



Comparative Pharmacokinetic Study Among 3 Metformin Formulations in Healthy Mexican Volunteers: A Single-Dose, Randomized, Open-Label, 3-Period Crossover Study

Sandra Lucía Montoya-Eguía, DrC¹, Lourdes Garza-Ocañas, DrMed^{1,*}, Christian Tadeo Badillo-Castañeda, DrC¹, Eduardo Tamez-de la O, DrC¹, Teresa Zanatta-Calderón, DrC¹, Marco Vinicio Gómez-Meza, PhD², Humberto Garza-Ulloa, MC¹

¹ Departamento de Farmacología y Toxicología, Facultad de Medicina, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, CP 64460, Mexico

² Facultad de Economía, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, CP 64930, Mexico

ARTICLE INFO

Key words:
Bioequivalence
generic formulations
metformin
Mexican population
pharmacokinetic

ABSTRACT

Background: Type 2 diabetes mellitus is the most common form of diabetes. Metformin is a first-line drug for its treatment. In Mexico, there are 34 generic formulations of metformin, so brand-generic substitutions and generic-generic substitutions are a common practice. Generic products are compared only with their brand-name equivalents and not with the same product made by other manufacturers.

Objective: Our aim was to establish whether 2 generic formulations of 500 mg metformin available on the Mexican market fulfill the criteria for interchangeability.

Methods: This single-dose, randomized-sequence, open-label, 3-period crossover study was conducted in 12 healthy subjects in compliance with the Declaration of Helsinki and International Conference on Harmonization guidelines. A validated HPLC procedure coupled with a spectrum mass detector were used to analyze the metformin concentration in plasma samples. All pharmacokinetic analyses were performed using WinNonlin Professional Software version 6.3 (Pharsight Corporation, Sunnyvale, California).

Results: Twelve healthy Mexican volunteers were enrolled in the study. Their mean age was 24.33 years and mean weight was 62.54 kg. The mean body mass index was 23.02. The values obtained for the test and reference formulations were: C_{\max} 1163.5 (295.2) ng/mL for treatment A, 1184.6 (215.0) ng/mL for treatment B, and 1167.8 (176.8) ng/mL for treatment C. AUC_{0-t} was 6240.7 (1629.4) ng·mL/h for treatment A, 6433.7 (1249.8) ng·mL/h for treatment B, and 6567.1 (1145.5) ng·mL/h for treatment C. $AUC_{0-\infty}$ was 6837.3 (1618.5) ng·mL/h for treatment A, 6911.8 (1178.4) ng·mL/h for treatment B, and 7178.6 (1086.8) ng·mL/h for treatment C.

Conclusions: The test formulation 500-mg metformin tablets were bioequivalent to the reference formulation and to each other, according to the general laws of health care in Mexico.

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Introduction

Diabetes mellitus is a complex chronic illness requiring continuous medical care with multifactorial risk reduction strategies beyond glycemic control.¹ The worldwide prevalence of diabetes mellitus is currently estimated to be around 180 million and the World Health Organization predicts this number to double by 2030.² Type 2 diabetes mellitus is the most common form of the

disease (affecting 90%–95% of persons with diabetes) and is characterized by an underlying insufficiency of insulin.^{3,4}

Metformin is an orally administered antidiabetic drug from the biguanide class. It is recommended as a first-line drug for the treatment of type 2 diabetes mellitus.⁵

Metformin acts in the presence of insulin to increase glucose use and reduce glucose production, thereby counteracting insulin resistance. The effects of metformin include increased glucose uptake, oxidation and glycogenesis by muscle, increased glucose metabolism, and reduced hepatic gluconeogenesis.⁴ Metformin is mainly absorbed in the small intestine and has an oral bioavailability of 60% under fasting conditions. Its plasma protein binding is negligible, and it is not metabolized by the liver. Metformin is 90% excreted unchanged in urine.⁶

* Address correspondence to: Dr. Lourdes Garza-Ocañas, Departamento de Farmacología y Toxicología, Facultad de Medicina, Universidad Autónoma de Nuevo León, Gonzalitos 235 Norte, Colonia Mitrás Centro, Monterrey, Nuevo León, CP 64460, México.

E-mail address: logarza@live.com.mx (L. Garza-Ocañas).

