

Bioequivalence and pharmacokinetic comparison of two fixed dose combination of Metformin/Glibenclamide formulations in healthy subjects under fed condition

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ABSTRACT

Aim: This study is conducted to compare the pharmacokinetic profiles of two fixed dose combination of metformin/glibenclamide tablets (500mg/5 mg per tablet).

Materials and Methods: This is a single-center, single-dose, open-label, randomized, 2-treatment, 2-sequence and 2-period crossover study with a washout period of 7 days. All 28 adult male subjects were required to fast for at least 10 hours prior to drug administration and they were given access to water ad libitum during this period. Thirty minutes prior to dosing, all subjects were served with a standardized high-fat and high-calorie breakfast with a total calorie of 1000 kcal which was in accordance to the EMA Guideline on the Investigation of Bioequivalence. Subsequently, subjects were administered either the test or reference preparation with 240mL of plain water in the first trial period. During the second trial period, they received the alternate preparation. Plasma levels of glibenclamide and metformin were analysed separately using two different high performance liquid chromatography methods.

Results: The 90% confidence interval (CI) for the ratio of the AUC_{0-t}, AUC_{0-∞}, and C_{max} of the test preparation over those of the reference preparation were 0.9693–1.0739, 0.9598–1.0561 and 0.9220 – 1.0642 respectively. Throughout the study period, no serious drug reaction was observed. However, a total of 26 adverse events (AE)/side effects were reported, including 24 that were definitely related to the study drugs, namely giddiness (n=17), while diarrhoea (n=3), headache (n=2) and excessive hunger (n=2) were less commonly reported by the subjects.

Conclusion: It can be concluded that the test preparation is bioequivalent to the reference preparation.

KEY WORDS:

Bioequivalence, glibenclamide, metformin, generic

INTRODUCTION

Metformin is a biguanide which reduces blood glucose levels, predominately by improving hepatic and peripheral tissue sensitivity to insulin without affecting the secretion of this hormone.¹ Glibenclamide is a sulphonylurea and acts by

inhibition of ATP-sensitive potassium channels.² Metformin is an amphoteric compound with two pKa values of 2.8 and 11.5. The absolute oral bioavailability of metformin is 50–60% and the gastrointestinal absorption is apparently complete within six hours of ingestion. Metformin is rapidly distributed and no plasma protein binding is observed and it undergoes renal excretion due to its high hydrophilicity.³

Glibenclamide is a sulphurous compound with a pKa value of 4.32. It is readily absorbed from the gastrointestinal tract with peak plasma concentrations usually occurring within two to four hours and fall within 24 hours to less than 5% of the peak value. It is extensively bound to plasma proteins. It is metabolized, almost completely in the liver. Serum concentration of glibenclamide appears to decline in a biphasic manner with elimination half-life of approximately 2–5 hours after oral administration. Some reports indicate a longer half-life of 8 to 10 hours in diabetic patients. Approximately 50% of a dose is excreted in the urine and 50% via the bile into the faeces.

Complementary mechanism of actions of glibenclamide and metformin result in a synergistic therapeutic effect when these drugs were used together. Treatment with a combination of metformin and glibenclamide has been shown to significantly provide a greater reduction in fasting plasma glucose and postprandial plasma glucose compared to monotherapy that is using glibenclamide or metformin.⁴ Moreover, a combination tablet preparation of metformin and glibenclamide is beneficial in terms of its convenience and patient compliance.

Common undesirable effects reported with the combination of metformin and glibenclamide are hypoglycaemia, taste disturbance, gastrointestinal disorders such as nausea, vomiting, diarrhoea, abdominal pain and loss of appetite. Lactic acidosis is very rare, reported at a rate of less than 1 case per 10,000 patient-years.

Bioequivalence study is required by the National Pharmaceutical Regulatory Agency (NPRA), Ministry of Health before marketing of the generic drugs. While there are numerous generic metformin/glibenclamide formulation marketed over the years, publication of relevant bioequivalence trials remain scarce. The objectives of this

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study is to investigate the safety profile, rate and extent of absorption of the test preparation, Diamide 5mg/500mg Film-Coated Tablet (manufactured by Hovid Bhd) and an equivalent oral dose of a reference preparation (Glucovance 500mg/5mg Tablet, manufactured by Merck Sante, S.A.S, France) under fed condition.

