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Drug–Herbal Interaction Study Of Glibenclamide And Phyllanthus Emblica: Pharmacokinetic Alterations And Enhanced Hypoglycemic Effect In Rats

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ABSTRACT

Background: Patients with Diabetes Mellitus (DM) often combine herbal and modern medicine to enhance therapy and reduce side effects, but this may cause drug interactions. Amla fruit extract (EBM) has hypoglycemic activity and is potentially used with DM drugs such as glibenclamide.
Objective: This study aimed to obtain scientific data on the safe co-use of glibenclamide and EBM by evaluating their pharmacodynamic and pharmacokinetic interactions.
Methods: For pharmacodynamic studies, 18 rats were divided into 6 groups: normal and glibenclamide controls, EBM doses of 500 and 750 mg/kg, and combinations of glibenclamide with EBM at both doses. Glucose tolerance test was used to evaluate glibenclamide effects. For pharmacokinetic studies, 15 rats were divided into 3 groups: glibenclamide control, and combinations of glibenclamide with EBM at 500 and 750 mg/kg. Glibenclamide levels were measured at specified intervals using a spectrophotometer, and analyzed with PKSolver via compartmental methods.
Results and Conclusions: The results showed that glibenclamide experienced drug interactions when used with EBM at a dose of 750 mg/kg. The onset of drug interactions was observed for 90 minutes and the results showed that the hypoglycemia effect increased 7.5 times. Moreover, the mechanism of drug interaction occurs in the pharmacokinetic process but not absorption, Cmax and AUC glibenclamide increase significantly after being used with EBM. Based on these results, health workers are recommended to monitor a patient who uses EBM and glibenclamide simultaneously.

Keywords: drug interaction, glibenclamide, Phyllanthus emblica.

INTRODUCTION

Currently, the Indonesian community can freely obtain and use herbal medicines without consulting a pharmacist, doctor, or other medical personnel. A study showed that 63% of traditional medicinal plants in Indonesia can cause pharmacokinetic interactions with conventional medicines when consumed simultaneously (Subronto, 2006). This is because modern medicine has failed to provide adequate treatment.

Diabetes mellitus (DM) is one of several diseases with poor management because patients are expected to continuously take medication to maintain blood glucose levels. This causes non-adherence to medication consumption due to its characteristics, which require long-duration therapy, and some patients who receive complex drug regimens (Hannan, 2013). Furthermore, this condition raises concerns about the side effects of drugs to promote the use of alternative treatments simultaneously with the assumption that these side effects can be avoided (Hamzah, 2019). An example of antidiabetic drugs is glibenclamide, a sulfonylurea group, which has a hypoglycemic effect by stimulating the beta cells of the islets of Langerhans to secrete insulin (Tjay & Rahardja, 2007).

The tendency to look for and use alternative drugs simultaneously is very possible. One of the natural medicine with the potential to be used for DM management is Amla fruit (Phyllanthus Emblica). This

plant originates from India, but generally grows in tropical and sub-tropical areas, including in Indonesia (Charoenteeraboon et al., 2010). Traditionally, it is used to treat various kinds of complaints including constipation, diabetes, diarrhea, headaches, and rheumatic pain (Sampath Kumar et al., 2012). It contains bioactive compounds such as tannins, flavonoids, phenols, saponins, terpenoids, and ascorbic acid, which are responsible for several pharmacological activities such as antimicrobial, antioxidant, radio-protective, hepatoprotective, antitussive, immunomodulatory, antihyperlipidemic, and several others. Amla is also reported to have anticancer, anti-HIV-reverse transcriptase, antidiabetic, antidepressant, and antiulcerogenic properties (Hasan et al., 2016).

The potential of amla fruit to be utilized in DM management has been studied extensively, including the report of Sultana that the fruit showed a hypoglycemic effect in male Long Evans rats through inhibition of glucose intake into the blood (Sultana et al., 2014). The water extract then showed hypoglycemic effects in rats caused by diabetes using alloxan at a level of 200 mg/kg BW (Qureshi et al., 2009). Morino explained that the condition of insulin resistance occurs due to high levels of total fat in the body (Morino et al., 2006). Amla fruit also showed anti-obesity activity in an obese rat model (Ardiansyah et al., 2018). Furthermore, flavonoid and polyphenolic are thought to be responsible for the antidiabetic activity of the amla fruit (Cahyaningrum et al., 2019). Based on the previous studies, it is necessary to examine drug interactions using the combination of glibenclamide and amla fruit extract (EBM) considering the safety of using both drugs simultaneously.

