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Glucose Regulation Effect by the Functional Bitter Melon Compound BmpP® in Palmitic Acid-Induced Insulin Resistance HepG2 Cells

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Abstract

Background: *Insulin resistance is one of the major causes and manifestations of diabetes. Previous studies have demonstrated the glucose-regulating effects of bitter melon. Therefore, this study employs a PA-induced insulin-resistant cell model to investigate the potential of functional bitter melon compound BmpP® in glycemic regulation.*

Methods: *Insulin resistance was induced in HepG2 cells by exposure to 0.25 mM palmitic acid (PA) for 18 hours. The effects of BmpP® on cellular glucose oxidase activity, glycogen accumulation, and ono-esterified fatty acid (NEFA) levels were then evaluated.*

Results: *BmpP® reversed the PA-induced reduction in glucose oxidase activity and glycogen accumulation in HepG2 cells. Additionally, PA-induced NEFA accumulation was alleviated following BmpP® treatment.*

Conclusion: *These findings suggest that BmpP® enhances glucose utilization and exhibits potential as a functional food supplement for glycemic regulation.*

Keywords: *Bitter Melon, Functional Food, İnsulin Resistant, HepG2.*

INTRODUCTION

Type 2 diabetes mellitus (T2DM) was estimated to affect 415 million people by 2015, with projections predicting the number of diabetes cases will reach 642 million globally by 2040 (Moridpour et al., 2024). Key factors that contribute to the exacerbation of cardiovascular disease (CVD) in T2DM include hyperinsulinemia, obesity, hypertension, hypertriglyceridemia, hypercholesterolemia, and homocysteinemia (Mokgalaboni, Mashaba, Phoswa, & Lebelo, 2024).

Insulin resistance (IR) is a condition in which the effectiveness of insulin is impaired in insulin-targeted tissues, such as skeletal muscle, liver, and adipocytes. In IR, the liver fails to properly produce glycogen and suppress gluconeogenesis, which contributes to elevated blood glucose levels (Y. Sun et al., 2021). The relationship between free fatty acids (FFAs) and IR is widely recognized (Song, Li, & Xu, 2024). Palmitic acid (PA), the most common circulating saturated fatty acid (SFA), has been shown to induce hepatic IR by inhibiting insulin signaling, reducing glycogen synthesis, decreasing glucose uptake, and increasing the accumulation of lipid metabolites in the liver, thereby promoting insulin resistance (Shou, Zhou, Hu, Zhu, & Luo, 2023). Long-term exposure to saturated FFAs is commonly used to induce IR in experimental models.

The *Momordica charantia L.* plant has been documented for its anti-diabetic properties, with its fruit, commonly known as bitter melon, bitter gourd, or balsam pear, being the most frequently consumed part for both culinary and medicinal purposes (Chang et al., 2021). Previous studies have shown that bitter melon extract can alleviate PA-induced insulin resistance in HepG2 cells by enhancing glucose uptake, increasing glycogen content, and reducing triglyceride accumulation, thereby exhibiting potential blood glucose regulation effects (Zhu et al., 2021).

In India, China, and many other countries, the use of herbal medicine has been a longstanding practice and is considered a safer and more cost-effective approach for managing various disease (Takahashi et al., 2023; Yadav et al., 2010). Several plants have been reported to contribute to blood glucose regulation, including *Gymnema sylvestre (Gaytan Martinez, Sanchez-Ruiz, Zuniga, Gonzalez-Ortiz, & Martinez-Abundis, 2021)* and mulberry leave (Takahashi et al., 2023). Although numerous studies have highlighted the antidiabetic

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potential of these plants, comprehensive investigations comparing their individual and complementary effects remain limited. Therefore, this study aimed to examine the potential synergistic effects of the functional bitter melon compound BmpP® on its antihyperglycemic properties in PA-induced IR HepG2 cells.

