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INFLUENCE OF *Momordica charantia (L.)* ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF GLICLAZIDE IN ALLOXAN-INDUCED DIABETIC RATS MODEL

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ABSTRACT

Diabetes mellitus is a metabolic disorder present in the top ten diseases that cause death. Diabetes treatment usually uses conventional drugs, such as gliclazide, which is the first choice in patients with type 2 diabetes mellitus who are intolerant to metformin. In addition to conventional medicine, herbal medicines are in great demand and have become a focus of research to obtain an alternative treatment for type 2 diabetes mellitus. One herb that has been widely used and studied is the bitter melon. This study aimed to determine the effects of bitter melon extract (BME) on the pharmacokinetics and pharmacodynamics of gliclazide when used simultaneously. The study was conducted on three groups of rats with alloxan-induced diabetes. Group 1 received a single dose of gliclazide (33 mg/kg BW, n = 3), group 2 received aqueous extracts of bitter melon fruit (341 mg/kg BW, n = 4), and group 3 received a combination of gliclazide and an aqueous extract of bitter melon (n = 4). The pharmacokinetic profile of gliclazide is affected by BME, where interactions occur during the absorption phase. The blood glucose levels were measured using a glucometer. Decreased blood glucose levels following administration of gliclazide, extract of bitter melon, and a combination of gliclazide and extract of bitter melon 6 hours after dosing were 74.73%, 82.977%, and 86.457%, respectively. This study demonstrated the interactions between gliclazide and BME in the absorption phase of gliclazide and its effect on blood glucose levels.

Keywords: Gliclazide, *Momordica charantia*, Diabetes mellitus, Pharmacokinetics, Pharmacodynamics, Drug-Herbs Interaction

INTRODUCTION

A deficiency in insulin secretion, function, or both characterizes diabetes mellitus (DM), a group of metabolic illnesses defined by chronic hyperglycemia. Microvascular and macrovascular disorders can also occur. With the changing lifestyles and increased physical inactivity worldwide, its prevalence is increasing. In 2021, diabetes will affect 537 people worldwide, and the number is predicted to increase by 46% by 2045, so that the number of diabetics will reach 783 million people (International Diabetes Federation, 2021).

Currently, insulin and oral antidiabetics are used to treat DM. Gliclazide is a second-generation sulfonylurea antidiabetic drug that is the first choice for the treatment of DM in patients intolerant to metformin (Khunti *et al.*, 2020; Karla *et al.*, 2021). DM is a chronic disease whose treatment requires a long time; therefore, many DM sufferers use alternative treatments in the form of herbs or as a complementary treatment to DM therapy (Ilhan *et al.*, 2016; Joeliantina *et al.*, 2016; Grossman, Roscoe and Shack, 2018). Herbal medicines contain a variety of bioactive compounds and have multiple effects on insulin activity, insulin production, or both (Tran, Pham and Le, 2020).

One of the plants used for the treatment of diabetes is the bitter melon (Joseph and Jini, 2013). An in vitro study has shown that bitter melon can increase insulin secretion from pancreatic β-cells (Shimada *et al*., 2022). Bitter melons can also inhibit glucose reabsorption in the intestine, increase peripheral glucose utilization, and suppress gluconeogenic enzymes (Chang *et al.*, 2021). Momorcharin and momordicin are compounds contained in bitter melon that lower blood glucose because both have a chemical structure similar to that of insulin (Richter *et al.*, 2023). The use of herbs as complements in the treatment of DM must be considered because they can potentially cause side effects or interactions between herbs and antidiabetic drugs (Gupta *et al.*, 2017).

RESEARCH METHODS

Materials

Micronized gliclazide (Kimia Farma), sterile aquabides (IPHA Laboratories), urethane (Merck), Alloxan (Sigma Aldrich), 70% ethanol (Ikapharmindo), 0.9% sodium chloride (Otsuka), heparin 5000 IU/ml (Inviclot), acetonitrile pro HPLC (Merck), methanol pro HPLC (Merck), glucometer test strips (Easy Touch), bitter melon water extract (Materia Medika Indonesia).

