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Bitter Gourd (*Momordica charantia* L.) Affects the Pharmacokinetics Profile of Metformin in Rabbits' Plasma

Asri Dwi Endah Dewi Pramesthi[1], Endang Lukitaningsih[2], Agung Endro Nugroho[3]

Master Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia[1]
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, Yogyakarta 55281, Indonesia[2]
Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Sekip, Utara, Yogyakarta, 55281, Indonesia[3]

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Drug-herb interaction;
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Metformin; Bitter Gourd;
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ABSTRACT

The drug-herb combination between metformin and bitter gourd needs further study of the benefits and risks. This study aims to explore metformin in plasma samples using the HPLC method and its pharmacokinetic profiles affected by the bitter gourd. This experimental method study with a post-test randomized controlled group design. Healthy albino rabbits were divided into three groups (n=3). They were administered with bitter gourd juice 100% (4 ml/kg BW) and metformin (BM1), bitter gourd juice 50% (4 ml/kg BW), and metformin (BM2), and metformin 26 mg/kg (M). BM1 and BM2 were given bitter gourd juice for 14 days, and then a single dose of metformin was given to all groups on the 15th day before metformin pharmacokinetic parameters were measured. Blood samples were collected from marginal ear vein punctures at 0, 10, 30, 60, 120, 240, 360, and 480 minutes. The plasma was analyzed using HPLC methods, and the concentration vs. time was used for a 1-compartmental open model pharmacokinetics analysis. Bitter gourd juice with 100% (4 ml/kg BW) concentration decreased the V/F and CL/F, also increased Ka, $T^1/_2$, C_{max}, MRT, and $AUC_{0\text{-}inf}$, also significantly increased $AUC_{0\text{-}480}$, and decreased T_{max} ($p < 0.05$). The pharmacokinetic interaction of metformin and *Momordica charantia* L. is presumably because of the competitive interaction between phytochemical constituents of bitter gourd and metformin on the OCT and MATE transporter.

1. Introduction

Drug-drug (DDI) and drug-herb interactions (DHI) can trigger various risks, good or bad, and even cause death. The complexities involved in the phytochemicals of herbs and the different potential targets become a significant challenge of the research [1], [2]. DHI must be detected earlier and studied further during the

early stages of herb-drug development. Research on DHI has been documented in several herbal medicines, such as Traditional Chinese Herbal Medicines (TCM), Western Herbal Medicines, and Ayurveda Medicines [3]. TCM used with drugs could affect the pharmacokinetic profile and pharmacodynamics activity. It can be affected by various pathological and physical conditions [4].

Metformin, a strong base from the biguanide group, is a protonated cation in physiological pH. It has high hydrophilicity and can be transported to membranes via organic cation transporters (OCT) [2]. Metformin is slowly absorbed in the proximal small intestine, and the higher the dose could decrease its absorption and bioavailability. Plasma concentrations of metformin depend on the quantity and rate of absorption. Concentrations will be higher in the hepatic portal vein and liver [5], [6]. Metformin is not metabolized once distributed nor binds to plasma proteins and will be excreted in its active form. The distribution and elimination process of metformin depends on OCT, so the presence of competitive interactions and polymorphisms in OCT will affect metformin transport [2].

Various DHI between metformin and herbs in pharmacokinetics have been carried out. For example, metformin and Nisha Amalaki, a combination of turmeric and malacca, could increase metformin's Cmax, AUC, and Tmax values ld reduce the Vd, K_{el}, and Cl values of metformin [7]. Phytochemicals constituents in malacca (*Phyllanthus emblica*) include vitamin C, chebulagic acid, coumaric acid, caffeic acid, gallic acid, and ellagic acid reported to have antidiabetic activity. The mechanism of action is similar to that of metformin, which is in the AMPK activity pathway [8]. Another example of a drug-herb combination, such as Green Rooibos Extract (GRT) and *Cassia auriculata*, can reduce the C_{max}, T_{max}, and AUC values of metformin [9], [10]. Alkaloids berberine are known to affect the pharmacokinetics of metformin. Berberine competitively inhibits the uptake of prototypic cations on tetraethylammonium and 1-methyl-4-phenylpyridinium, OCT substrates. Berberine also significantly inhibited the OCT1- and OCT2-mediated uptake of metformin observed in HEK293/OCT1 and HEK293/OCT2 cells [11], [12].