METHODS & MATERIALS

Experimental Compound

The functional bitter melon compound BmpP® supplement, provided by Tsa International Co., Ltd. (500 mg per tablet), included bitter melon extract, soluble fiber, chromium yeast, zinc yeast, *Gymnema sylvestre* leaf extract, mulberry leaf extract, and barley extract.

Cell Culture

HepG2 cells (BCRC No. 60025) were seeded into T75 flasks and cultured in DMEM medium containing 4 μM L-glutamine and 4.5 g/L glucose (Catalog No. 12800017), with 1.5 g/L $NaHCO_3$, 1% penicillin-streptomycin (PSN), and 10% fetal bovine serum (FBS). Cell culture was maintained at 37°C in a humidified incubator with 5% CO_2, with the culture medium replaced approximately every two days.

Cell Viability Assay

96-well plates were seeded with a cell density of 2000 cells/mL to assess cell viability. The cells were exposed to different concentrations of BmpP® (5–80 μg/mL) for 48 hours under standard incubation conditions (37°C, 5% CO_2). After incubation, the culture medium was removed and replaced with 100 μL fresh medium and 10 μL CCK-8 assay solution. The plates were then incubated for an additional 4 hours at 37°C. The absorbance at 450 nm was measured using a microplate reader. Cell viability (%) was calculated using the formula:

Cell viability (%) = [(OD of experimental group - OD of blank) / (OD of control group - OD of blank)] × 100%.

Insulin Resistance Cell Model and Treatment

Palmitic acid (TargetMo, T2908) was conjugated to bovine serum albumin (BSA) (Sigma, A0281) to prepare a stock solution. Specifically, 0.02 mL of 10 mM palmitate, 0.0098 g of BSA, and 0.98 mL of ddH_2O were combined to generate a 0.2 mM palmitate stock solution. When HepG2 cells reached 80–90% confluence in a 12-well plate, the culture medium was replaced with serum-free DMEM and incubated for 12 hours. Then treated with serum-free medium containing 0.25 mM palmitic acid and varying concentrations of the BmpP® (40 and 80 μg/mL) or metformin (10 mM) for 18 hours, and the cellular glucose oxidase activity, the level of glycogen, and NEFA were detected.

Glucose Oxidase Activity Assay

The activity of glucose oxidase was measured using the Glucose Oxidase Activity Assay Kit (Abcam, Cat. No. ab219924) in accordance with the manufacturer's instructions.

Glycogen Levels Detection

The level of cellular glycogen was measured using the Glycogen Assay Kit (Abcam, cat: ab169558) in accordance with the manufacturer's instructions.

Non-Esterified Fatty Acid (NEFA) Level Detection

The level of cellular glycogen was measured using the NEFA assay kit (Randox, cat: FA115) in accordance with the manufacturer's instructions.

Data analysis

SPSS 22.0 statistical software was used for data analysis. We utilized a Student's *t*-test to evaluate the statistical significance between the two groups. The measurement data were presented as the mean ± standard deviation, with $p < 0.05$ indicating statistical significance.

RESULTS

Effect of BmpP® on Cell Viability in HepG2 Cells

The results of the BmpP® treatment on HepG2 cells are presented in Figure 1. No significant differences were observed across the 5–80 μg/mL BmpP® treatments. Based on these findings, BmpP® concentrations of 40 and 80 μg/mL were selected for the subsequent PA-induced IR experiment.

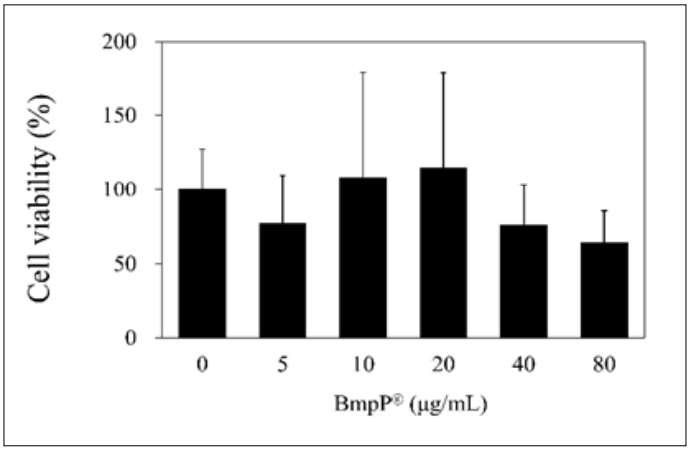

Figure 1. Effect of BmpP® on cell viability in HepG2 cells.