Animals

Animal handling and all the surgical procedures described in this study were approved by The Research Ethics Committee Padjadjaran University and followed the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). Male Wistar rats aged 8–12 weeks (Animal Laboratory, PT. Biofarma, Indonesia), with a weight range of 180–300 g, was used. All rats had free access to food and water and were acclimatized under a 12-h light/12-h dark cycle at a temperature of 20-25°C and relative humidity of 48-52% for at least 5 days prior to the experiment. A diabetic type 2 rat model was established by intraperitoneal injection of alloxan monohydrate (140 mg/kg BW). After 72 hours blood sugar levels were assessed, and rats with fasting blood sugar levels > 250 mg/dL were chosen for the experiment (Ighodaro Adeosun and Akinloye, 2017).

Pharmacokinetic Studies

Alloxan-induced diabetic rats were divided into 3 groups. Group 1 received a single dose of gliclazide (33 mg/kg BW, n = 3), group 2 received BME (341 mg/kg BW, n = 4), and group 3 received a combination of gliclazide and BME (n = 4).

Rats that had fasted for eight–ten hours the previous night underwent surgery (Jespersen Knupp and Northcott, 2012). A 1.25 g/kg intraperitoneal injection of urethane (250 mg/mL in physiological saline) was used to induce general anesthesia. Rats were placed under deep anesthesia and underwent minor surgery to isolate the femoral vein and artery, followed by insertion of polyethylene cannulas (Microtube Extrusions, NSW, Australia; PE8050 and PE6128 connected to PE9650 for the femoral vein and artery, respectively). The cannula inserted into the femoral artery was used to draw blood, while the cannula in the vein was used to inject gliclazide and administer physiological saline (to restore fluid loss). Urethane was administered intravenously. At the end of the experiment, the rats were euthanized by administering high doses of urethane (Adiwidjaja and Sasongko, 2021).

Blood samples from all groups of rats were collected via the femoral artery at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 8, 12, 18, and 24 h after drug administration. Blood samples taken at each sampling point amounted to 0.3 ml. After each blood draw, the tubing was flushed with 50 IU of heparin solution. The blood was placed in a microcentrifuge tube containing 10 µL of 5000 IU heparin solution. The sample was vortexed for 30 seconds and centrifuged for 3 minutes at 12,000 rpm. Plasma was taken and stored in the freezer -20°C.

Plasma samples were removed from the freezer at-20°C, allowed to thaw at room temperature, and then vortexed for 30 seconds. Plasma (50 µL) was placed in a microcentrifuge tube, 100 µL of pro-HPLC acetonitrile was added for protein precipitation, the sample was vortexed for 30 seconds and then centrifuged at 12,000 rpm for 10 minutes.

The supernatant was separated and 50 µL of the supernatant was injected into the HPLC system. Separation by HPLC (Jasco) was carried out at room temperature using a C18 Luna column (250 mm x 4.6 mm i.d., 5 µm, Phenomenex, USA), 0.01M phosphate buffer mobile phase pH 2.7 (A): acetonitrile (B), and isocratically eluted at a ratio of 35:65. The elution time was 7 minutes with a flow rate of 1 ml/min. Injection volume 50 µl. The chromatogram was monitored using a UV detector at a wavelength of 229 nm.

Pharmacodynamic Studies

Plasma (10 µL) was added to 1 ml of working solution from the GOD-PAP kit, vortexed for 10 seconds then incubated at 37°C for 20 minutes. After that, it was put into a semi-micro cuvette tube. Absorbance was determined using a colorimetric method at a wavelength of 500 nm.