METHOD

The study was carried out at the Pharmacology Laboratory, Indonesian School of Pharmacy, Bandung. The test materials used include simplicia of Amla fruit (Amlavita®) and standard BP/Ph glibenclamide.Eur (Cadila Pharmaceuticals®). Other materials used include 95% ethanol, Chloroform pro analysis (Full Time ACS®), and Na-CMC.

Experiments were carried out on test animals namely healthy male rats of the Wistar strain with a weight of 200-250g. Before the test, the animals were acclimatized for 15 days and kept in cages having a capacity of 5 individuals each with a setting of 12 hours of light and 12 hours of darkness. The bedding was carried out every 3 days and the test animals were given adequate drinks and a standard feed of 5-10 g/100 g of rat weight per day. The tools used included UV-Vis Spectrophotometer (Shimadzu UV-1800®), Centrifugator (Gemmyco PLC-01®), Micropipette, Blood Glucose Test Meter (Easy Touch®).

The study was divided into three stages, namely the preparation of the test material, pharmacodynamics glibenclamide interactions with EBM, and the pharmacokinetics of glibenclamide drug interaction with EBM. This research protocol involving experimental animals was approved by the Research Ethics Committee of Universitas Padjadjaran under approval number 778/UN6.KEP/EC/2023.

Test simplicia

Amla fruit simplicia was obtained from Amlavita®, which is a product with composition packaged in tea packs produced by CV. Eshal Herbs.

Preparation of Test Materials

A total of 400 g of Amla fruit Simplicia was extracted using the maceration method, which involved soaking the fruit in 95% ethanol for 3 x 24 hours and stirring every 8 hours. The fiber was concentrated at a temperature of 45°C and Na-CMC 1% suspension was used as a carrier when the test materials (EBM and glibenclamide) were administered orally to test animals. The 1% Na-CMC suspension was made by placing 1 g of Na-CMC in a mortar containing 20 ml of hot water, allowed to stand for 15 minutes until a transparent mass was obtained, crushed to form a gel, then diluted with distilled water to 100 ml.

Pharmacodynamic Study of Drug Interaction of Glibenclamide with EBM

The study aimed to determine the incidence of drug interactions of glibenclamide consumed simultaneously with EBM. At random, 18 male Wistar rats were divided into 6 groups, namely normal and 0.45 mg/kg glibenclamide control group, 2 control doses of Malacca fruit extract (EBM) 500 and 750 mg/kg, and 2 test groups given glibenclamide and EBM with 2 different doses of 500 and 750 mg/kg, respectively. The test animals were checked for blood glucose levels (T0), then treatment was

administered according to the group. After 30 minutes, all groups were given a glucose load of 0.09g/kg orally. Then, blood glucose levels were measured at 30, 60, 90, 120, and 150 minutes using a Blood Glucose Test Meter.

Pharmacokinetic Study of Drug Interaction of Glibenclamide with EBM

This study was designed to screen the mechanism of drug interaction between glibenclamide and EBM in pharmacokinetic processes. At random, 15 male Wistar rats were divided into 3 groups, namely the control group given glibenclamide 10 mg/kg and 2 test groups given glibenclamide 10 mg/kg and EBM with 2 different doses of 500 and 750 mg/kg, respectively. Serum glibenclamide levels were measured at 0.5, 1, 1.5, 2, 2.5, 3, 5, 7, and 9 hours using the UV spectrometric method developed by Eapen (Eapen et al., 2012). The pharmacokinetic profile of glibenclamide was determined by linear pharmacokinetic analysis of the compartment method using the PKSolver program.

RESULTS AND DISCUSSION

Test Material Preparation Results

The simplicia used was obtained from the Amlavita®, which is a product with a pure composition of Amla fruit in a tea package. This product was used as a material to get closer to the real conditions of the study of glibenclamide drug's interaction with herbal products. The results of the extraction of amla fruit Simplicia by the maceration method with 95% ethanol solvent are shown in Table 1.

