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In vivo Effect of *Momordica charantia* on Dipeptidyl Peptidase-4 Activity in Diabetic Rats

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a metabolic disease characterised by prolonged high blood glucose levels due to insulin action, production, and resistance issues. In 2015, the prevalence was estimated at 415 million individuals aged between 20 and 79. The number of deaths is expected to rise by 578 million in 2030 and 700 million in 2045, ranking the disease as the 6th most significant cause of death globally. Therefore, this study aimed to examine the pathophysiological mechanisms of T2DM using dipeptidyl peptidase. A total of 30 Wistar rats were separated into 6 groups namely (1) control, (2) STZ-NA-induced, (3) STZ-Na-induced group treated with DPP-4i, (4) STZ-Na-induced group treated with MCE 75 mg/kg bw, (5) STZ-Na-induced group treated with MCE 150 mg/kg bw, and (6) STZ-Na-induced group treated with MCE 300 mg/kg bw. DPP-4 levels were quantified using an Enzyme-Linked Immunosorbent Assay (ELISA). The results showed that the Kruskal-Wallis DPP-4 test indicated a non-significant difference among the groups (p=0.192), with the DPP-4 level in the MCE 300 mg/kg bw group being lower than the vildagliptin group. In T2DM model rats, *Momordica charantia* ethanol extract at 300 mg/Kg bw was more effective than vildagliptin in reducing dipeptidyl peptidase-4 (DPP-4).

Keywords: *Momordica charantia*; type 2 diabetes mellitus; DPP-4; vildagliptin

Introduction

Type 2 diabetes mellitus (T2DM) is characterised by persistently high glucose levels due to reduced insulin secretion and function.[1] Insulin resistance reduces glucose absorption in skeletal muscle and adipose tissue, causing a greater need for production from pancreatic beta cells. However, the beta cells cannot meet this heightened demand for insulin secretion.[2] In 2015, there were approximately 415 million cases of T2DM in individuals aged 20 to 79. This figure is projected to reach 578 million by 2030 and 700 million by 2045. T2DM is currently the 6th most significant cause of mortality globally[3], and Indonesia ranks third worldwide among countries with the highest number of T2DM cases in individuals aged 20-79. Approximately 10.7 million cases were reported in 2019, and this figure is projected to reach 13.7 million by 2030 and 16.6 million by 2045.[4]

The diagnostic criteria for T2DM rely on assaying venous blood samples. A fasting blood glucose level of 7.0 mmol/L is most closely associated with a 2-hour postprandial blood glucose value of > 11.1 mmol/L in a 75 g oral glucose tolerance test (OGTT).[5] Glycated haemoglobin (HbA1c) test is highly specific but less sensitive than standard glucose criteria for diagnosing T2DM. The test can be carried out any day and is more convenient than GDP or 2JPP in 75 g OGTT. Furthermore, the HbA1c test circumvents the daily fluctuations in glucose levels by providing an average blood glucose measurement for the preceding 2 to 3 months.[6]

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T2DM has a complex aetiology, including multiple factors, and understanding the intricate biology can guide the appropriate treatment strategy. In 2009, DeFronzo described the pathophysiological course of T2DM, which includes the Ominous Octet, insulin resistance in the liver and muscle, and loss of pancreatic beta cells. The Ominous octet strategy entails targeting the digestive tract and pancreatic beta cells. The digestive system is linked to incretin hormones and fructose transportation through glucose transporter 5 (GLUT5), while pancreatic beta cells are crucial for insulin secretion.[7] Incretin is a hormone secreted by intestinal cells in response to meal consumption, with Glucagon-like peptide-1 (GLP-1) being the most effective type.[8] GLP-1 is a 30-amino acid endocrine hormone produced in enteroendocrine L cells. It plays a crucial role in regulating metabolism and increases insulin release from pancreatic cells. GLP-1 activity is terminated by the DPP-4 enzyme, which degrades the active hormone in the kidney brush border membrane, hepatocytes, and capillary endothelial cells, necessitating DPP-4 inhibitor medications.[9] Furthermore, GLP-1 hormone and GLP-1R receptor are targeted for treating T2DM with exenatide, liraglutide, vildagliptin, and sitagliptin. Aside from the therapeutic benefits, these medications might cause side effects such as fluid retention, weight gain, and headaches.[10] Due to the harmful effects of these medicines and high costs, finding alternate treatments for managing T2DM is necessary. Medicinal plants and derivatives, including bitter melon (*Momordica charantia*), have been historically used as the primary treatment for diabetes.[9]