METHODS

The bioequivalence study was carried out in accordance to the current ICH Guidelines for Good Clinical Practice (GCP),⁵ Malaysian Guideline for Good Clinical Practice⁶ and Declaration of Helsinki. The study protocol was reviewed and approved by the Malaysian Medical Research and Ethics Committee and the study was initiated after MREC approval [(NMRR-17-2205-38031, KKM/NIHSEC/P17-1599 (23)]. All investigators involved were certified under Malaysia Good Clinical Practice Guideline.⁶

The study was conducted at the Ambulatory Care Centre, Raja Permaisuri Bainun Hospital, Perak, Malaysia while the bioanalysis was conducted at Attest Research Sdn Bhd, Bayan Lepas, Penang, Malaysia.

Study Population

Prior to enrolment into the study, potential subjects were required to undergo screening not earlier than 30 days before first dosing. Informed consent was taken from the subjects prior to initiation of the screening. A complete medical history and the vital signs were recorded during the screening process, which included sitting position blood pressure, pulse rate, oral temperature and respiratory rate. A 12-lead electrocardiogram was also performed. Laboratory test performed included full blood count (red blood cell count, hemoglobin, hematocrit, platelets and white blood cell count), sodium, potassium, chloride, creatinine, urea, total protein, albumin, globulin, serum bilirubin, alkaline phosphatase (ALP), aspartate transaminase (AST/SGOT), alanine transaminase (ALT/SGPT), fasting blood glucose and serology tests (hepatitis B, hepatitis C and HIV).

Inclusion criteria: Male adult subjects aged between 18 to 55 years old, with body mass index between 18.5 - 29.9 kg/m² or within 20% ideal body weight for height and build according to the Metropolitan Life Insurance Company Standards, in good health condition dictated by the medical history and laboratory tests.

Exclusion criteria: Subjects who were unable to provide informed consent or unable to comply with the study protocol, with significant abnormal laboratory tests, with history or presence of organ dysfunction or other comorbidities, with history or suspicion of drug dependence and alcohol abuse, with the requirement of pre-specified medication (i.e., tranquilizers, sedatives, chronic medications for hypertension and diabetes, anti-platelet agents, anti-epileptics, analgesics, opioids, psychotropics, antibiotics, MAO inhibitors), with hypersensitivity to metformin or glibenclamides or their derivatives, experienced difficulty while donating blood or swallowing tablets/capsules, donate blood within the past eight weeks, have contracted any clinically significant illnesses within the past four weeks,

taken any gastrointestinal motility-altering drugs within the past one week, smoked more than 10 cigarettes per day or currently on e-cigarette.

Sample size calculation

Sample size was calculated using intrasubject coefficient of variation (CV).⁷ To date, very little information was available regarding the intrasubject CV of glibenclamide and metformin in bioequivalence study. Ghazzi et al., reported a bioequivalence study on glibenclamide using 24 subjects with bioequivalence successfully conducted and capable of achieving a statistical power of 80%.⁸ Another study by Flores-Murrieta FJ et al., also reported 24 subjects recruited into their study and bioequivalence was successfully concluded for glibenclamide.⁹

Previous study conducted on metformin immediate release and extended release tablets as well as findings reported by Yuen et al., have shown that the intrasubject CV values obtained were less than 25% for C_{max}, AUC_{0-t} and AUC_{0-∞}.¹⁰ Based on the above, the present study was conducted with 28 subjects and the statistical power of the study were determined post-study. A total of 28 subjects recruited for the present bioequivalence study fulfilled the requirements of ASEAN Guidelines for The Conduct of Bioequivalence Studies (2015) and European Medicines Agency (2010).^{11,12}

Test and reference preparation

The test preparation (Diamide® 5mg/500mg film-coated tablet) was manufactured by Hovid Bhd., Perak, Malaysia; while the reference preparation (Glucovance® 500mg/5mg tablet) was manufactured by Merck Sante, S.A.S, France. Both preparations contained 5mg of glibenclamide and 500mg of metformin hydrochloride per tablet and they were supplied by Hovid Bhd. to the study site. The preparations were stored and repacked at the study site prior to study commencement.

Study design

This was an open-label, randomized trial with two-way crossover design involving 28 healthy adult male subjects to be randomly divided into two groups of 14 people. On the first trial period, group 1 was given one tablet of the reference preparation (Glucovance 500mg/5mg Tablet) and group 2 was given one tablet of the test preparation (Diamide 5mg/500mg Film-Coated Tablet). After a seven-day washout period, the two groups received the alternate preparation during the second trial period.