Table 1. Results of amla fruit Simplicia maceration

Extraction Process	Results
Simplicia	400 g
Maserate	152.34 g
Yield	19.04%

Results of Pharmacodynamic Study of Drug Interaction of Glibenclamide with EBM

The study determines drug interactions of glibenclamide when consumed simultaneously with EBM and the analysis of the effects of hypoglycemia follows the Riasari method (Riasari et al., 2018). Furthermore, the occurrence of drug interactions was assessed by comparing blood glucose levels between glibenclamide control animal groups, two control doses of EBM with two test groups given glibenclamide together with EBM. The two test groups were given different doses to determine the effect of the amount of EBM on drug interactions with glibenclamide. The results showed that glibenclamide experienced drug interactions when the blood glucose level of the test animal group was lower than the control.

Table 2. Blood glucose levels (KGD) at each observation time in each treatment group

Group	Average KGD (mg/dL) at time					
	0 Minutes	30 Minutes	60 Minutes	90 Minutes	120 Minutes	150 Minutes
Normal Control	91.67±5.13	123.67±7.09^{bcef}	124.00±3.46^{bcdef}	127.67±8.02^{bcdef}	129.00±5.20^{bcdef}	129.33±4.93^{bcdef}
Glibenclamide Control	72.33±2.36	106.67±1.25^{ad}	101.00±4.24^{a}	96.67±7.41^{af}	93.67±4.78^{a}	94.00±5.35^{a}
EBM 500 mg/kg Control	81.00±5.66	112.33±2.05^{ae}	107.00±2.83^{a}	101.33±9.46^{af}	99.33±6.5^{a}	101.00±7.07^{a}

EBM 750 mg/kg Control	83.67±9.74	120.33±7.13^{bef}	109.33±8.22^{a}	92.00±4.32^{a}	94.67±1.25^{a}	95.67±3.09^{a}
Glibenclamide+ EBM 500 mg/kg	85.67±2.87	99.33±4.78^{acd}	101.33±4.50^{a}	95.33±4.92^{a}	97.33±3.77^{a}	98.00±11.22^{a}
Glibenclamide +EBM 750 mg/kg	84.33±6.55	103.33±6.24^{ad}	102.00±4.90^{a}	81.33±5.44^{abc}	93.00±8.64^{a}	94.67±7.32^{a}

Description;
EBM: Amla Fruit Extract
a: significantly different from the normal control group (P<0.05)
b: significantly different from the Glibenclamide control group (P<0.05)
c: significantly different from the EBM 500 mg/kg control group (P<0.05)
d: significantly different from the EBM 750 mg/kg control group (P<0.05)
e: significantly different from the Glibenclamide +EBM 500 mg/kg group (P<0.05)
f: significantly different from the Glibenclamide +EBM 750 mg/kg group (P<0.05)

The measurement of blood glucose levels using the glucose tolerance test method as presented in Table 2 shows that glibenclamide and EBM at both doses has a hypoglycemic effect at each observation time. The blood glucose levels in each group were smaller than the normal control, except for the 750 mg/kg dose at 30 minutes of observation time. However, there was no significant difference in the effect of hypoglycemia in the two groups of test animals compared to the glibenclamide, except for 750 mg/kg dose of glibenclamide and amla fruit at the 90th minute of observation, with a lower glucose and blood pressure. During the observation time interval, there was no significant difference in blood glucose levels between the groups at the dose of EBM except at the 30th and 90th-minute observations.
The result showed that glibenclamide experienced drug interactions when used simultaneously with EBM at a dose of 750 mg/kg, and the onset of drug interactions was observed at 90 minutes. However, at 120 minutes, there were no glibenclamide drug interactions by observing blood glucose levels. This was expected to occur because the animals used were normal, not induced by diabetes, thus, their body system carried out homeostatic regulation in controlling blood glucose levels. Furthermore, the effect of the amount of extract on the incidence of drug interactions was assessed by the percentage decrease in blood glucose levels at each observation time. The figure below shows blood glucose levels relative to the initial condition in each treatment group and the values will be used to determine its percentage reduction.