RESULTS AND DISCUSSION

Momordica charantia fruits were purchased from local markets in Bandung, and identification was confirmed by Herbarium Jatinangor, Padjadjaran University, and extracted by Materia Media Indonesia. The experimental protocol was approved by the Ethics Committee (protocol no. 1348/UN6).KEP/EC/2018) by the Research Ethics Committee of Padjadjaran University. In this study, the samples were prepared to unitize protein precipitation (PP), because PP is the most commonly used sample preparation method for drug discovery and pharmacokinetics, which is the simplest method that involves little method development and eliminates most of the protein from the sample (Alshammari *et al*., 2015).

Male Wistar rats aged 8–12 weeks. The advantages of rats include their small size, ease of maintenance, brief life cycle, and extensive genetic resources, allowing the outcomes of investigations using humans as subjects to be connected or contrasted. Rats have traditionally been the preferred species for biomedical research animal models because of their anatomical, physiological, and genetic resemblance to humans (Bryda, 2013). The alloxan induction method was used to establish a rat model of diabetes mellitus. Alloxan's selective suppression of glucose-stimulated insulin secretion by the inactivation of glucokinase and selective necrosis of the beta cells via generated ROS highlight its diabetogenic effects (Ighodaro, Adeosun and Akinloye, 2017). The alloxan dose of 140 mg/kg BW for induction-stable diabetes resulted in rats with blood glucose levels > 250 mg/dL (Rahman *et al*., 2017).

Pharmacokinetic Studies

The curves of plasma gliclazide concentration versus time for the group administered a single dose of gliclazide and the group administered gliclazide in combination with BME in alloxan-induced diabetic rats are shown in Figure 1, while the curve on a logarithmic scale is shown in Figure 2.

In a previous study conducted by Adiwidjaja and Sasongko (2021), the pharmacokinetic profile of gliclazide followed two-compartment oral kinetics, either in the gliclazide group administered alone or in the gliclazide group co-administered with herbs. Two-compartment modeling more accurately describes the profile of gliclazide because the concentration of gliclazide in the plasma decreases biphasically or in two phases. where the first and second phases are the distribution and elimination phases, respectively.

This has also been proven in a study conducted by Miyazaki *et al.* (1983), who used radioactivity to determine the disposition of gliclazide, and found that gliclazide decreased biphasically after absorption. In this study, the pharmacokinetic parameters were difficult to determine because of the fluctuations in the curve. It can be seen from the rough picture that

the decrease in plasma gliclazide occurred biphasically in either the single gliclazide administration curve or co-administration with bitter melon aqueous extract.