For both trial periods, the subjects were admitted one day before dosing. They were required to fast for at least 10 hours prior to drug administration, provided with *ad libitum* water access. Thirty minutes prior to dosing, all subjects were served with a standardised high-fat and high-calorie breakfast with a total calorie of 1000 kcal, adhering to the EMA Guideline on the Investigation of Bioequivalence. Subsequently, subjects were administered either the test or reference preparation with 240mL of plain water.

Other than the water administered during dosing, water access was restricted for one hour before and after drug administration, while food was restricted for 4 hours after dosing. Nonetheless, all subjects were given 100 mL of 20%

oral glucose solution hourly until three hours post dosing. The study team monitored the subjects closely to ensure no additional food or drinks apart from those allotted for the study.

Subjects were monitored for any adverse reaction, within the sight of study team for four hours after dosing. They were also advised to inform the attending study clinician if they experienced any discomfort or adverse events. Vital signs (i.e., body temperature, blood pressure, pulse rate and respiratory rate) were monitored within 30 minutes of 2, 6, 10 and 14 hours post dosing. In the event of a conflict between blood sampling and vital signs monitoring, the former was performed before the latter. In view of the hypoglycaemic nature of the preparations, blood glucose level of the subjects was monitored during each blood sampling interval until six hours post dosing.

Blood samples of 8 ml volume each were drawn at times 0 (predose), 30 mins, 1, 1.5, 2, 2.33, 2.67, 3, 3.33, 3.67, 4, 5, 6, 8, 10, 12, 16 and 24 hours after drug administration via an in-dwelling cannula placed in the forearm or direct venipuncture. The blood samples were centrifuged for 15 minutes at 3500 rpm at the temperature of 25°C to obtain the plasma samples. The plasma samples were separated promptly and transferred to their respective cryovials and kept frozen until assayed.

Analysis of plasma glibenclamide and metformin concentration

Plasma level of glibenclamide and metformin were analysed separately using two different high performance liquid chromatography (HPLC) methods. The former was analysed using HPLC with fluorescence detector while the latter using HPLC with photodiode array detector (PDA). The HPLC system for the analysis of glibenclamide comprised a Waters 600E Multisolute Delivery System (Waters, Maple Street, Milford, USA), a Waters 2475 Multiλ Fluorescence Detector (Waters, Maple Street, Milford, USA), a Waters 717Plus Autosampler (Waters, Maple Street, Milford, USA) and a data acquisition and analysis software, Waters Empower™ 2 Data Software (Waters, Maple Street, Milford, USA). A C18 analytical column (150 x 4.6 mm id, 4 μm) (Grace Davison Discovery Sciences, Illinois, USA) was used for the chromatographic separation while the mobile phase consisted of a mixture of acetonitrile and 0.05M disodium hydrogen phosphate (pH5.8) at a ratio of 42:58 (v/v). Analysis was performed isocratically at a flow rate of 1.2 ml/min. The fluorescence detector was operated at excitation wavelength 308 nm and emission wavelength 350 nm.

On the other hand, the HPLC system for the analysis of metformin comprised a Waters Alliance e2695 Separation Module (Waters, Maple Street Milford, USA), a Waters 2998 Multiλ Photodiode Array Detector (PDA) (Waters, Maple Street, Milford, USA), a data acquisition and analysis software, Waters Empower® 3 Data Software (Waters Maple Street, Milford, USA). A C18 analytical column (150 x 4.6 mm id, 5 μm) (Agilent Technologies, USA), was used for the chromatographic separation while the mobile phase consisted of a mixture of acetonitrile, 0.01 M potassium dihydrogen phosphate and 0.01 M sodium dodecyl sulfate at a ratio of 36:36:28 (v/v). Analysis was performed isocratically

at a flow rate of 1.2 ml/min. The PDA detector was operated at a wavelength of 234 nm.

Both bioanalytical methods were validated according to the European Medicines Agency's Guideline on Bioanalytical Method Validation (2011) to demonstrate the reliability of the methods for quantification of glibenclamide and metformin in the plasma samples.¹³

Pharmacokinetic and statistical method

The four pharmacokinetic parameters for metformin and glibenclamide included maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}), area under the plasma concentration-time curve from time zero to the last measurable concentration (AUC_{0-t}) and total area under the plasma concentration-time curve ($AUC_{0-\infty}$), were estimated from the plasma concentration-time data. The values of C_{max} and T_{max} were obtained directly from the plasma values.¹⁴ The $AUC_{0-\infty}$ was calculated by adding the area from time zero to last sampling time t (AUC_{0-t}) and the area from time t to infinity ($AUC_{t-\infty}$). The former was calculated using the trapezoidal formula; and the latter by dividing the last measurable plasma drug concentration with the elimination rate constant (k_e).