HepG2 cells were exposed to varying concentrations of BmpP® (5–80 μg/mL) for 48 hours. Cell viability was assessed using the CCK-8 assay, and the results were expressed as the mean ± SD (n = 3). There is no significant difference compared to 0 μg/mL.

Effect of BmpP® on Glucose Oxidase Activity in PA-Induced IR HepG2 Cells

The effect of BmpP® treatment on glucose oxidase activity in PA-induced IR HepG2 cells is presented in Figure 2. The results indicate that PA induction significantly reduced glucose oxidase activity in HepG2 cells. However, treatment

with 10 mM metformin (positive control) or 40 and 80 μg/mL BmpP® significantly increased glucose oxidase activity compared to the IR group.

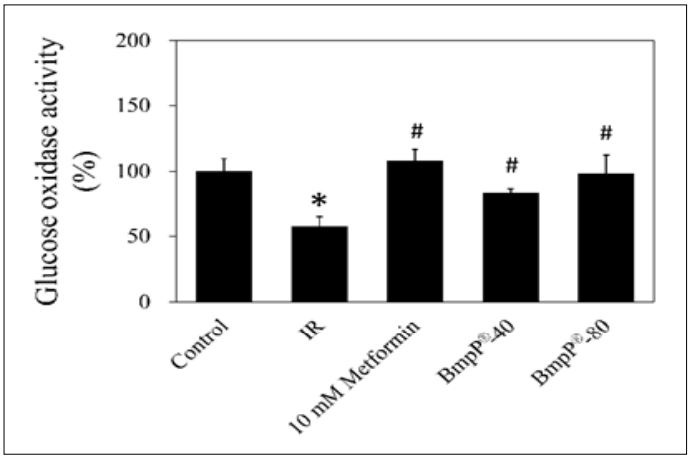

Figure 2. Effect of BmpP® on glucose oxidase activity in PA-induced IR HepG2 cells.

The insulin resistance (IR) was induced by 0.25 mM palmitic acid. HepG2 cells were exposed to BmpP® (40 and 80 μg/mL) or Metformin (10 mM) for 18 hours. The results were expressed as the mean ± SD (n = 3). * indicates a significant difference compared to the control group ($p < 0.05$). # indicates a significant difference compared to the IR group ($p < 0.05$).

Effect of BmpP® on Cellular Glycogen Level in PA-Induced IR HepG2 Cells

The cellular glycogen level in PA-induced IR HepG2 cells after BmpP® treatment is presented in Figure 3. PA induction resulted in a significant reduction in glycogen levels in HepG2 cells. However, treatment with 10 mM metformin (positive control) or 40 and 80 μg/mL BmpP® significantly increased glycogen accumulation compared to the IR group.

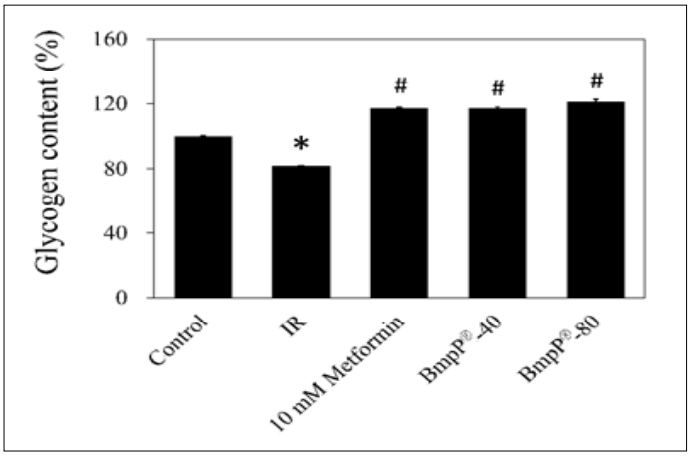

Figure 3. Effect of BmpP® on glucose oxidase activity in PA-induced IR HepG2 cells.