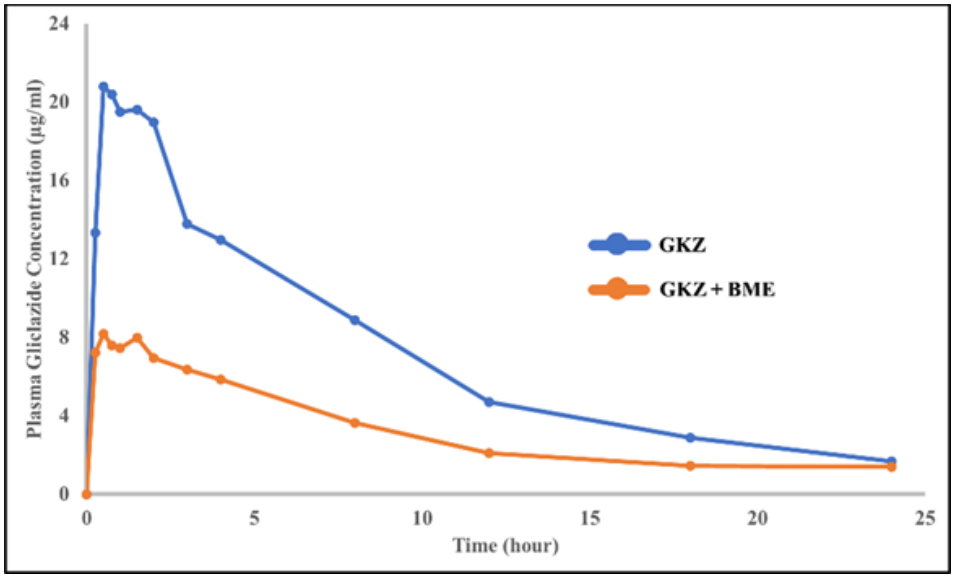

Figure 1. Curve of plasma gliclazide concentration

In direct observation, the tmax values were obtained for the single gliclazide administration group and the group given together with BME, which were approximately half an hour or 30 minutes after the rats were given the drug. These results were faster than those of previous studies, which stated that the tmax of gliclazide occurred 1-2 hours after drug administration (Adiwidjaja and Sasongko, 2021). In addition, it can be seen that the maximum plasma gliclazide concentration (Cmax) in the group given gliclazide together with BME only reached half of the Cmax in the group given gliclazide alone. This indicated that there was a pharmacokinetic interaction between gliclazide and BME. This interaction is thought to occur in the absorption phase because the concentration of absorbed gliclazide is only about half, and it can also be observed that in the distribution and elimination phases, the pattern of decline is almost the same; therefore, it is suspected that there is no interaction between the two phases.

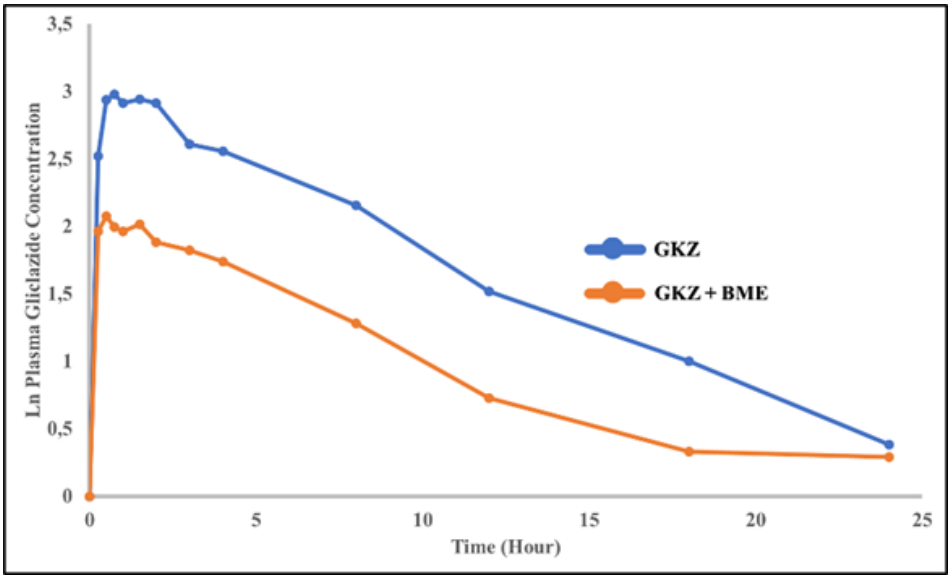

Figure 2. Curve of plasma gliclazide concentration in logarithmic scale

In this study, the BME used was an aqueous extract of bitter melon fruit, although in the market there is more of it in the ethanol extract of bitter melon. This is because, in a previous study conducted by Pratiwi and Sasongko (2019), it was stated that the water extract of bitter gourd had better activity in lowering blood glucose levels compared to the ethanol extract, where the water extract of bitter gourd gave a percent reduction in blood glucose levels of 61.04%, while the extract bitter gourd ethanol only provided a decrease in blood glucose percentage of approximately 15.07%. This study was reinforced by other studies conducted by Virdi *et al.* (2003) and Yadav *et al.* (2010), who found that the antihyperglycemic activity of aqueous BME was better than that of the bitter melon ethanol extract.