Bitter melon, a tropical plant belonging to the Cucurbitaceae family, is used for managing T2DM. It safeguards pancreatic beta cells, enhances insulin production, blocks intestinal α-glucosidase, impeding glucose transfer, stimulates AMPK, and decreases gluconeogenesis.[11] Bitter melon extract containing charantin demonstrated hypoglycemic effects in healthy and diabetic rabbits. The methanol extract, at 375 mg/kg, reduced fasting blood glucose levels in alloxan-induced diabetic rats after 12 hours. Other investigations have shown that administering bitter melon juice at a 6 mL/kg dose reduced blood glucose, cholesterol,

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and triglycerides by decreasing PKC-β activity in streptozotocin-induced diabetic rats.[12] The n-hexane fractionation and ethanol extract from bitter melon fruit can reduce malondialdehyde levels and ameliorate streptozotocin-induced liver damage in rats.[13,14] The protein extract inhibited alpha-amylase and alpha-glucosidase and reduced blood sugar levels in diabetic rats with similar effects as acarbose.[15] Therefore, this study aimed to examine the impact of bitter melon ethanol extract on dipeptidyl peptidase-4 (DPP-4) activity in T2DM model rats.

Materials and Methods

Ethical approval

The protocol received ethical approval from the Research Ethics Committee of the Faculty of Medicine at Sebelas Maret University, Surakarta, with approval number 91/UN27.06.11/KEP/EC/2023.

Experimental design and protocol

This study used an experimental method, specifically The Post Test Only Control Group Design. This study was conducted over 6 months using a sample of 30 male Wistar rats, aged between 3 and 4 months, with a body weight ranging from 200 to 250 grams. Rats were subsequently separated into 6 separate groups.

The parameters were analysed by examining the blood serum using specific ELISA kits for blood glucose (MyBioSource, catalogue number MBS7233226) and DPP-4 (BT Lab, code E0226Ra). The ethanol extract of bitter melon fruit was acquired, processed, and analysed by PT Lansida, Yogyakarta, Indonesia, under reference number 78/LH/05/2023. The fruit was crushed and soaked for 9 days in 96% ethanol and filtered. The macerate was evaporated using a rotary evaporator, maintaining a temperature below 40°C until a viscous and concentrated extract was obtained. Rats were acclimatised for 1 week in the Animal House to aid adaptation to a controlled environment that was pleasant, devoid of noise, and maintained at a temperature of 27°C. Each rat was individually housed in clean cages lined with husks. Rats were exposed to a 12-hour light and 12-hour dark regimen. Standard feed (AIM-93M) was given, and the rats had access to distilled water for consumption. In addition, dead or diseased rats were excluded from the study.

The induction process was carried out through the intraperitoneal injection of nicotinamide (NA) at a dose of 230 mg/kg bw, followed by STZ at a dose of 65 mg/kg bw after 20 minutes. After 5 days, the extract was administered.[16–18] Subsequently, 30 rats were randomly assigned to 6 groups. Rats were weighed and systematically evaluated to verify agility and the absence of disease indicators.

Group I, designated as the control group, received standard feed. Group II, the positive control group, was induced with STZ-NA and subsequently provided with standard feed. The negative control group III was induced with STZ-NA, and vildagliptin was administered along with standard feed. Group IV, V, and VI were induced using STZ-NA, provided with standard feed, and received the test material, specifically the ethanol extract of bitter melon, at 75 mg/kg, 150 mg/kg, and 300 mg/kg body weight, respectively. The experiment lasted 28 days. The procedure for blood collection entailed the insertion of a hematocrit capillary tube into the retroorbital plexus area, situated posterior to the eye socket. Blood was collected in an Eppendorf tube and centrifugated at 12,000 rpm for 10 minutes to separate the serum from other components. The serum was used for the different measurements.

