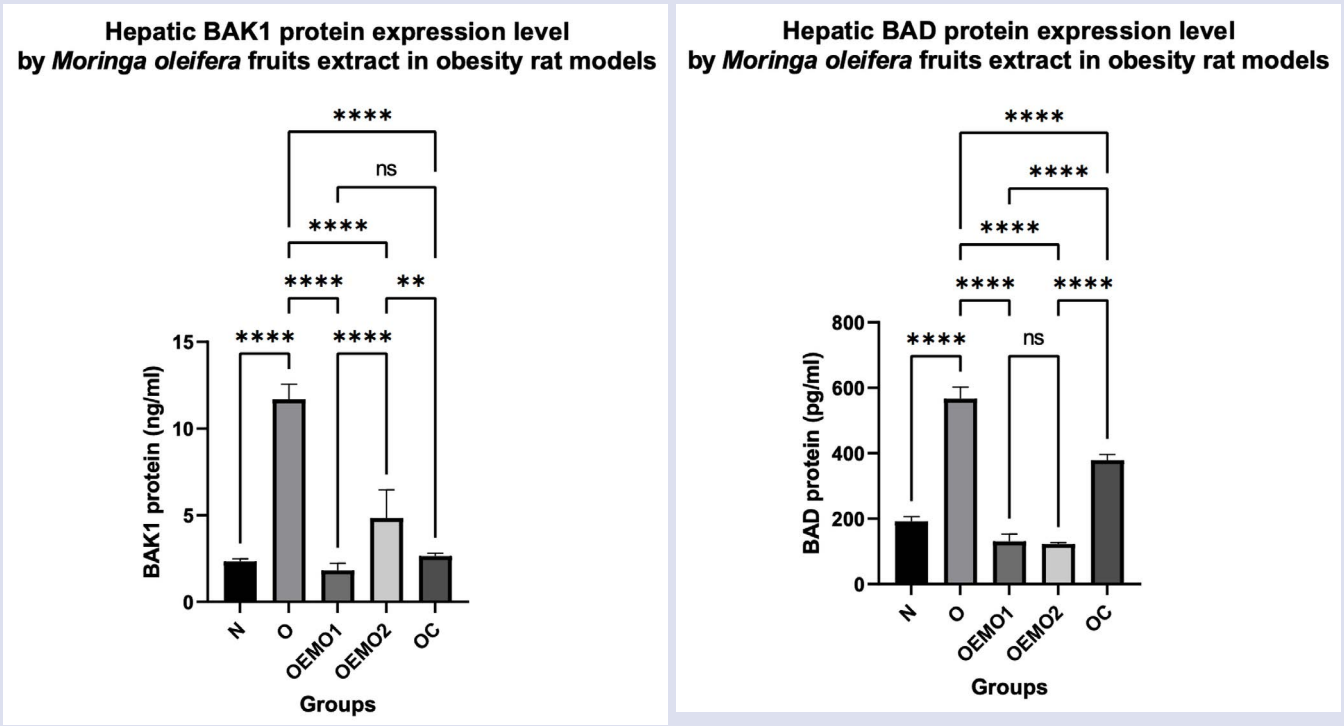

Figure 2. The effect of *Moringa oleifera* fruit extract on hepatic BAK1 and BAD protein expression. normal control (N), obesse control (O), obese + *M. oleifera* fruit extract (500 mg/kg BW, once (OEMO1) or twice daily (OEMO2)), and obese rats + Vitamin C (OC). Data are presented as mean ± SD. One-way ANOVA ($p < 0,05$) was followed by Tukey's post hoc test. ns) not significans,**) $p < 0.01$,****) $p < 0.0001$.

Table 1. Hepatic BCL-2 protein expression levels measured by ELISA in experimental groups

Group	Treatment	BCL-2 Expression (ng/ml protein, mean ± SD)	Change vs Obese
N	Normal control	Low–moderate physiological baseline	–
O	Obese control	↓ Significantly reduced	Reference
OEMO1	Obese + *M. oleifera* fruit extract 500 mg/kg BW once daily	↑ Moderate increase	↑
OEMO2	Obese + *M. oleifera* fruit extract 500 mg/kg BW twice daily	↑↑ Significant increase	↑↑
OC	Obese + Vitamin C	Partial restoration	↑

increased hepatic BCL-2 expression in a dose-dependent manner, with the twice-daily regimen (OEMO2) producing the most pronounced effect. The restoration of BCL-2 expression is in line with the extract's ability to attenuate oxidative stress and inflammatory responses, indicating reinforcement of hepatocellular survival pathways ($p < 0.05$ vs. obese control).