The k_e of metformin and glibenclamide were estimated from the terminal slope of the individual plasma concentration-time curves after logarithmic (\ln) transformation of the plasma concentration values (at least three concentration values were used) and application of linear regression.¹⁵ The half-life ($t_{1/2}$) was calculated by inserting k_e in the following equation: $t_{1/2} = \ln 2/k_e$.

The statistical analysis was performed using the commercial software, Phoenix™ WinNonlin 6.4 from (Certara™, USA) except T_{max} which was analysed using EquivTestPK (Cork, Ireland). The values of C_{max} , AUC_{0-t} , $AUC_{0-\infty}$, k_e and $t_{1/2}$ obtained with the two preparations were analyzed using an analysis of variance (ANOVA) procedure, which distinguishes effects due to subjects, periods, and treatment.¹⁶ The AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} values were logarithmic transformed before analysis. The T_{max} values were analysed using the Wilcoxon Signed Rank Test for paired samples.

Bioequivalence was concluded based on the 90% confidence interval for the ratio of the C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ values of the test preparation (Diamide 5mg/500mg Film-Coated Tablet) over that of the reference preparation (Glucovance 500mg/5mg Tablet). The type 1 (alpha) error was set at the level of 5%.¹⁷ In order to conclude bioequivalence, as stipulated by the EMA (2010) and ASEAN Guideline for the Conduct of Bioequivalence Studies (2015), the accepted range for AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} is 80.00 – 125.00%.

RESULTS

Subject Demographics

Of the 28 subjects enrolled into this study one voluntarily withdrew his participation during the first trial period due to blood taking phobia. Eventually, a total of 27 subjects completed both trial periods and only data obtained from these 27 subjects were included in the pharmacokinetic

Table I: Demographic characteristics of subjects (n=27)

Age, years, median (IQRa)	21 (6)
BMI, kg/m ² , median (IQRa)	22.8 (5.3)
Ethnicity, n (%)	
Malay	26 (96.3)
Indian	1 (3.7)
Normal Head and ENT	27 (100)
Normal Eyes	27 (100)
Normal Lymph Glands	27 (100)
Normal Musculoskeletal and Skin	27 (100)
Normal Electrocardiogram	27 (100)
Normal Full Blood Count	27 (100)
Normal Renal Function	27 (100)
Normal Liver Function	27 (100)
Normal Fasting and Random Glucose	27 (100)
Negative Hepatitis B, Hepatitis C and HIV	27 (100)

IQRa, Interquartile range

Table II: Adverse event profiles of test and reference product

Adverse events	Test product (n)	Reference product (n)	Not related to both test and reference product
Giddiness	7	10	-
Excessive hunger	1	1	-
Headache	2	-	1
Diarrhea	1	2	-
Mild flu-like symptoms	-	-	1

Table III: Metformin pharmacokinetic parameters of the test preparation (Diamide) versus the reference preparation (Glucovance) under fed condition

Pharmacokinetic parameters	Test Preparation Diamide 5/500mg tablet Mean (SD) ^a	Reference Preparation Glucovance 5/500mg tablet Mean (SD) ^a
AUC _{0-t} (hr.ng/ml)	5547.68 (1248.05)	5487.00 (1367.86)
AUC _{0-∞} (hr.ng/ml)	5773.77 (1275.95)	5767.07 (1366.08)
C _{max} (ng/ml)	895.40 (228.11)	904.34 (216.24)
T _{max} (hr)	2.26 (0.73)	2.41 (0.95)

SDa, Standard deviation

Table IV: Glibenclamide pharmacokinetic parameters of the test preparation (Diamide) versus the reference preparation (Glucovance) under fed condition

Pharmacokinetic parameters	Test Preparation Diamide 5/500mg tablet Mean (SD) ^a	Reference Preparation Glucovance 5/500mg tablet Mean (SD) ^a
AUC _{0-t} (hr.ng/ml)	1157.01 (294.84)	1118.08 (261.20)
AUC _{0-∞} (hr.ng/ml)	1218.02 (296.92)	1174.94 (269.06)
C _{max} (ng/ml)	242.10 (82.27)	258.29 (77.08)
T _{max} (hr)	3.97 (1.56)	3.65 (1.59)