In Mexico, there are 34 generic formulations of metformin, so brand-to-generic substitution and generic-generic substitutions are a common practice.^{7,8} It is important to keep in mind that generic products are only compared with their brand-name products and not with the same product made by other manufacturers.

Some studies^{9–11} have warned about the lack of pharmacokinetic bioequivalence among generic drugs in the postmarketing setting. Therefore, based on these considerations, the aim of our work was to establish whether 2 generic formulations of 500 mg metformin available on the Mexican market fulfill the criteria for interchangeability.

Subjects and Methods

Materials

Metformin chloride USP was used for the study. HPLC-grade acetonitrile was purchased from Tedia High Purity Solvents (Fairfield, Ohio), HPLC-grade methanol and formic acid were purchased from Fermont (Monterrey, Nuevo León, Mexico). Deionized water was purchased from Laboratorios Monterrey (Monterrey, Nuevo León, Mexico).

Products evaluated

Reference A tested 500-mg Dabex tablets (Merck S.A. de C.V. Naucalpan de Juárez, Estado de México, México). Batch M33801, expires June 2017). Test B utilized 500-mg Pre-Dial tablets (Laboratorios Silanes S.A. de C.V. Toluca Estado de México, México). Batch 13LI07VI, expires November 2015. Test C utilized 500-mg Dimefor tablets (Siegfried Rhein Querétaro, Qro. México). Batch 310507, expires September 2015.

Study subjects

Twelve healthy adult (male and female) Mexican volunteers participated in the study. All were in good health based on their medical histories, complete physical examination, vital signs (eg, heart rate, systolic and diastolic blood pressure, and body temperature), and routine laboratory tests performed before and after the study (eg, complete blood count, blood chemistry, urinalysis, pregnancy test for women, renal and liver function tests, antibody testing for HIV, hepatitis B surface antigen, and hepatitis C virus). None had a history of any allergy to metformin and related compounds. Subjects did not receive any other medication during the study. All volunteers abstained from any xantine-containing food or beverages or alcoholic products for 48 hours before dosing and throughout the sampling schedule during each period.

Study design

The study was conducted in the Departamento de Farmacología y Toxicología, Facultad de Medicina, Universidad Autónoma de Nuevo León.

This is a single-dose, randomized-sequence, open-label, 3-period crossover study that was carried out under fasting conditions with a 1-week washout period.

Subjects were admitted and housed in our clinical pharmacology unit for 12 hours before the dose and were discharged 24 hours after the dose during each period. A single 500-mg tablet of the formulations was administered with 250 mL water after an overnight fast. A total of 15 venous blood samples (5 mL each) were collected predose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4,

6, 8, 12, and 16 hours. Serum was separated by centrifugation at 12,500 rpm for 10 minutes, and stored at –40°C until analysis.

A standardized breakfast and lunch were given at 4 and 8 hours, respectively, after medication administration.

Ethical considerations

The study protocol and the informed consent form were approved on May 6, 2014, by our institutional ethics and research committee (study No. FA14-001) and the study was conducted in accordance with the principles of the Declaration of Helsinki¹² and its amendments, the International Conference on Harmonization Guidelines for Good Clinical Practice,¹³ and the general laws of health care in Mexico.¹⁴ The order of treatment sequence (reference or test drugs) was randomized using Excel (version 2010, 2010, Microsoft Corp, Redmond, Washington).

Bioanalytic methods

The sample preparation process was accomplished by protein precipitation using acetonitrile. A 200-μL aliquot of each plasma sample was transferred to a polypropylene tube (Eppendorf, Hamburg, Germany), and 1000 μL acetonitrile was added. After brief vortex mixing, the tubes were centrifuged at 12,500 rpm for 10 minutes. Then the supernatants were evaporated to dryness under a nitrogen stream at 60°C, reconstituted with 400 μL assay mobile phase, vortexed for 2 minutes, and then centrifuged at 12,500 rpm for 4 minutes. The supernatant was transferred to a vial with a flat bottom glass insert.

Plasma concentrations of metformin were determined using HPLC-MS/MS using an Agilent Technologies (Palo Alto, California) Model 1100 series instrument equipped with a degassing unit, a high pressure binary pump, an autosampler, and a mass spectrometer detector (6410 B; Agilent Technologies), using a method based on that published by Kandhwal et al¹⁵ and Harahap et al.¹⁶ Separations were performed on a Zorbax HILIC Plus Rapid Resolution 4.6 × 100 mm, 3.5 μm column (Agilent Technologies), and eluted with a mobile phase consisting of 2.65 mM acetonitrile and formic acid solution (40%/60% v/v). The eluate was filtered through a 0.45 μm pore size cellulose membrane. The chromatographic separation was performed isocratically at 21°C at a flow rate of 0.8 mL/min.