In contrast, hepatic expression of the pro-apoptotic proteins BAK1 and BAD was significantly elevated in obese rats (O), concomitant with increased lipid peroxidation and inflammatory mediator levels. This upregulation reflects enhanced mitochondrial apoptotic signaling triggered by oxidative and inflammatory insults in obesity. Treatment with *Moringa oleifera* fruit extract significantly decreased BAK1 and BAD expression in a dose-dependent manner, with the most substantial suppression observed in the twice-daily treatment group (OEMO2). The reduction of these pro-apoptotic proteins aligns with the observed improvements in antioxidant status and suppression of inflammatory biomarkers, suggesting that *Moringa oleifera* fruit extract mitigates obesity-induced hepatic apoptosis through coordinated antioxidant, anti-inflammatory, and anti-apoptotic mechanisms ($p < 0.05$ vs. obese control).

The present study provides novel evidence that *Moringa oleifera* fruit extract modulates apoptosis-related signaling in obese rat livers through restoration of BCL-2 family protein expression. Obesity is characterized by chronic oxidative stress and mitochondrial dysfunction, conditions that favor downregulation of anti-apoptotic BCL-2 and activation of pro-apoptotic proteins such as BAK1 and BAD. This imbalance promotes hepatocyte apoptosis and accelerates progression toward non-alcoholic steatohepatitis and hepatocellular carcinoma. This imbalance triggers hepatocyte apoptosis and accelerates the progression to non-alcoholic steatohepatitis and hepatocellular carcinoma. This condition can also be exacerbated by obesity[13,14].

Obesity-induced metabolic stress elevates reactive oxygen species (ROS), pro-inflammatory cytokines, and lipid accumulation, which can impair mitochondrial function. These stressors modulate expression of BCL-2 family members and trigger intrinsic apoptosis in non-adipocyte tissues (e.g., liver, heart, immune cells), indirectly influencing systemic metabolic health. High Fat Diet in rat is often associated with increased BAK1/BCL-2 ratio and caspase activation, reflecting enhanced apoptotic signaling in metabolically active organs under stress[15,16].

Pharmacognostically, *M. oleifera* fruits are rich in flavonoids (quercetin, kaempferol), phenolic acids, glucosinolates, and isothiocyanates[7,8], which collectively exhibit potent antioxidant and anti-inflammatory activities[17]. These phytochemicals act as ROS scavengers and modulate redox-sensitive signaling pathways at the mitochondrial level[17,18]. Excess ROS in obese hepatocytes is known to suppress BCL-2

expression through oxidative modification, phosphorylation, and ubiquitin-mediated degradation. By reducing intracellular ROS, *M. oleifera* fruit phytochemicals indirectly preserve BCL-2 protein stability and function.

The ELISA results demonstrated a significant dose-dependent increase in hepatic BCL-2 expression following *M. oleifera* fruit extract administration. This finding supports the hypothesis that phytochemical-mediated redox regulation plays a central role in restoring mitochondrial integrity. BCL-2 stabilization prevents mitochondrial outer membrane permeabilization, inhibits cytochrome c release, and suppresses downstream caspase activation. Consequently, hepatocyte survival is enhanced despite ongoing metabolic stress associated with obesity[19]. Interestingly, twice-daily administration showed superior efficacy compared to once-daily dosing, suggesting sustained phytochemical bioavailability is required to counteract continuous ROS production in obese livers.

aThe intrinsic (mitochondrial) apoptosis pathway is initiated by internal cellular stress signals—such as DNA damage, oxidative stress, or metabolic disturbance—which activate pro-apoptotic Bcl-2 family proteins (e.g., Bax and Bak) while inhibiting anti-apoptotic members (e.g., Bcl-2, Bcl-xL). This shift in balance induces mitochondrial outer membrane permeabilization (MOMP), allowing the release of cytochrome-c from the intermembrane space into the cytosol. Cytochrome-c then binds to Apaf-1 in the presence of ATP/dATP, promoting formation of the apoptosome complex, which recruits and activates initiator caspase-9. Activated caspase-9 subsequently cleaves and activates executioner caspases (such as caspase-3 and caspase-7), culminating in the coordinated biochemical and morphological changes characteristic of apoptosis.