Bitter gourd contains various phytochemicals such as vitamins, minerals, flavonoids, phenolic compounds, saponins, peptides, and alkaloids [13]. The antidiabetic efficacy and mechanisms of bitter gourd play a role in increasing glucose uptake and utilization through the PPARα and PPARγ pathways, increasing glucose biogenesis and translocation through GLUT4, inhibiting glucose reabsorption in the jejunum and kidney, and directly or indirectly stimulating the AMPK pathway to decrease gluconeogenesis [14]. Thus, the pharmacodynamic interactions of metformin and bitter gourd can be predicted based on a similar mechanism of action.

The alkaloids in bitter gourd, momordicine, chorine, and vicine [15], interact with metformin on the OCT works for metformin transport. Organic cation transporters (OCTs) are found in the intestines, kidneys, liver, and brain. Alkaloids act as both substrates and inhibitors at OCT, so when consumed with compounds that OCT also facilitates, it is suspected to affect the activity of the compounds [12], [16]. Bitter gourd contains several phytochemicals similar to *Phyllanthus emblica* and *Cassia auriculata*, which is one of the reasons for alleviating the pharmacokinetic profile of metformin.

The concentration of metformin in plasma needs to be known and time points to analyze the pharmacokinetic parameters [17]. The analysis of metformin could be done with HPLC with a 1-compartmental open model analysis that has widely been used. One-compartmental open model is the most uncomplicated modeling with a single disposition phase.

2. MATERIALS AND METHODS

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2.1 Materials and Instrumentations

Bitter gourd from Desa Sedayu, Muntilan, Magelang, East Java, metformin HCl (99.5% purity, from PT Phapros), atenolol (100.0% potency, from EP), acetonitrile (grade HPLC, from JT Baker), potassium dihydrogen phosphate (for analysis, from Merck), sodium dodecyl sulfate (for analysis, from Merck), and water (grade aquadest, from PT. Brataco).

HPLC (Hitachi D-2000), pH meter (Ohaus STATER 3100), analytical balance (Ohaus PAJ1003), semi-micro balance (Ohaus Explorer EX225D), Centrifuge (Dynamic Velocity 18R), Column (Comosil C18, 250 x 4.6 mm, 5 µm). As for the chromatographic conditions, HPLC with detector UV λ 233 nm, flow rate 1.0 ml/minute, loop injection 20 µl, needle wash with water: methanol (90:10), and run time for 13 minutes.

2.2 Methods

2.2.1 Extraction

Bitter gourd was used with the prior approval of the Pharmaceutical Biology Department, Faculty of Pharmacy, Universitas Gadjah Mada (No. 21.21.06/UN1/FFA/BF/PT/2021). One kg of fresh fruit was mashed using a Kirin KJE-K98 *juice extractor*. It was filtered, and the supernatant was 100% juice. Bitter gourd juice was mixed in the water (1:1) to get a 50% juice concentration.

2.2.2 Laboratory and Animal Groupings

In this experimental study, nine male Albino rabbits weighing 2.5-3.0 kg (4-5 months) were obtained from a local farmer in Jetis, Bantul, Yogyakarta, Indonesia, and maintained with free access to similar *ad libitum* food and water. Rabbits were used with the prior approval of the Animal Ethics Committee, LPPT, Universitas Gadjah Mada (No. 00012/04/LPPT/VI/2021).

Rabbits were divided into three groups (n = 3), BM1 and BM2 groups were given the 100% (4 ml/kg BW) and 50% (4 ml/kg BW) juice orally for 14 days. On the 15th day, metformin (26 mg/kg) was given orally to all groups, and blood samples (1.5 mL each) were collected at 0, 10, 30, 60, 120, 240, 360, and 480 minutes. Blood samples were collected from marginal ear vein punctures using a NAF vacutainer. The blood samples were centrifuged for 10 minutes at 5000 rpm to isolate plasma and were stored at -20 ^{o}C until the samples were analyzed [16], [18].

2.2.3 Sample Plasma, Standard, and Concentration Series Analysis Procedure

Sample plasma was spiked with metformin 1000 ng/ml standard solution, and the internal standard solution was injected into the chromatography system for each validation parameter. The chromatograms were recorded, and the areas of the peak responses were analyzed for system suitability requirement and metformin concentration measurement [19], [20].