SDa, Standard deviation

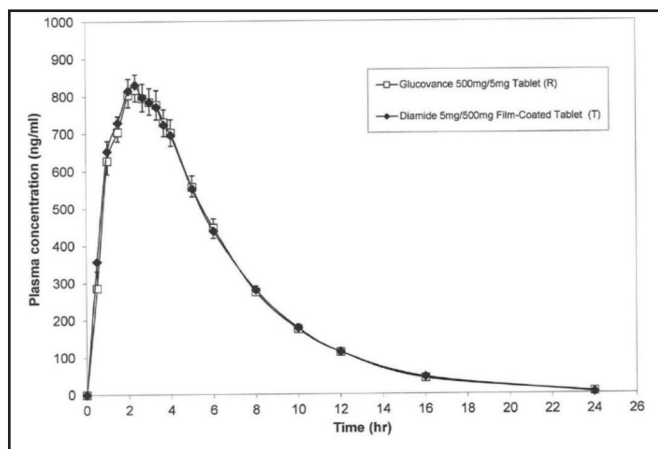


Fig. 1: Mean plasma metformin concentration versus time profiles obtained with Glucovance 500mg/5mg Tablet and Diamide 5mg/500mg Film-Coated Tablet.

analysis. The majority of the subjects were of Malays, with a median of 21 years old (IQR: six years) and body mass index (BMI) of 22.8 kg/m² (IQR: 5.3).

Test / reference Preparation Safety

Throughout the study period, no serious drug reaction was observed. However, a total of 26 adverse events (AE)/side effects were reported, including 24 that were definitely related to the study drugs, namely giddiness (n=17), while diarrhoea (n=3), headache (n=2) and excessive hunger (n=2) were less commonly reported by the subjects. All adverse events that were definitely related to the study drugs and were attributed to their hypoglycemic activity (Table II).

Metformin

Figure 1 depicts the mean metformin plasma concentration versus time profile for both the test and reference preparations. Overall, the two plasma profiles showed that the mean peak plasma metformin concentrations was attained at approximately 2.33 hours after the administration of Glucovance 500mg/5mg Tablet as well as after the administration of Diamide 5mg/500mg Film-Coated Tablet. The profiles of the two products were almost superimposable and displayed comparable C_{max} values (Figure 1).

The AUC_{0-t}, AUC_{0-∞} and C_{max} values of Diamide 5mg/500mg Film-Coated Tablet and Glucovance 500mg/5mg Tablet were relatively similar. No statistically significant differences were observed between the values of AUC_{0-t} (p = 0.5247), AUC_{0-∞} (p = 0.8222), and C_{max} (p = 0.7803) of the two products when analysed using ANOVA for two-way crossover design. Similarly, there was no statistically significant difference between the ke values (p = 0.1492) values of the two preparations.

The 90% confidence interval (CI) for the ratio of the AUC_{0-t}, AUC_{0-∞}, and C_{max} of the test preparation over those of the reference preparation were 0.9693 – 1.0739, 0.9598 – 1.0561 and 0.9220 – 1.0642 respectively, which were all within the acceptable bioequivalence limit of 0.8000 – 1.2500.

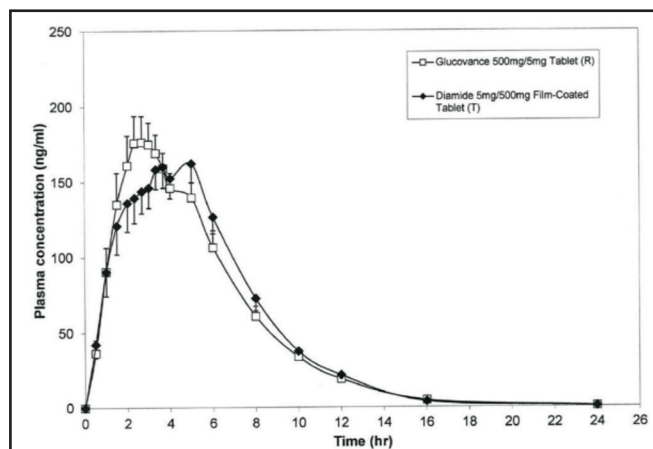


Fig. 2: Mean plasma glibenclamide concentration versus time profiles obtained with Glucovance 500mg/5mg Tablet and Diamide 5mg/500mg Film-Coated Tablet.