Tolerability

Tolerability was determined by clinical assessment and monitoring vital signs (eg, blood pressure, heart rate, and body temperature) at baseline, 3 times during the study, and at the end of the periods. Laboratory analyses were also performed before and after the study. In addition, subjects were required to report to the investigators any adverse effects that occurred at any time during the study, including during the washout period.

Pharmacokinetic analysis

The following pharmacokinetic values for each subject and for each treatment were determined: C_{\max} , T_{\max} , AUC_{0-t} , $AUC_{0-\infty}$, and $T_{1/2}$. AUC_{0-t} was calculated using the linear trapezoidal rule¹⁷ and $AUC_{0-\infty}$ was calculated as the sum of AUC_{0-t} and the extrapolated area under the concentration time curve ($C_{\text{last}}/\text{elimination rate constant } [K_e]$). C_{\max} and T_{\max} were obtained directly from the original data set and $t_{1/2}$ was calculated as \ln_2/K_e . K_e was obtained by linear regression from the best-fit slope of the terminal log-linear decay in plasma concentrations versus time profile. All pharmacokinetic analyses were performed using WinNonlin

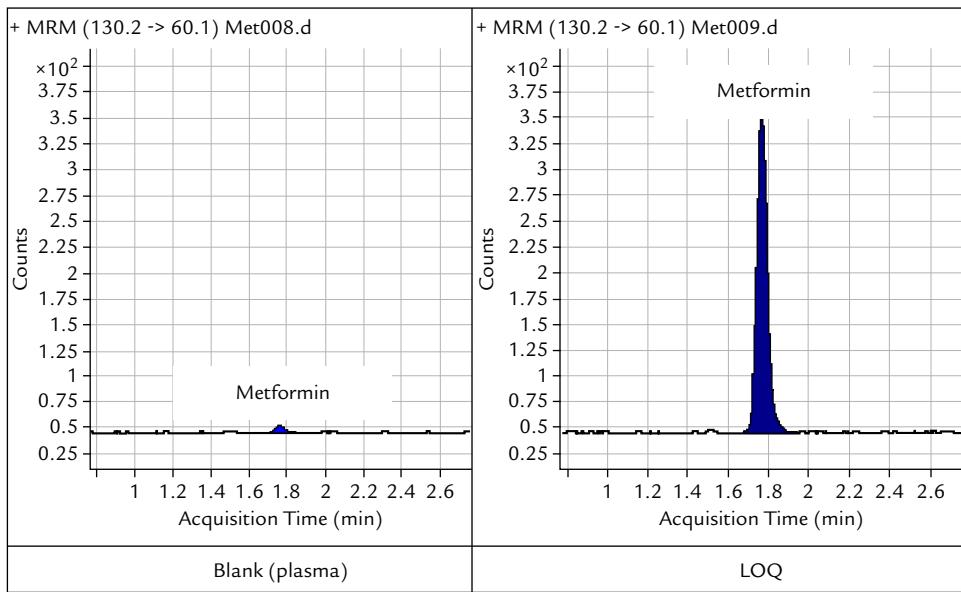


Figure 1. Comparison of limit of quantification versus blank (plasma).

Professional Software version 6.3 (Pharsight Corporation, Sunnyvale, California).

Statistical analysis

All statistical analyses were carried out using SPSS software version 13.0 (IBM-SPSS Inc, Armonk, New York).

Using a noncompartmental analysis, ANOVA was performed to test the significant differences between formulations with the logarithmically transformed values of AUC_{0-t} , $AUC_{0-\infty}$, and C_{max} .¹⁸ In addition, Schuirmann's 2 1-sided *t* test procedure was used to assess the bioequivalence of the pharmacokinetic characteristics of the 2 medications.¹⁴

As recommended by Food and Drug Administration guidance, data were log transformed. Using logarithmic transformation, the general linear statistical model employed in the analysis of bioequivalence data allows inferences about the difference between the 2 means on the log scale, which can then be retransformed into inferences about the ratio of the 2 averages (means or medians) on the original scale. Logarithmic transformation thus achieves a general comparison based on the ratio rather than the differences.¹⁹ The formulations were considered bioequivalent when the logarithmically transformed values of C_{max} , AUC_{0-t} , and $AUC_{0-\infty}$ were within the predetermined equivalence range of 80% to 125% with a 90% CI. Differences were considered significant at $P < 0.05$.