Figure 1. Graph of blood glucose levels relative to the measurement time

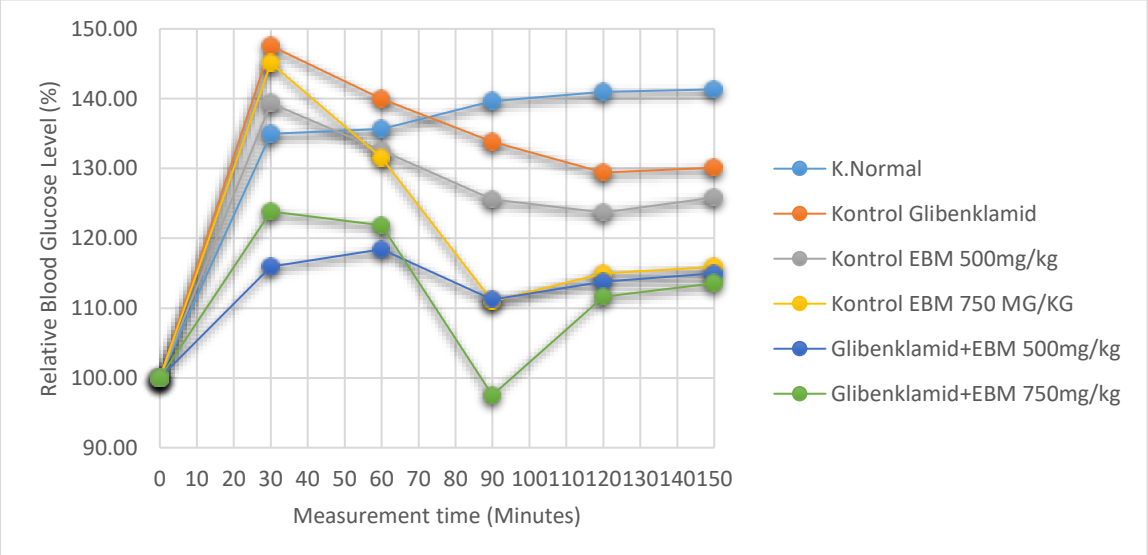


Figure 1 above depicts the patterns of the glibenclamide drug interactions. The graphic area for the two groups of test animals given the combination of glibenclamide and EBM was the lowest compared to the control group with glibenclamide. In addition, the lowest graphic area, which is observed at the 90th minute, is the test group given the largest dose. Conclusively, drug interaction patterns of glibenclamide occurred when used simultaneously with EBM, and the effect depends on the dose.