2.2.4 Data Analysis

Every quantitative data from each group were analyzed by Microsoft Office Excell 2016. Qualitative data supported the main data stated in tables, graphs, diagrams, and figures. Pharmacokinetic parameters were analyzed with One-Compartmental Open Model Analysis with PK Solver 2.0 add-ins in Microsoft Office Excell 2016. Its parameters and unit were ka (1/min), V/F (mL/kg), CL/F (mL/min/kg), $T^1/_2$ elimination (min), Cmax (ng/mL), Tmax (min), MRT (min), AUC0-480 (ng.min/mL), AUC0-inf (ng.min/mL). Statistical analysis was done with the ANOVA method on IBM SPSS Statistics 22.

3. FINDINGS AND DISCUSSION

HPLC method was used in this study to determine metformin assay in plasma. Atenolol was used as an

internal standard, and partial validation was carried out, including system suitability, calibration curve performance, accuracy, and precision. Based on the g, method validation should be performed entirely or partially to demonstrate the reliability of particular analysis methods. This study did not perform full validation because the analytical methods were fully validated before, including selectivity, the lower limit of quantification (LLOQ), calibration curve performance, accuracy, precision, and stability [19].

The system suitability requirement for assay determination of metformin in plasma was studied by analyzing standard solutions. The equipment, electronics, operational analytics, and samples must be evaluated. It was done by optimizing the method based on the acceptance of selectivity, sensitivity, and chromatographic parameters. It could be seen based on the produced peaks in peak sharpness, symmetry, tailing factor, and resolution results between two peaks, it could be seen [21]. The results shown in Table 1, the system met specification by %RSD of retention time was 0.21 with 0.66 area of metformin standard and retention time was 0.16 with 0.47 area of atenolol, asymmetry or tailing factor result was less than 2, theoretical plates were more than 2000, and resolution result was 5.03. The validation methods stated that system suitability meets the specification if %RSD area and retention time for six replicate injections of standard solution is ≤ 2%, tailing factor for the main peak is ≤ 2%, the theoretical plate is ≥ 2000, and resolution is ≥ 2.0 [19].

Table 1. System Suitability Test (SST) as part of system methods validation for metformin (1 ppm) and atenolol (1 ppm) in plasma to evaluate the system before analyzing the samples

No	**RT of metformin**	**Asymmetry of metformin**	**Area of metformin**	**N plate of metformin**	**RT of atenolol**	**Asymmetry of atenolol**	**Area of atenolol**	**N of atenolol**	**Resolution**
standard-1	6.13	1.23	80040	8134	7.66	1.2	23920	10059	5.31
standard-2	6.13	1.19	79750	8164	7.66	1.21	24075	10045	5.29
standard-3	6.13	1.2	79644	8138	7.66	1.21	24130	-	4.49
standard-4	6.14	1.18	79710	8113	7.67	1.18	24114	-	4.48
standard-5	6.15	1.21	79085	8188	7.68	1.18	24275	9965	5.29
standard-6	6.16	1.2	78606	8236	7.69	1.23	24106	10095	5.31
average	6.14	**1.20**	79472.50	**8162.17**	7.67	**1.20**	24103.33	**10041.00**	**5.03**
SD	0.01		526.47		0.01		113.77		
RSD	**0.21**		**0.66**		**0.16**		**0.47**		

%RSD area and retention time for all replicate standards are less than 2.0% (0.21 for metformin and 0.47 for atenolol). The tailing factor or asymmetry of the main peak is not more than 2.0% (1.20 for both metformin and atenolol). The theoretical plates are not less than 2000 (8162.17 for metformin and 10041.00 for atenolol). The resolution should not be less than 2.0, and the result showed 5.03 for the resolution. The data demonstrate the system's suitability within acceptance criteria, so the system was suitable.