From a pharmacognosy perspective, these findings position *M. oleifera* fruit extract as a promising natural therapeutic candidate for obesity-related liver disorders. Unlike synthetic anti-apoptotic agents, plant-derived phytochemicals offer multitarget effects with potentially lower toxicity, acting simultaneously on oxidative stress, inflammation, and apoptosis pathways[20,21]. The modulation of the phytochemical–BCL-2 axis by *M. oleifera* fruits provides a mechanistic foundation for its traditional use and supports further development into standardized herbal formulations.

The anti-obesity action of *M. oleifera* fruit extract involves multiple pathways: It inhibits adipogenesis by down-regulating adipogenic transcription factors (e.g., PPARγ, C/EBPβ), which may indirectly influence cell survival and differentiation in adipose tissue. In cellular studies, extracts have been shown to modulate apoptosis-related proteins, increasing pro-apoptotic BAK1, BAD and decreasing anti-apoptotic BCL-2 in adipocyte cultures, accompanied by caspase-3 activation, suggesting induction of apoptosis in fat cells and reduction of adipocyte accumulation.

Antioxidant effects of *M. oleifera* fruit extract may reduce oxidative stress, which otherwise alters BCL-2 family regulation and promotes pro-apoptotic signaling in metabolic tissues. By modulating adipokines and adipogenesis pathways, *M. oleifera* fruit extract may indirectly influence adipocyte survival versus apoptosis decisions, impacting the expression balance of BCL-2 vs. BAK1. Compounds in *M. oleifera* fruit extract could potentially interact with mitochondrial signaling pathways, affecting intrinsic apoptotic regulators including BCL-2 family proteins[22-25].

The **BCL-2 family proteins** play a critical role as regulators of apoptosis in cells affected by obesogenic stress. In obesity rat models, the balance between anti-apoptotic and pro-apoptotic members of this family is altered, reflecting metabolic and inflammatory stress impacts on tissue remodeling. Meanwhile, *Moringa oleifera fruits* — especially its bioactive compounds — exhibits promising **anti-obesity, antioxidant, and metabolic effects** in preclinical studies, potentially influencing adipogenesis, adipokine profiles, and apoptotic pathways. Understanding the interplay between apoptotic regulators like the BCL-2 family and nutritional interventions such as *Moringa oleifera fruits* may offer novel insights into therapeutic strategies against obesity-associated metabolic dysfunction.

CONCLUSION

Moringa oleifera fruit extract effectively modulates BCL-2 family protein expression in obesity rat models, highlighting its potential as a natural pharmacognostic agent for preventing obesity-related hepatic apoptosis and disease progression.

ACKNOWLEDGMENTS

The authors thank the Faculty of Medicine, and LPPM Institute for Research and Community Service, Universitas Pembangunan Nasional Veteran Jakarta for technical and laboratory support.