The intra-subject variation, estimated using the mean square error of the ANOVA analysis for AUC_{0-t}, AUC_{0-∞} and C_{max} (Diletti et al., 1991), has coefficient of variation values of 11.03%, 10.32%, and 15.52%, respectively and the respective power of the study calculated using PhoenixTM WinNonlin was 100%, 100%, and 99.93%. The inter-subject variation for AUC_{0-t}, AUC_{0-∞} and C_{max} has coefficient of variation values of 23.60%, 22.70%, and 23.05%, respectively.

Glibenclamide

Figure 2 depicts the mean glibenclamide concentration versus time profile for both the test and reference preparations. The two plasma profiles showed that the mean peak plasma glibenclamide concentrations was attained at approximately 2.67 hours after administration of Glucovance 500mg/5mg Tablet and at approximately 5 hours after the administration of Diamide 5mg/500mg Film-Coated Tablet (Figure 2).

The AUC_{0-t}, AUC_{0-∞} and C_{max} values of Diamide 5mg/500mg Film-Coated Tablet and Glucovance 500mg/5mg Tablet were relatively similar. No statistically significant differences were observed between the logarithmic transformed values of AUC_{0-t} (p = 0.3135), AUC_{0-∞} (p = 0.2257), and C_{max} (p = 0.2239) of the two products when analysed using ANOVA for two-way crossover design. There was no statistically significant difference between the ke values (p = 0.9503 for glibenclamide) and t_{1/2} (p = 0.8994 glibenclamide) values of the two preparations.

The 90% confidence interval the ratio of AUC_{0-t}, AUC_{0-∞}, and C_{max} of the test preparation over the reference preparation were 0.9788 – 1.0759, 0.9856 – 1.0761 and 0.8388 – 1.0268 respectively, which were all within the acceptable bioequivalence limit of 0.8000 – 1.2500.

The intra-subject variation, estimated using the mean square error of the ANOVA analysis for AUC_{0-t}, AUC_{0-∞} and C_{max} (Diletti et al., 1991), has coefficient of variation values of 10.18%, 9.46%, and 21.98%, respectively while the respective power of the study calculated using PhoenixTM WinNonlin was 100%, 100%, and 97.51%. Besides, the inter-subject

variation estimated using the mean square error of the ANOVA analysis for AUC_{0-t} , $AUC_{0-\infty}$, and C_{max} has coefficient of variation values of 21.17%, and 21.49%, respectively.

DISCUSSION

As per US FDA (2001), EMA (2010) as well as ASEAN Guideline for the Conduct of Bioequivalence Studies (2015), the conclusion of bioequivalence is based on the 90% CI of the test preparation over the reference preparation. This study found that, all the ratios AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} of Diamide 5mg/500mg Film-Coated Tablet over those of Glucovance 500mg/5mg Tablet were within 80.00 – 125.00%, thus, the test preparation is bioequivalent to the reference preparation.

In this study, hypoglycemic symptoms occurred at a rate of 38.8%. These included seventeen cases of giddiness, two cases of excessive hunger and two cases of headaches after administration of the study drug. This was comparable with the occurrence rate of hypoglycemic symptoms (30-35%), reported by another study that treated diabetic patients with Glucovance 2.5mg/500mg. In this study, the reported hypoglycemic symptoms included dizziness, shakiness, sweating and hunger (FDA, 2018).

Specifically, it was observed in this study that giddiness occurred slightly more frequently after the administration of Glucovance 5mg/500mg (n=10) when compared with Diamide 5mg/500mg (n=7), in which such difference was deemed as clinically insignificant. Excessive hunger was observed at a same rate between two brands of the drug preparation (n=1 in each group). All adverse events were resolved with administration of 20% w/v oral glucose solution. Similar finding was observed in another study by Marather (2000) wherein only one incidence of hypoglycemia was unresolved even with administration of 20% w/v oral glucose solution.

CONCLUSION

On the basis of the results obtained, it can be concluded that the test preparation, Diamide 5mg/500mg Film-Coated Tablet (manufactured by Hovid Ltd, Malaysia) is bioequivalent to the reference preparation, Glucovance 500mg/5mg Tablet (manufactured by Merck Sante, S.A.S, France).

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DECLARATION ON CONFLICT OF INTEREST

Chang Chee Tao and Ang Ju Ying did not have any conflict of interest to declare. Wong Jia Woei, Tan Siew Siew, Chin Siaw Kuen, Lim Ai Boey, Tan Weng Hong were employees to Attest Research Pte Ltd, an independent research company and a subsidiary of Hovid Bhd. Kah Hay Yuen was the R&D Director of Attest Research Sdn Bhd.

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