Statistical Analysis

Data were presented as the mean ± SD and subjected to the Shapiro-Wilk test followed by one-way analysis of variance (ANOVA) to compare differences between the test groups and the controls. Results were considered significant at a 95% confidence interval.

Results and Discussion

Based on the normality test results using the Shapiro-Wilk test (Table 1), the data followed a normal distribution. The homogeneity test indicated non-homogeneous results. Hence, the Kruskal-Wallis test was carried out, and a p-value of less than 0.05 was obtained after treatment. This implied a significant difference in blood glucose levels between groups. The induction of diabetes in the rats (Figure 1) shows a rise in blood glucose levels, confirming the effective establishment of T2DM model rats. DPP-4 is an enzyme in the body that acts on incretin hormones, particularly GLP-1 (glucagon-like peptide-1) and GIP (gastric inhibitory peptide).

Group	Pre Induction		Post Induction		Post Treatment	
	Mean (mg/dL)	SD	Mean (mg/dL)	SD	Mean (mg/dL)	SD
1	61	22.87	136.2	4.76	134.2	38.96
2	71.2	21.74	218.8	72.71	467.0	95.04
3	73.8	8.44	324.2	138.64	83.6	26.02
4	94.2	17.94	226.2	50.18	100.2	20.69
5	89.4	14.38	351.4	142.96	95.8	16.42
6	96.6	23.52	270.2	147.43	80.8	37.99

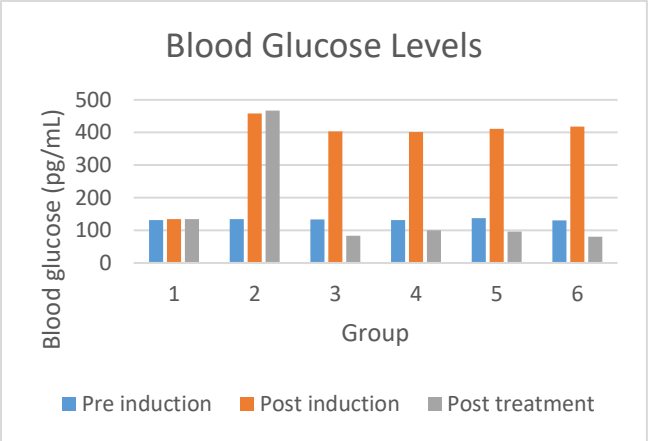
Table: 1 Blood glucose levels pre-induction, post-induction, and post-treatment results

Table 2: DPP-4 Levels (pg/mL)

Group	Mean	SD	Sig. [a]	Sig. [b]
1	125.357	14.627	0.728	0.518
2	133.687	20.008	0.723	
3	120.109	17.750	0.976	
4	124.791	16.463	0.947	
5	129.326	21.136	0.125	
6	106.487	12.357	0.081	

[a]normality test results
[b]homogeneity test results

Figure 1. Graph showing the rise in blood glucose levels in each group pre-induction, post-induction, and post-treatment



It helps regulate glucose levels by enhancing insulin release and reducing glucagon release. DPP-4 levels were assessed for normality using the Shapiro-Wilk test. The results showed that the data in each group followed a normal distribution (p>0.05) and homogeneous results (p>0.05), leading to the use of a parametric test, namely One-way Analysis of Variance (ANOVA). The results showed no significant differences in either group, with a p-value greater than 0.05, as shown in Table 3.