Figure 2. Graph of the percentage decrease in blood glucose levels over time

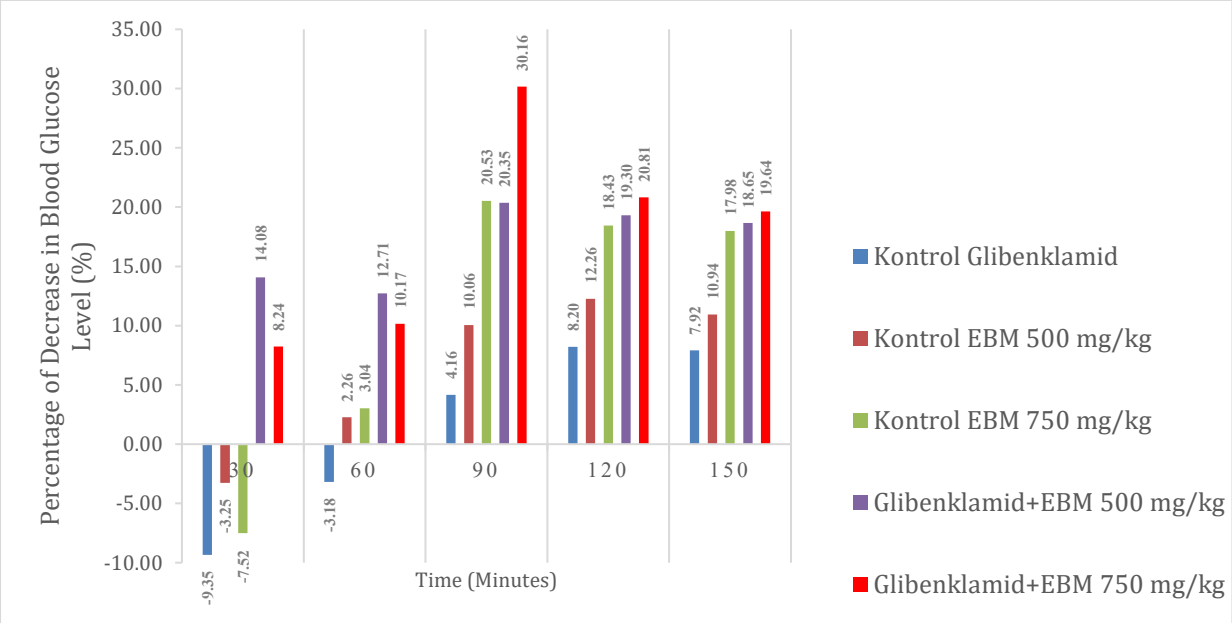


Figure 2 above shows the percentage decrease in blood glucose levels in each treatment group. The hypoglycemic effect of glibenclamide increases up to 7.5 times at the 90th minute when used simultaneously with EBM at a dose of 750 mg/kg. The pharmacodynamic data showed patterns of a drug interaction. To determine the mechanism of drug interaction, a pharmacokinetic study of glibenclamide after being used simultaneously with EBM needs is required.

Results of Pharmacokinetic Study of Drug Interaction of Glibenclamide with EBM

This study identifies a screening mechanism for the drug interaction of glibenclamide when used simultaneously with EBM. Several pharmacokinetic profiles of glibenclamide were used as analytical parameters, including the absorption rate constant (Ka), the time when the drug reached its maximum concentration (Tmax), the maximum level of the drug in the body (Cmax), and AUC_t^0 (Area Under Curve observation), which represents the total drug concentration during the observation time interval. Furthermore, the occurrence of drug interactions was assessed by comparing these parameters between the groups that were given glibenclamide simultaneously with EBM. The two test groups were given different doses of EBM to determine the effect of the amount of extract on the pharmacokinetic profile of glibenclamide.

Table 3. Pharmacokinetic of glibenclamide in each treatment group

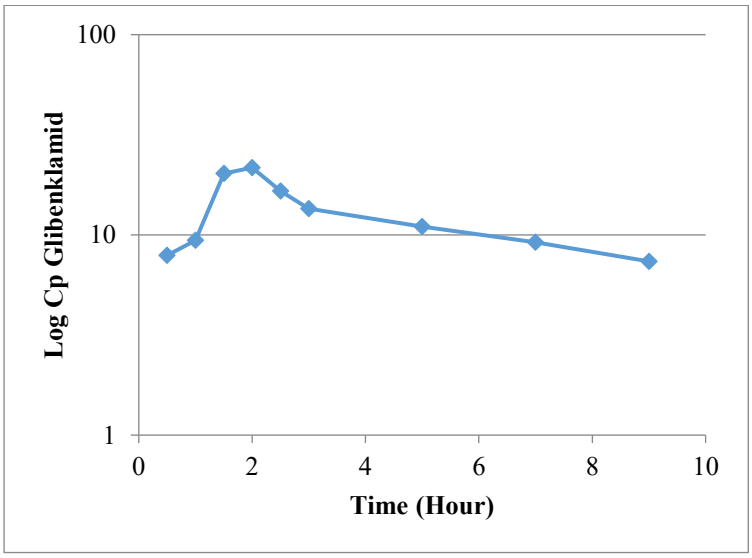
Time (Hour)	Glibenclamide levels in serum(µg/ml)		
	Glibenclamide Control	Glibenclamide +EBM 500 mg/kg	Glibenclamide +EBM750 mg/kg
0.5	7.89 ± 3.39^{bc}	17.85 ± 7.87^{a}	22.04 ± 2.31^{a}
1	9.39 ± 2.50^{bc}	18.97 ± 7.83^{a}	23.47 ± 2.79^{a}
1.5	20.25 ± 7.06	23.75 ± 4.11	27.12 ± 2.41