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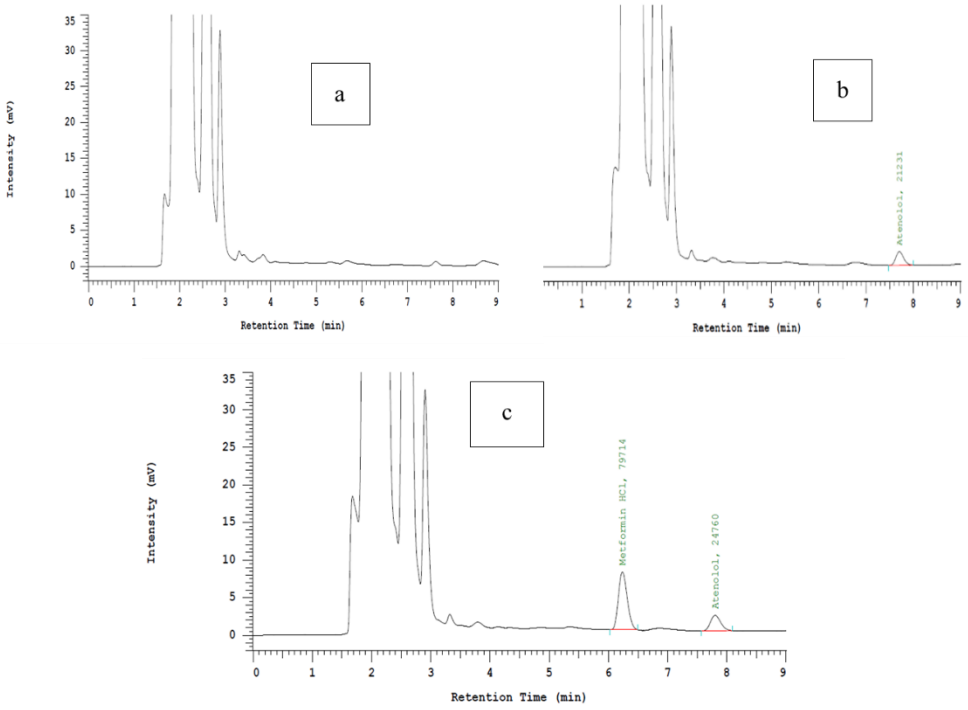

Figure 1. External calibration curved from chromatograms of metformin over chromatograms of atenolol with metformin concentrations in plasma

Analytical methods should have acceptance criteria for analysis sample study, such as accuracy for recovery analysis and precision to analyze the sample repeatedly. The accuracy and precision tests were performed with two replications of three different concentrations of metformin, namely Low QC-1, Low QC-2, Med QC-1, Med QC-2, High QC-1, and High QC-2. As stated in the guideline, accuracy and precision must meet the Guideline on Bioanalytical Method Validation requirement. Accuracy and precision are part of quality control before analyzing the samples. Metformin solution standards were spiked into the plasma at four concentration levels which cover the calibration range: LLOQ, within three times the LLOQ (Low QC), around 30-50% calibration curve range (Medium QC), and at least 75% of the upper calibration curve range (High QC). The accuracy study meets the specification if the mean concentration should be within 15% of the nominal values for the QC samples, except for the LLOQ, which should be within 20% of the nominal value. At the same time, the precision meets specification if the coefficient variation (CV) value should not exceed 15% for the QC samples, except for the LLOQ, which should not exceed 20% [19]. The results of QC samples shown in Table 2 for Low QC were 13.54, Med QC was 2.72, and High QC was 0.25. All of the results met the criteria since the results were less than 15%. After the system was confirmed by the suitability test and quality control analysis, plasma samples were analyzed to get the plasma concentration of metformin vs. the sampling times.

Table 2. Percentage of concentration of metformin chromatograms from the study as part of accuration and precision analysis of the methods before analyzing the samples

No	Nominal Concentration (ng/ml)	Metformin area	Atenolol area	Ratio	Obtained concentration (ng/ml)	% diff	% recovery
Low QC-1	44.7929	3883	24845	0.1563	47.3624	5.74	105.74
Low QC-2	44.7929	3675	28186	0.1304	39.0865	-12.74	87.26

Average							96.4984
CV							13.0645
% CV							**13.54**
Med QC-1	497.6990	38632	23989	1.6104	511.9109	2.86	102.86
Med QC-2	497.6990	39439	23569	1.6733	532.0176	6.90	106.90
Average							104.8755
CV							2.8567
%CV							**2.72**
High QC-1	746.5485	58955	23190	2.5423	809.6119	8.45	108.45
High QC-2	746.5485	57568	22566	2.5511	812.4344	8.83	108.83
Average							108.6364
CV							0.2673
%CV							**0.25**