Bitter melon has antidiabetic properties such as protecting the islet of Langerhans cells, enhancing insulin production, blocking α-glucosidase

in the intestine, enhancing hepatic glucose clearance, and decreasing gluconeogenesis. Additionally, it elevates GLP-1 levels in the intestines. Sub-chronic treatment of bitter melon increases GLP-1 by enhancing enteroendocrine L cell receptors responsible for GLP-1 production.[8] The charantin, cucurbitacin, and momordicoside D compounds from bitter melon fruit have activated the TGR5 and GLP1 receptors, simultaneously inhibiting DPP4.[19] The chemical constituents present in bitter melon help in minimising oxidative damage by neutralisation of free radicals activity and performing prompt actions to control the death of β-cells.[20] *M. charantia* exhibits its antidiabetic effects via the suppression of MAPKs and NF-κβin pancreatic cells, promoting glucose and fatty acids catabolism, stimulating fatty acids absorption, inducing insulin production, ameliorating insulin resistance, activating AMPK pathway, and inhibiting glucose metabolism enzymes.[21]

Table 3: One-way ANOVA test for DPP-4 levels

	Sum of Squares	df	Mean Square	F	p
Between Groups	2217.733	5	443.547	1.479	0.234
Within Groups	7199.416	24	299.976		
Total	9417.150	29			

The results showed no significant changes between groups in terms of DPP-4 levels. However, the value in group 6, given the highest dose treatment, was lower than in group 3, given typical DPP-4 inhibitor medicine. Perumal (2022) reported a mixture of crude extracts from *T. officinale* and *M. charantia* inhibited DPP-4. DPP-4 inhibitory activity of the crude extract was evaluated using a non-Cayman cell-based assay. This test evaluates DPP-4 enzyme function by measuring the activity with a fluorescent substrate called Gly-Pro-Aminomethylcoumarin. DPP-4 enzyme breaks peptide bonds in the substrate, generating fluorescent Gly-Pro-Aminomethylcoumary groups detected and studied using specific excitation and emission wavelengths. Vildagliptin had the maximum inhibition of 79.95 ± 0.35% ($p < 0.05$) compared to all crude extracts of *T. officinale and M. charantia*. The crude extract of *T. officinale* had the maximum inhibitory efficacy with acetone (44.85 ± 0.44%), ethanol (43.69 ± 0.56%), and water solvent (28.15 ± 0.31%).[22] A study used computational methods to identify the most effective antidiabetic peptide derived from the hypoglycemic P-polypeptide of bitter melon. The study assessed the peptide's binding strength and interaction characteristics with four receptor proteins, specifically as an insulin receptor agonist and an inhibitor of sodium-glucose cotransporter 1, DPP-4, and glucose transporter-2 through a molecular docking method. Based on the results, 37 peptides were connected to this receptor. The 8 best ligands selected were LIVA (Leucine, Isoleucine, Valine, Alanine), TSEP (Threonine, Serine, Glutamate, Proline), EKAI (Glutamate, Lysine, Alanine, Isoleucine), LKHA (Leucine, Lysine, Histidine, Alanine), EALF (Glutamate, Alanine, Leucine, Phenylalanine), VAEK (Valine, Alanine, Glutamate, Lysine), DFGAS (Aspartate, Phenylalanine, Glycine, Analine, Serine), and EPGGGG (Glutamate, Proline, Glicine, Glicine, Glicine, Glicine). These ligands comply with Lipinski's rule of five and have high quality.[23]