Pharmacodynamic Studies

In this study, pharmacodynamic studies were carried out on rats that were divided into 3 groups, namely group 1 alloxan-induced diabetes rats that were administered a single dose of gliclazide (p.o), group 2 alloxan-induced diabetes rats that were administered a single dose of BME (p.o), and group 3 alloxan-induced diabetes rats that were administered a single dose of gliclazide and BME (p.o). Pharmacodynamic testing showed that all groups had a significant decrease in blood glucose levels. Changes in glucose levels are shown in **Figure 3**.

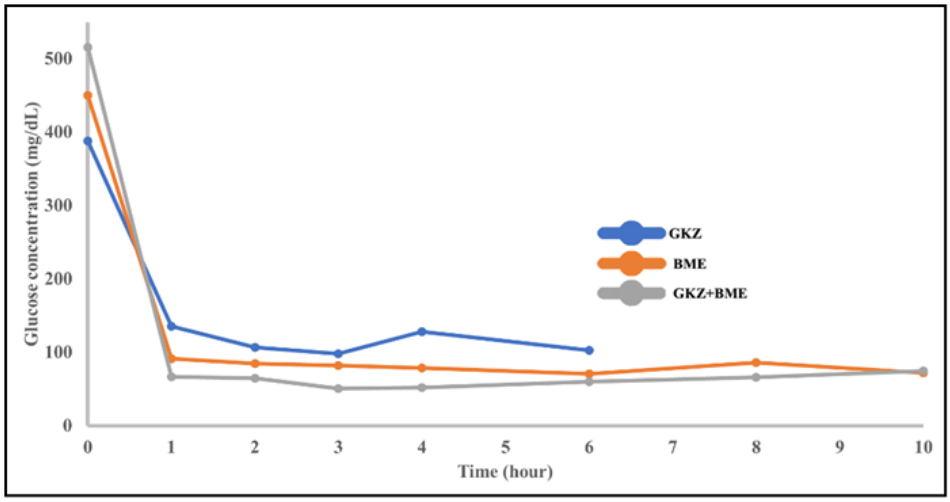

Figure 3. Curve of plasma gliclazide concentration in logarithmic scale

The profile of decreased blood glucose levels in insulin-resistant rats administered a single dose of gliclazide showed a very sharp decrease, especially in the first hour after administration of the drug. The sulfonylurea group causes hypoglycemic shock, but gliclazide as a 2nd generation sulfonylurea lower causes severe hypoglycemia (Bolanle Ademolu, 2019). In another study by Landman *et al.* (2014), hypoglycemic events were observed in 2387 diabetic patients using gliclazide; only 25 hypoglycemic events were mild.

Gliclazide stimulates insulin secretion via β-cell sulfonylurea receptors (Seino *et al.*, 2017) and inhibits pancreatic β-cell adenosine triphosphate-dependent potassium channels (Hu and Jia (2019). In this way, gliclazide acts to increase abnormal first-phase insulin release in type 2 diabetes and also has an effect on the second phase. This pattern of insulin release is thought to result in lower hypoglycemia levels.

Administration of BME resulted in a reduction in blood glucose levels similar to that of gliclazide, and the percentage decrease could offset the percentage decrease in blood glucose in the single gliclazide group. Some of the mechanisms responsible for the antihyperglycemic action of bitter melon are facilitating fatty acid transport and fat catabolism in tissues and increasing carnitine palmitoyltransferase (CPT) and acyl-CoA dehydrogenase enzyme systems in the mitochondria, which can increase fatty acid oxidation.

Components of bitter melon water extract, such as insulin-like peptides, carotenes, and alkaloids, exert a hypoglycemic effect with insulin sensitivity. In patients with T2DM, there is an excessive expression of CPT-1, cytokine-3 signaling, cJun N-terminal kinase (JNK), and Akt at protein and mRNA levels in the liver, which can increase insulin resistance. In addition, the components of BME have also been shown to reduce leptin and resistin levels in adipose tissue, which can reduce insulin resistance (Vani, Chekka and Mantipelly, 2020).