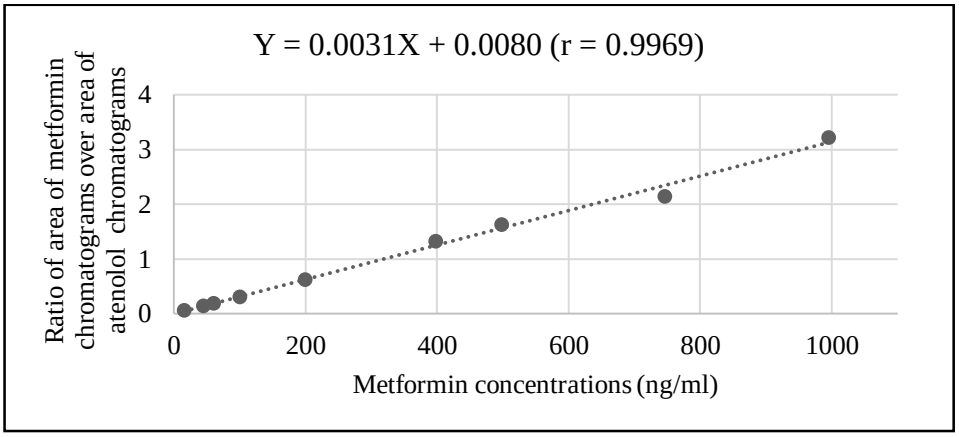

Figure 2. Chromatogram from HPLC analysis. (a) blank plasma, (b) zero sample consisting of Atenolol (RT 7.71 and area 21231) as internal standard, (c) Metformin HCl 1000 ng/ml (RT 6.23 and area 79714) and Atenolol (RT 7.80 and area 24760) as internal standard. KH2PO4 + SDS pH 6.0 and ACN as the mobile phase.

The analyte's instrument response must be evaluated over a specific concentration range before sample analysis. The calibration concentration range must be within six concentrations and can be replicated [20], [22]. The mobile phase was used as a solvent for every sample to minimize the noise and eliminate the unwanted solvent peaks [21]. This internal standard curve of metformin by the linearity analysis was used for quantitative analysis of metformin within rabbits' plasma [23]. The system methods had been evaluated and met the required criterion, indicating that analysis of the samples was ready to be done reported in **Error! Reference source not found.**. The system was precised by repeatedly showing the differentiation between the samples and internal standard with no intervention of the sample's chromatogram by the chromatogram of the internal standard. The previous study stated that metformin reached its maximum concentration two to 3 hours after the given dose. Based on this study, metformin reached its maximum concentration at 120 minutes or 2 hours after a single dose was given [24], [25]. Profile exposure time must be considered essential and not based on clinical application. If it is necessary for rapid-acting, T_{max} must be achieved faster. For chronic diseases, such as diabetes mellitus, it's crucial to maintain the plasma

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concentration above the defined measurable minimum pharmacologic response [26]. To assess differences in exposure by measuring AUC, $C_{max,}$ and $T_{max.}$ The result showed that the AUC and C_{max} of BM1 are higher than BM2 and M groups. T_{max} of BM1 group achieved faster than BM2 and M group. These results indicated that bitter gourd exposure for 14 days affected metformin exposure while given on the day 15^{th}. This result should be considered to adjust the dose of metformin combined with bitter gourd since prolonged exposure to the drug might increase the adverse effects.

As seen in Figure 3, the curved of metformin increased in BM1 compared with other groups. Physiologic and physicochemical factors, surface area and motility of gastrointestinal tract, metabolism of intestine microflora, and the blood flow in absorption site that vary might determine drug transport through the membrane. It affects the rate of absorption and the number of absorbed drugs [27]. Across the membrane, a substance's movement depends on its molecular size, lipophilicity, charge, degree of ionization, blood flow, and protein binding [28], [29]. Metformin absorption predominantly occurs in the small intestine by microvilli [30], [31]. Metformin is a weak acid that would easily absorb in weak acid conditions or the small intestine [32]. Bitter gourd fruit juice given for 14 days might affect the gastrointestinal pH and increase the absorption of metformin in the GI tract. Bitter gourd fruit juice pH was around 5.1 to 5.4 in range, which were weak acid might change the pH of the gastrointestinal tract [33].