As determined through computational studies, Bitter melon compounds have a strong binding affinity to 3 confirmed diabetes-related targets, namely Takeda G-Protein-Coupled Receptor 5 (TGR5), Glucagon-Like Peptide-1 (GLP-1), and Dipeptidyl Peptidase-4 (DPP-4). The extract enhanced GLP-1 expression by 295.7%. The compound derived from the plant increased the expression of the GLP-1 gene. *In silico* results suggest that the phytochemical compound responsible for this effect is either mormodicoside (TGR5-mediated secretion of GLP-1) or the GLP-1 analogue cucurbitacin. Mormodicoside has the highest binding affinity with TGR5, while cucurbitacin has the highest affinity with GLP-1. DPP-4 expression decreased by 87.2% when 20% extract was introduced into the diet of rats, and the decline was concentration-dependent. Bitter melon reduced DPP-4 expression, increasing GLP-1 expression by inhibiting its cleavage. This action prolonged the half-life of GLP-1 and insulin production. The computational simulations suggest that charantin, a type of saponin, may act as a DPP-4 inhibitor.[24] In the molecular docking simulation and toxicity assessment of polyphenolic compounds from bitter melon as inhibitors of DPP-4 enzyme for the management of T2DM, the results showed that these compounds had better docking scores compared to synthetic inhibitors, including Sitagliptin, Vildagliptin, and Saxagliptin.[25] In another study on the Momordica family, a combination of effective doses, namely 400 mg/kg *Leptadenia hastata* and 200 mg/kg *Momordica balsamina*, significantly reduced blood glucose levels by 63.89% in 28 days, nearly comparable to the standard drug (Glucovance, 66.94%). It also increased serum insulin levels, total haemoglobin, and the activity of metabolic enzymes such as hexokinase while reducing HbA1c levels and glucose-6-phosphatase activity.[26]

Conclusion

In conclusion, this study found that different doses of ethanol extract from *Momordica charantia* inhibited DPP-4 in T2DM Wistar rats induced by streptozotocin-nicotinamide. The results support the need for additional studies to confirm the effectiveness of bitter melon fruit ethanol extract as a treatment for T2DM and its application in clinical settings.

Conflict of Interest

This research has no conflict of interest.

Acknowledgment

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References

1. Perkeni. Guidelines for the management and prevention of type 2 diabetes mellitus in Indonesia 2021. Published online 2021:46.
2. Ling C. Epigenetic regulation of insulin action and secretion – role in the pathogenesis of type 2 diabetes. J Intern Med. 2020;288(2):158-167. doi:10.1111/joim.13049
3. International Diabetes Federation. IDF Diabetes Atlas. 2021;102 doi:10.1016/j.diabres.2013.10.013
4. Kementrian Kesehatan Republik Indonesia. Infodatin 2020 Diabetes Mellitus. Published online 2019.
5. Goyal R, Jialal I. Diabetes Mellitus, Type 2. 2020. doi:10.1016/b978-3-437-42502-8.00154-6
6. Punthakee Z, Goldenberg R, Katz P. Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome. Can J Diabetes. 2018;42:S10-S15. doi:10.1016/j.jcjd.2017.10.003
7. Kelly SD, Neary SL. Ominous Octet and Other Scary Diabetes Stories: The Overview of Pathophysiology of Type 2 Diabetes Mellitus. Physician Assist Clin. 2020;5(2):121-133. doi:10.1016/j.cpha.2019.11.002
8. Bhat GA, Khan HA, Alhomida AS, Sharma P, Singh R, Paray BA. GLP-I secretion in healthy and diabetic Wistar rats in response to aqueous extract of *Momordica charantia*. BMC Complement Altern Med. 2018;18(1). doi:10.1186/s12906-018-2227-4
9. Singh R, Bhat GA, Sharma P, Bhat A. GLP-1 secretagogues potential of medicinal plants in management of diabetes. J Pharmacogn Phytochem. 2015;4(1):197-202.
10. St. Onge EL, Miller S, Clements E. Sitagliptin/metformin (Janumet) as combination therapy in the treatment of type-2 diabetes mellitus. P and T. 2012;37(12):699-708.
11. Peter EL, Nagendrappa PB, Hilonga S, Tuyiringire N, Ashuro E, Kaligirwa A, Sesaazi CD. Pharmacological reflection of plants traditionally used to manage diabetes