Research on obese animal models has shown that BME can reduce body weight and fat accumulation, and obesity is an early sign of T2DM (Dwijayanti *et al.*, 2020). A recent study also demonstrated that BME can increase adipocyte death via cyclic adenosine monophosphate-activated protein kinase-mediated apoptosis in white adipose tissue. In addition, BME reduces fat accumulation during the differentiation process (from preadipose to adipose tissue) and downregulates peroxisome proliferator-activated receptor γ (PPARγ), a transcription factor that affects insulin sensitivity and glucose metabolism by regulating the expression and secretion of adipocytokines., which includes adiponectin levels. This suggests that the mechanism of action of BME is similar to that of thiazolidine, which is anti-diabetic and specifically works as a PPAR-γ ligand that can increase glucose uptake and adiponectin secretion in 3T3-L1 adipocytes. In addition, BME can also increase adiponectin release by 75% after 1 hour of administration, and by decreasing PPAR-γ regulation, BME can improve lipid profiles and reduce blood glucose levels (Yuan, Chen and Li, 2015).

In T2DM patients, dyslipidemia is a risk factor for insulin resistance, but furthermore, dyslipidemia in diabetes can be caused by the release of free fatty acids in insulin-resistant adipose cells (Moreno-Navarrete and Fernández-Real, 2019). BME has a lipid-lowering effect in animal and human studies and can reduce hepatic and serum total cholesterol and triglyceride levels and increase high-density lipoprotein cholesterol concentrations in the serum (Doosti-Moghaddam *et al.*, 2022). In addition, BME can also reduce oxidative stress by increasing the activity of glutathione peroxidase and superoxide dismutase enzymes; therefore, BME may be effective in reducing complications from diabetes (G *et al.*, 2017).

The decrease in blood glucose in the group administered gliclazide together with a single dose of BME showed a pattern of decrease similar to that of a single dose of gliclazide and a single dose of BME, where hypoglycemic shock occurred after one hour of administration. There was also an increase in the decrease in blood glucose levels when compared to single doses of gliclazide and single doses of BME. This allows herbal interactions with conventional drugs because the receptors work together to cause synergistic, antagonistic, and additive effects. Based on the results obtained, it is suspected that there is a synergistic interaction in which the combination is given. Synergistic drug effects can cause serious or even fatal problems.

The parameter used to calculate the magnitude of the decrease in blood glucose levels, regardless of the onset and duration of action, is the curve (AAC) of glucose levels over time. The AAC values for a single dose of gliclazide, a single dose of BME and a single dose of a combination of gliclazide and BME respectively were 1448.33±85,56 ; 1913±530,82 and 2568.87±464,24 mg.hour/dL. Based on the AAC results, the antihyperglycemic effect of gliclazide changed significantly ($P<0.05$) when combined with BME. Single-dose gliclazide showed a decrease in blood glucose levels of 74.73%, which increased to 86.457% after being combined with BME. These results indicate that there is an interaction that occurs pharmacodynamically because the decrease in blood glucose levels can be increased when gliclazide is administered together with BME; however, when compared to the percentage decrease in the group administered BME (82.977%), there was no significant difference.

The results of calculating insulin levels for the group administered a single dose of gliclazide, a single dose of BME, and a single dose of a combination of gliclazide and BME were respectively 10,18±0,99; 3,57±1,77; 7,06±1,52 µU/ml. The gliclazide and combination groups showed normal insulin levels, whereas the normal fasting serum insulin levels were 5-20 µU/ml. If the serum insulin level is > 20 µU/ml, it can be classified as T2DM, which

does not receive treatment. It can be concluded that the use of gliclazide, gliclazide, and BME can increase insulin sensitivity, so that the serum insulin levels of rats induced by diabetes can return to normal levels.

CONCLUSION

There was a pharmacokinetic interaction between gliclazide and bitter melon aqueous extract, which caused the absorption of gliclazide to be halved compared with the single administration of gliclazide. Bitter melon aqueous extract can be used as a companion treatment for patients with diabetes mellitus who use gliclazide as the primary therapy.

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