As the AUC and C_{max} increased after bitter gourd fruit juice 100% were given, the half-life elimination and mean residence time increased. Extending the concentration of metformin in plasma reduced the clearance, distribution volume, and elimination rate constant. Table 3 and Figure 3 show that the previous parameters correlated with the result. It might be because of the interaction between metformin and bitter gourd in the body. Drug-herb interaction mainly occurs in the metabolism phase. Since metformin is un-metabolized in the body and excreted in its active form [24], metformin and bitter gourd do not interact in this phase.

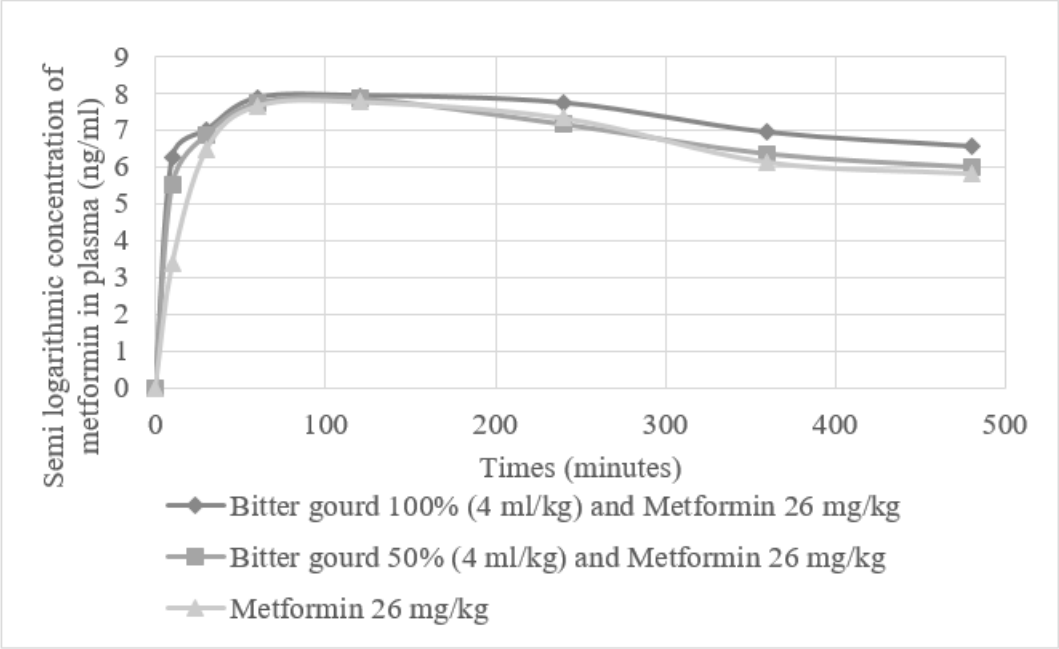

Figure 3. Semi-logarithmic curved of average metformin concentration in plasma (ng/ml) over time (minutes) after given bitter gourd juice with 100% (4 ml/kg BW) concentration and 26 mg/kg of metformin (for the first group), given bitter gourd with 50% (4 ml/kg BW) concentration and 26 mg/kg of metformin (for the second group), and given 26 mg/kg of metformin only (for the third group). Bitter gourd juice was administered for 14 days before being given metformin on the fifteenth day before sampling was done for

analysis.

The alteration of primary parameters in the BM1 group would affect the secondary and derived parameters. Primary parameters depend on biological factors like biochemistry and anatomic also could be affected by age, genetics, gender, pathological condition, etc [27]. Clearance and volume distribution was vital in defining secondary and derived parameters such as the area under curved, plasma half-life, and mean residence time. AUC alteration in the BM1 group is affected by its clearance. While metformin clearance decreased in the BM1 group, the AUC value increased and vice versa in the M group. Mean residence time and plasma half-life of metformin in the BM1 group increased due to V/F and CL/F alteration. Alteration of Vd/F in the BM1 group affected the maximum concentration of metformin in plasma (C_{max}).

The mechanism, presumably because of its phytochemical constituent with metformin on their transporters to transport metformin through the cell [1], [12], [34]. In this study, metformin was measured in rabbits' plasma, and the interaction might occur mainly in OCT transporters. Competitive interaction between metformin and bitter gourd's alkaloids and phenols inhibited metformin uptake in the cell and stayed more in the plasma. Hence, its measurement was more than the group that only administered metformin [11]. Pharmacokinetics parameters of metformin changed while combined with bitter gourd shown in Table 3.