The insulin resistance (IR) was induced by 0.25 mM palmitic acid. HepG2 cells were exposed to BmpP® (40 and 80 μg/mL) or Metformin (10 mM) for 18 hours. The results were expressed as the mean ± SD (n = 3). * indicates a significant difference compared to the control group ($p < 0.05$). # indicates a significant difference compared to the IR group ($p < 0.05$).

Effect of BmpP® on Cellular NEFA Level in PA-Induced IR HepG2 Cells

Figure 4 illustrates the cellular NEFA levels in PA-induced IR HepG2 cells after BmpP® treatment. PA induction significantly increased NEFA levels in HepG2 cells. Treatment with 10 mM metformin (positive control) or 80 μg/mL BmpP® significantly reduced NEFA levels compared to the IR group.

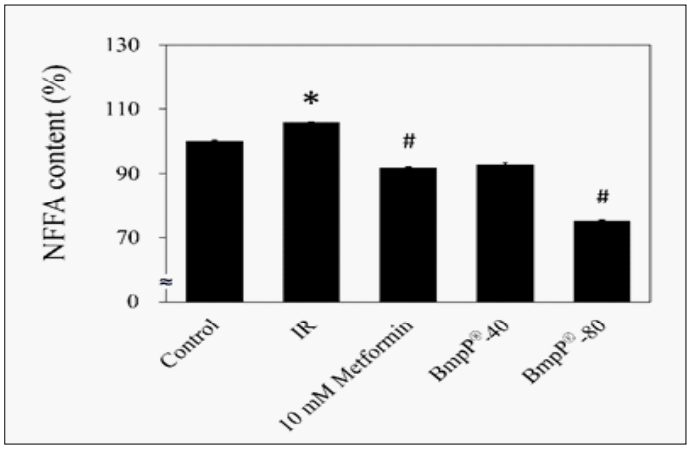

Figure 4. Effect of BmpP® on glucose oxidase activity in PA-induced IR HepG2 cells.

The insulin resistance (IR) was induced by 0.25 mM palmitic acid. HepG2 cells were exposed to BmpP® (40 and 80 μg/mL) or Metformin (10 mM) for 18 hours. The results were expressed as the mean ± SD (n = 3). * indicates a significant difference compared to the control group ($p < 0.05$). # indicates a significant difference compared to the IR group ($p < 0.05$).

DISCUSSION

Adipose tissue releases FFAs through increased lipolysis, leading to elevated circulating FFA levels. This contributes to the development of IR in muscle and liver tissues while also impairing insulin secretion from pancreatic β-cells (Veit, van Asten, Olie, & Prinz, 2022). Consequently, IR reduces glucose uptake in muscle cells, disrupts the suppression of hepatic glucose production, and promotes excessive glucose accumulation. These metabolic disturbances result in hyperglycemia and an increased influx of FFAs (Won, Choi, Kang, & Kim, 2021). In this study, an in vitro model of PA-induced IR in HepG2 cells was used to investigate the role of the BmpP® in glucose regulation. BmpP® was found to enhance glucose uptake, promote glycogen synthesis, and reduce NEFA accumulation, suggesting its potential to improve insulin resistance.