mellitus in Tanzania. J Ethnopharmacol. 2021;269:113715. doi:10.1016/j.jep.2020.113715
12. Saeed F, Sultan MT, Riaz A, Ahmed S, Bigiu N, Amarowicz R, Manea R. Bitter melon (*Momordica charantia* l.) fruit bioactives charantin and vicine potential for diabetes prophylaxis and treatment. Plants. 2021;10(4). doi:10.3390/plants10040730
13. Parawansah P, Sudayasa IP, Syarifin AN, Eso A, Nuralifah N, Fathanah WO, Sandra F. *Momordica charantia* L. fruit fractions inhibit malondialdehyde level and regenerate hepatic damage of hyperglycemic rats. Indones. Biomed. J. 2020;12(1):57-61. doi:10.18585/INABJ.V12I1.963
14. Surya O, Hendro W, Yudhanto E, Himawan AB, Adespin DA. Effect of *Momordica charantia* fruit ethanolic extract on malondialdehyde (MDA) level in blood of Sprague-Dawley rats induced by 7, 12-dimethylbenz [a] anthracene. Diponegoro Med. Jour. 2022;11(1):32-6.
15. Poovitha S. *In vitro* and *in vivo* α-amylase and *α*-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia* L.). BMC Complement Altern Med. 2016;16. doi:10.1186/s12906-016-1085-1
16. Ayoub SM. Evaluation of hypoglycemic effect of *Momordica charantia* extract in distilled water in streptozotocin-diabetic rats. Braz. J. Vet. Pathol. 2013;6(2):56-64. https://api.elsevier.com/content/abstract/scopus_id/84881620563
17. Raza H, John A. Streptozotocin-induced cytotoxicity, oxidative stress and mitochondrial dysfunction in human hepatoma HepG2 cells. Int J Mol Sci. 2012;13(5):5751-5767. doi:10.3390/ijms13055751
18. Zhu Y. Effects of bitter melon (*Momordica charantia* L.) on the gut microbiota in high-fat diet and low dose streptozocin-induced rats. Int J Food Sci Nutr. 2016;67(6):686-695. doi:10.1080/09637486.2016.1197185
19. Kusniawati MA. Systematic Review of the Activity of Bitter Gourd Fruit (Momordica charantia L.) Against Antidiabetic Molecular Targets. Indones. J. Pharm. Published 18(2):141-151. DOI:10.31001/jfi.v18i2.974
20. Mahwish. Bitter melon (*Momordica charantia* l.) fruit bioactives charantin and vicine potential for diabetes prophylaxis and treatment. Plants. 2021;10(4). doi:10.3390/plants10040730
21. Oyelere SF. A detailed review on the phytochemical profiles and antidiabetic mechanisms of *Momordica charantia*. Heliyon. 2022;8(4). doi:10.1016/j.heliyon.2022.e09253
22. Perumal N, Nallappan M, Shohaimi S, Kassim NK, Tee TT, Cheah YH. Synergistic antidiabetic activity of *Taraxacum officinale* (L.) Weber ex F.H. Wigg and *Momordica charantia* L. polyherbal combination. Biomed Pharmacother. 2022;145. doi:10.1016/j.biopha.2021.112401
23. Arif R. Molecular Docking and Simulation Studies of Antidiabetic Agents Devised from Hypoglycemic Polypeptide-P of *Momordica charantia.* Biomed Res Int. 2021;2021. doi:10.1155/2021/5561129
24. Elekofehinti OO. *Momordica charantia* nanoparticles potentiate insulin release and modulate antioxidant gene expression in pancreas of diabetic rats. Egypt. J. Med. Hum. Genet. 2022;23(1). doi:10.1186/s43042-022-00282-0
25. Latigui A, Bouzidi NK, Hassini N, Benmetir S. Exploration of Novel Type 2 Antidiabetic Agents: Molecular Docking and Toxicity Assessment of Polyphenolic Compounds for Improved Therapeutic Potential. Trop. J. Nat. Prod. Res.. 2024;8(7):7755-7758. doi:10.26538/tjnpr/v8i7.19
26. Kabir N, Umar IA, James DB, Inuwa HM, Atiku MK. Antidiabetic potentials of aqueous leaf extracts of *Momordica balsamina linn* and *Leptadenia hastata* (Pers) decne alone and in combination in streptozotocin-induced diabetic rats. Trop. J. Nat. Prod. Res. 2019;3(1):10-16. doi:10.26538/tjnpr/v3i1.3