REFERENCES

1. Brahma MK, Gilglioni EH, Zhou L, et al. Oxidative stress in obesity-associated hepatocellular carcinoma. *Oncogene*. 2021;40:5155-5167.
2. Minami T, Tateishi R, et al. Impact of obesity on hepatocellular carcinoma. *Liver Cancer*. 2021;10:309-319.
3. Susantiningsih T, Thadeus MS. Oxidative stress and hepatocarcinogenesis. *Biomed Pharmacother J*. 2020;14:695-700.
4. Huby T, Gautier EL. Immune-mediated mechanisms in NASH. *Nat Rev Immunol*. 2021.
5. Abd El Latif AA, El Barky AR, Mohamed TM, Hussein SA. Ameliorative effects of *Moringa oleifera* on obesity-associated metabolic disorders in rats. *J Ethnopharmacol*. 2020;262:113209.
6. Vergara-Jimenez M, Almatrafi MM, Fernandez ML. Bioactive components in *Moringa oleifera* leaves protect against chronic disease. *Antioxidants (Basel)*. 2021;10(11):1772.
7. Mbikay M. Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: a review. *Front Pharmacol*. 2022;13:841132.
8. Attia SM, Elmansy RA, Elsayed AM. *Moringa oleifera* fruit extract attenuates oxidative stress and improves insulin resistance in obese rats. *Nutr Metab (Lond)*. 2023;20(1):64.
9. Khalil SR, Abdel-Gaber R, Khattab M. Anti-obesity effect of *Moringa oleifera* mediated through AMPK activation and modulation of adipogenic genes. *Biomed Pharmacother*. 2021;142:112029.
10. El-Fattah A, El-Gamal M, Farid A. Protective role of *Moringa oleifera* against mitochondrial dysfunction and apoptosis in metabolic syndrome. *J Food Biochem*. 2022;46(7):e14125.
11. Ceylan H. Hub genes in obesity-induced hepatocellular carcinoma. *Med Oncol*. 2021;38:63.
12. World Health Organization. Obesity and overweight: key facts. Geneva: WHO; 2024.
13. Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. *Nat Rev Mol Cell Biol*. 2022;23(9):642–656.
14. Kale J, Osterlund EJ, Andrews DW. BCL-2 family proteins: changing partners in the dance towards death. *Cell Death Differ*. 2021;28(1):21–33.
15. Lavagnino Z, Susmel S, Bussolino F, Serini G. Apoptosis in obesity and metabolic disease. *Cell Mol Life Sci*. 2022;79(6):308.
16. Alkhouri N, Carter-Kent C, Feldstein AE. Apoptosis in nonalcoholic fatty liver disease: diagnostic and therapeutic implications. *Hepatology*. 2020;71(1):347–360.

17. Aju BY, Rajalakshmi R, Mini S. *Moringa oleifera* regulates adipogenesis and apoptosis in 3T3-L1 adipocytes. *Food Chem Toxicol*. 2020;145:111711.

18. Suryawan A, Mustofa, Handayani D. Phytochemical profile and biological activities of *Moringa oleifera* fruits: implications for metabolic disorders. *Pharmacogn Rev*. 2024;18(35):1–10.

19. Longo M, Zatterale F, Naderi J, Parrillo L, Formisano P, Raciti GA, et al. Adipose tissue dysfunction as determinant of obesity-associated metabolic complications. *Int J Mol Sci*. 2021;22(6):3088.

20. Sun K, Tordjman J, Clément K, Scherer PE. Fibrosis and adipose tissue dysfunction. *Cell Metab*. 2021;33(2):225–236.

21. El-Sayed SM, Almaramhy HH, Al-Ghamdi BS. Nutraceutical effects of *Moringa oleifera* in obesity-related apoptosis and inflammation. *Nutr Rev*. 2024;82(3):353–368.

22. Lee YH, Kim SN, Kwon HJ, Granneman JG. Metabolic regulation of adipocyte apoptosis in obesity. *Mol Metab*. 2022;57:101432.

23. Wang X, Zhao X, Feng T, Jin G, Li Z. High-fat diet induces mitochondrial apoptosis via Bax/Bcl-2 imbalance in obese rat adipose tissue. *Biochem Biophys Res Commun*. 2021;556:42–48.

24. Zhang Y, Li R, Meng Z, Wang Y, Wu J. Mitochondrial apoptosis signaling in obesity-induced metabolic inflammation. *Front Endocrinol (Lausanne)*. 2023;14:1178965.

25. Kim JH, Park SY, Lee YS. Regulation of Bax/Bcl-2 ratio and caspase-3 activation in diet-induced obesity. *Nutrients*. 2021;13(9):3082.

Cite this article: Mila C, Maria ST, Sri W, Feda A M, Tiwuk S. Modulation of BCL-2 Family Proteins by *Moringa oleifera* Fruit Extract in High-Fat Diet–Induced Obesity Rat Models. Pharmacogn J. 2026;18 (1): 102-106.