2	21.66 ± 9.71	24.22 ± 7.49	29.45 ± 1.63
2.5	16.57 ± 6.06	18.57 ± 9.23	25.33 ± 3.25
3	13.51 ± 6.10	17.02 ± 8.75	19.47 ± 5.00
5	10.98 ± 4.37	11.95 ± 5.79	17.58 ± 4.59
7	9.17 ± 3.92	10.75 ± 6.66	15.64 ± 4.37
9	7.37 ± 3.63	7.37 ± 7.48	16.17 ± 7.16

Description;
EBM: Amla Fruit Extract
a: significantly different from the Glibenclamide control group (P<0.05)
b: significantly different from the Glibenclamide +EBM 500 mg/kg group (P<0.05)
c: significantly different from the Glibenclamide +EBM 750 mg/kg group (P<0.05)

Table 3 above shows that EBM only affects glibenclamide levels at 0.5 and 1 hour after both drugs are used simultaneously. The effect patterns these times show that the use of a greater EBM results in higher glibenclamide levels. Furthermore, the measurement results for each treatment group were used to examine the screening of glibenclamide interaction mechanism at these times.
The pharmacokinetic profile was determined by linear pharmacokinetic analysis by compartment method using the PKSolver program. PKSolver is a Microsoft Excel Add-in that helps to analyze the pharmacokinetics and pharmacodynamics of a drug (Zhang et al., 2010).
Based on the data of the control group depicted on a graph in a logarithmic scale (Figure 3), glibenclamide showed linear pharmacokinetics in test animals with the corresponding compartment analysis depicted in a two-compartment model, which follows Rambiritch (Rambiritch et al., 2016). The graph shows a biexponential linear line, therefore, it was concluded that glibenclamide has two different distributions, with the quicker and slower rate in the first/central and second/tissue compartment, respectively.

Figure 3. Graph of pharmacokinetic data for the glibenclamide control group on a logarithmic scale



The linear pharmacokinetic analysis described in the two-compartment model (Table 4) showed that the administration of EBM only affected the pharmacokinetic profile of Cmax and AUC_9^0 of the four pharmacokinetic profiles of the determined glibenclamide. Its maximum and total levels during the observation time increased because it was used simultaneously with EBM. Furthermore, the changes in the glibenclamide levels during the observation time were affected by the doses of EBM.

Table 4. Pharmacokinetic profile of glibenclamide in each treatment group

Pharmacokinetics Parameter	Group		
	Glibenclamide Control	Glibenclamide +EBM 500 mg/kg	Glibenclamide +EBM 750 mg/kg
Ka (1/hour)	0.67 ± 0.20	2.02 ± 2.10	1,61 ± 1,02
Tmax (hour)	1.99 ± 0.45	1.42 ± 0.93	1,33 ± 0,18
Cmax (µg/ml)	17.51 ± 5.87[c]	23.57 ± 5.96	27,53 ± 1,61[a]
AUC_9^0 (µg/ml*hour)	106.1 ± 37.84[c]	127.00 ± 33.14[c]	173,42 ± 24,37[ab]

Description;
EBM: Amla Fruit Extract
a: significantly different from the Glibenclamide control group (P<0.05)
b: significantly different from the Glibenclamide +EBM 500 mg/kg group (P<0.05)
c: significantly different from the Glibenclamide +EBM 750 mg/kg group (P<0.05)

Based on the table above, the mechanism of drug interaction between glibenclamide and EBM occurs in the pharmacokinetic process, and the changes in the profile of Cmax and AUC_9^0. According to previous studies, the increased hypoglycemic effect of glibenclamide when given concurrently with EBM at a dose of 750mg/kg is due to its increased level. This effect is also due to the onset of drug interactions observed at 90 minutes since glibenclamide levels were at the maximum condition at that time. The mechanism of drug interaction is not in the absorption process because its rate does not change significantly after being used simultaneously with EBM. Therefore, health workers are required to monitor the simultaneous use of both in DM management both from the efficacy and unexpected events.

CONCLUSION

Conclusively, glibenclamide experienced drug interactions with EBM at a dose of 750 mg/kg. Furthermore, the onset of drug interactions was observed at 90 minutes and the hypoglycemic effect increased significantly up to 7.5 times. The administration of EBM at a dose of 500 mg/kg did not show a significant drug interaction when both drugs were used simultaneously.

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