Table 3. Primary, secondary, and derived pharmacokinetic parameters of metformin (mean±SD, n=3) after administered with a bitter gourd for 14 days for BM1 (100% (4 ml/kg)) and BM2 (50% (4 ml/kg)) groups and metformin (26 mg/kg) for all groups at day fifteenth, all administration were done orally.

Parameter (Unit)	**Value** (mean±SD)		
	BM1	**BM2**	**M**
Primary Parameters			
ka ($^{1}/_{min}$)	0.01703±0.018[a]	0.01349±0.006[a]	0.0094962±0.001
V/F ($^{mL}/_{kg}$)	4057.87±895.799[a]	5476.27±2463.51[a]	4273.7952±558.814
CL/F (mL/min/kg)	27.2676±8.734[a]	38.7273±6.407[a]	36.3306±2.581
Secondary Parameters			
$T^{1}/_{2}$ elimination (min)	105.36±12.302[a]	95.11±26.350[a]	82.21±10.079
Derived Parameters			
Cmax ($^{ng}/_{mL}$)	3002.47±381.209[a]	2326.97±384.891[a]	2174.96±65.626
Tmax (min)	27.2676±59.227[b]	38.7273±12.816[a]	39.4719±13.444
MRT (min)	260.989±87.350[a]	220.959±9.656[a]	224.969±26.866
AUC_{0-480} (ng.min/mL)	858950±163234[b]	632765±110359[a]	612310±4654
AUC_{0-inf} (ng.min/mL)	1013529±282514[a]	683044±106185[a]	663859±70953

[a] Insignificantly different compared with the metformin 26 mg/kg only group ($p>0.05$)

[b] Significantly different compared with metformin 26 mg/kg only group ($p<0.05$)

In the absorption phase, metformin depends on some transporters, such as PMAT, essential for metformin absorption. PMAT is a plasma membrane monoamine transporter expressed in human enterocytes' apical membrane. The substrate and inhibitor of PMAT mostly overlap with OCT which belongs to the SLC22 transporter family. Metformin, the positive charge transported by OCT and PMAT in membrane potential- and Na^{+}- dependent manners [35], [36]. The phytochemical constituent of bitter gourd juice consisted of flavonoids, alkaloids, terpenoids, and phenols. PMAT is sensitive to constituent flavonoids and seemed more sensitive to un-glycosylated flavonoids than glycosylated ones [37]. PMAT might also interact with phenol compounds from the juice involved in PMAT-mediated intestinal transport. It then affects the

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pharmacokinetics profile of metformin, increases the C_{max}, AUC_{0-480}, and elimination the half-life of metformin. The PMAT interaction can occur because of the mutations in the transporter itself or because of its pathological conditions [35], [36], [38].

Alkaloids, flavonoids, and phenols play a significant role in interaction with the transporters. Pharmacokinetic parameters such as AUC_{0-480} value and AUC_{0-inf} value were increased after being given bitter gourd juice co-administration. However, its CL/F was decreased with changing T_{max} by co-administration (Table 3). The results suggest that bitter gourd juice can cause pharmacokinetic alterations of metformin by reducing metformin distribution to other tissue because of the inhibition of OCT [11], [39]. Metformin does not undergo the metabolism phase, and the elimination phase depends on OCT2, MATE1, and MATE2-K transporters. The OCT and MATE transporter build a vectorial system to determine substances' renal secretory clearance. The MATE (multidrug and toxin extrusion protein) contains MATE1 and MATE2-K. The MATE1 is an antiporter for proton expressed in the liver's canalicular membrane. Organic cations and zwitterions excretion mediate it into bile. Alkaloids are their substrate and potent inhibitors with phenols [40], [41]. Both MATE1 and MATE2-K are localized in the kidney proximal tubules apical membrane. Its function is the final excretion step of drugs/toxins into the urine. Alkaloids and phenols might inhibit metformin transport [12], [40]. Metformin as OCT and MATE substrate while concurrent use with alkaloids will decrease metformin elimination from the kidney due to OCT2 inhibition by the inhibitors. Increased metformin concentration due to OCT2 inhibition may cause life-threatening lactic acidosis risks, especially in dehydrated or nephropathy diabetic patients.