Bitter melon is a widely consumed plant in both Asia and Africa and holds an important role in traditional medicine, where it is commonly used to manage diabetes in the form of dried fruit chips or infused tea (Mohkami et al., 2024). Previous studies have demonstrated that a 12-week

supplementation of bitter melon extract in individuals with prediabetes did not show adverse effects such as fatigue, vaginitis, periodontitis, or colon polyps, confirming its safety for consumption and its potential to support blood glucose regulation (Kim et al., 2023). Another study reported that treating HepG2 cells with 100–1000 μg/mL of bitter melon extract for 12 hours did not significantly affect cell viability (Zhu et al., 2021). In this study, cell viability assays were conducted to evaluate the potential cytotoxic effects of the BmpP® on HepG2 cells (Figure 1). The results indicated that treatment with 5–80 μg/mL of BmpP® for 48 hours had no significant impact on HepG2 cell growth, suggesting its safety in this cellular model. Based on these findings, concentrations of 40 and 80 μg/mL were selected for further intervention in the IR model.

Glucose uptake and glycogen synthesis are key indicators of glucose metabolism, with their regulation playing a crucial role in hepatic glucose output. Studies have shown that impaired glycogen synthesis and increased gluconeogenesis contribute to hyperglycemia in insulin-resistant rats (Fan et al., 2019). This study induced IR in HepG2 cells with PA and evaluated BmpP®'s effects on glucose metabolism after 18 hours (Figures 2 and 3). The results showed that treatment with 40 and 80 μg/mL of BmpP® increased glucose oxidase activity by 46% and 71%, respectively, and enhanced glycogen content by 43% and 49%. These findings suggest that BmpP® improves glucose metabolism in a dose-dependent manner. Previous research has reported that PA-induced IR in HepG2 cells can be alleviated by bitter melon extract, with significant improvements in glucose consumption observed at 100 and 250 μg/mL, whereas 50 μg/mL showed no significant effect (Bai et al., 2020). Similarly, another study demonstrated that mulberry leaf extract at 100 and 250 μg/mL significantly enhanced glucose consumption in HepG2 cells, with 250 μg/mL also increasing glycogen content, while concentrations ranging from 0.5 to 50 μg/mL had no significant effect (Yan, Zhang, Zhang, & Zheng, 2016). These findings are consistent with our results; however, in this study, BmpP® at a lower concentration of 40 μg/mL was sufficient to enhance glucose uptake and glycogen synthesis. This suggests that the compound formulation may achieve metabolic benefits at a lower effective dose compared to single extracts.

As a primary insulin-sensitive organ, the liver plays a crucial role in regulating energy metabolism and maintaining lipid and glucose homeostasis. Elevated levels of FFAs have been implicated in the pathophysiology of IR in T2DM (Malik, Inamdar, Acharya, Goel, & Ghaskadbi, 2024). The findings of this study indicate that PA-induced HepG2 cells exhibit a significant increase in NEFA levels. However, treatment with 40 and 80 μg/mL of BmpP® reduced NEFA concentrations by 9% and 29%, respectively (Figure 4). Previous studies have demonstrated that supplementation with bitter melon extract significantly decreases blood glucose and plasma triglyceride levels in HFD/STZ-induced T2DM rats (K. Sun et al., 2023). Similarly, supplementation with mulberry leaf extract has been shown to reduce hepatic lipid accumulation in HFD-induced obese rats (Lee et al., 2020). Furthermore, gymnema leaf extract has been reported to mitigate FFA and TG accumulation in HFD-induced metabolically disordered hamsters (Li et al., 2024). However, research on the regulatory effects of bitter melon, gymnema leaves, and mulberry leaves on lipid metabolism in the PA-induced HepG2 IR model remains limited. In this study, 80 μg/mL of BmpP® effectively alleviated NEFA accumulation caused by IR, suggesting its potential to improve insulin sensitivity and prevent lipid deposition.

CONCLUSION

BmpP® has been shown to enhance glucose oxidase activity and glycogen content in PA-induced insulin-resistant HepG2 cells while reducing NEFA accumulation. These findings suggest that BmpP® possesses regulatory effects on blood glucose levels and ameliorates insulin resistance, making it a promising candidate for the development of a functional food supplement for glycemic control.

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