Table 4. List of some *Momordica charantia's* Phytochemicals and its Mechanism as Antidiabetics

No	Phytochemicals	Mechanism of Action	References
1	Triterpenoids	Stimulation of glucose uptake, regulation of insulin secretion, and inhibition of α-glucosidase.	[44- 46]
2	Curcubitane triterpenes	Stimulation of GLUT4 translocation is regulated by phosphorylation of AMPK and IRS-1 activation and downstream proteins.	
3	Saponins	Increased insulin secretion in INS-1 pancreatic β-cells by PI3K, AKT, FoxO1 signaling pathway, and downstream molecule PDX-1. Improve pancreatic β-cells function by inhibiting serine 731 phosphorylation level of IRS-2.	[47]
4	Cucurbitacin	The immense affinity in binding with GLP-1r results in enhancement of β-cells proliferation and function, potentiating insulin secretion as glucose-dependent.	[48]
5	Momordicoside D	Has the highest binding affinity with TGR5. Its activation resulted in GLP-1r secretion to initiate insulin secretion, the proliferation of β-cells, and improving β-cells' function.	
6	Charantin	Decrease the expression of DPP-4, which is responsible for increasing the expression of GLP-1r to prevent the cleavage of GLP-1r.	
7	MCPIIa	Alleviate glucose metabolism by promoting and inhibiting the activities of Pdk4 and Hsd11β1, altered Fads2 levels to decrease the T2DM risks, and potentially inhibiting pancreatic islet apoptosis.	[49]
8	SMC	Prevent gluconeogenesis by promoting the activity of AMPK in the liver and reducing the NF-$_{K}$B levels.	[50]
9	Charantin	Increased GLUT4 translocation with no pathway involved explanation	[45]

GLUT4, Glucose transporter-4; GLP-1r, Glucagon-like peptide-1 receptor; DPP4, dipeptidyl peptidase IV; TGR5, Takeda-G-protein-receptor-5; Pdk4, Pyruvate dehydrogenase kinase; Hsd11β1, Hydroxysteroid 11-

beta dehydrogenase 1; Fads2, Fatty acid desaturase 2; T2DM, type 2 diabetes mellitus; MCPIIa, *Momordica charantia* polysaccharides IIa; SMC, *Momordica charantia* saponins; FoxO1, forkhead box O1; PI3K, phosphatidylinositol three kinases; AKT, protein kinase B.

This interaction's mechanism of action is presumably caused by inhibiting another transporter present in the brush border of the renal epithelium membrane by the bitter gourd's phytochemical constituent [12]. Rabbits were treated with bitter gourd 100% (4 ml/kg) for 14 days, then given metformin 26 mg/kg once on day 15th affected the renal histology of rabbits. This condition affects the elimination of metformin from the body since it's actively excreted from the body through transporters. Once the elimination progress affects this condition, metformin cannot leave the body after being distributed since its main excretion phase depends on the renal function [25], [42], [43]. This pharmacokinetics study is performed on rabbits' whole bodies and describes the overall activity of the body. Specific studies need to be performed to find the exact mechanism of the interaction, such as in vitro or study in an isolated organ.

4. CONCLUSIONS

Bitter gourd fruit juice with 100% (4 ml/kg BW) concentration affected the pharmacokinetic profile of the metformin insignificantly ($p>0.05$), decreased the V/F, CL/F, and Tmax, and insignificantly ($p>0.05$) increased Ka, $C_{max,}$ $T^1/_2$ elimination, MRT, and $AUC_{0\text{-}inf}$. In contrast, it significantly ($p<0.05$) increased T_{max} and $AUC0_{\text{-}480}$. Pharmacokinetics interaction between metformin and *Momordica charantia* L. presumably because of the competitive interaction between phytochemical constituents of bitter gourd and metformin on the OCT and MATE transporter.

5. ACKNOWLEDGEMENTS

Author contribution

All authors contributed to the concept and design making in this study. Asri Dwi Endah Dewi Pramesthi contributed to collecting data and writing the article. Agung Endro Nugroho and Endang Lukitaningsih contributed to the critical review and confirmed the publication of the final version.

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Conflict of interest

None to declare.

Ethics approval

Rabbits were used with the prior approval of the Animal Ethics Committee, LPPT, Universitas Gadjah Mada (No. 00012/04/LPPT/VI/2021).

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