Results

Subjects

Twelve healthy Mexican volunteers (5 men and 7 women) were enrolled and all completed all periods of the study. Their mean (SD) age and weight was 24.33 (6.34) years (range = 19–38 years), and 62.54 (9.34) kg (range = 48.2–82.0 kg), respectively. The mean body mass index was 23.02 (range = 18.59–26.50). A power analysis (value expected of at least $1 - \beta = 0.8$) determined that the power of the ANOVA was > 0.8 at a 90% CI, indicating that the number of subjects enrolled in the study was sufficient.

Method validation

This method was developed and validated for specificity, sensitivity, linearity, matrix effect, precision, accuracy, and stability in our laboratory under NOM-177-SSA1-2013 specifications. In this regard, metformin quantification was not interfered with by acetaminophen, aspirin, caffeine, diclofenac, ibuprofen, heparin, loratadine, or naproxen.

The retention time for metformin was found to be 1.78 minutes. The relationship between concentration and peak area ratio was found to be linear within the range of 78.88 to 2524.25 ng/mL ($r^2 > 0.991$). The limit of quantification was 78.88 ng/mL. Accuracy from control samples at 236.65, 473.30, and 1893.19 ng/mL concentrations was 14.37%, 3.94%, and 8.81%, respectively. The intra-assay coefficient of variation was 2.20%, 4.00%, and 3.07%, respectively; and the interassay coefficient of variation was 0.18%, 0.63%, and 2.59%, respectively. No significant degradation of metformin during freezing and thawing cycles, short and long storage, or processing conditions was noted. Absence of interfering components was accepted where the response is $< 20\%$ of

Table I
Mean plasma concentration for treatment A (reference), treatment B (test), and treatment C (test).

Time (h)	Treatment A (reference) (ng/mL)	Treatment B (test) (ng/mL)	Treatment C (test) (ng/mL)
0.00	Lower than LOQ	Lower than LOQ	Lower than LOQ
0.25	Lower than LOQ	Lower than LOQ	Lower than LOQ
0.50	346.66	316.62	226.48
0.75	611.80	591.12	515.23
1.00	805.59	769.42	707.65
1.50	883.94	897.81	902.75
2.00	963.10	1034.39	993.84
2.50	1005.47	1067.74	1036.33
3.00	1022.89	1088.17	1057.12
3.50	1023.70	1073.87	1069.77
4.00	1022.28	1019.25	1048.18
6.00	553.60	562.80	603.43
8.00	302.43	297.76	334.99
12.00	Lower than LOQ	80.60	90.44
16.00	Lower than LOQ	Lower than LOQ	Lower than LOQ

LOQ = limit of quantification.

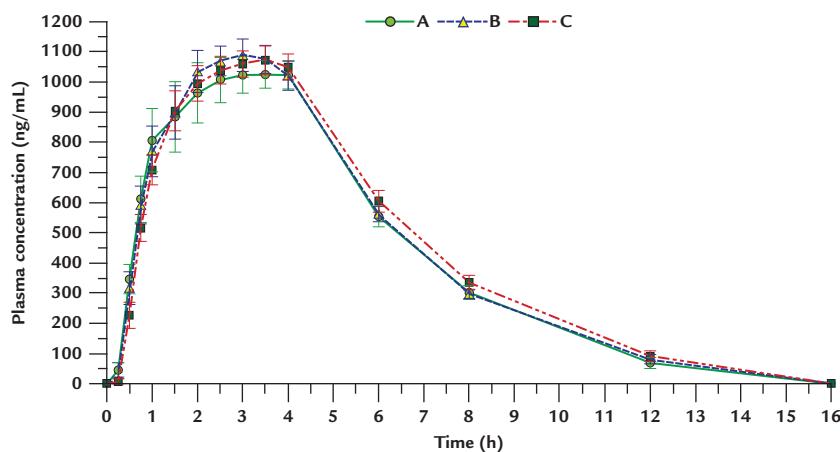


Figure 2. Plasma concentration versus time. Treatment A (reference), treatment B (test), and treatment C (test).

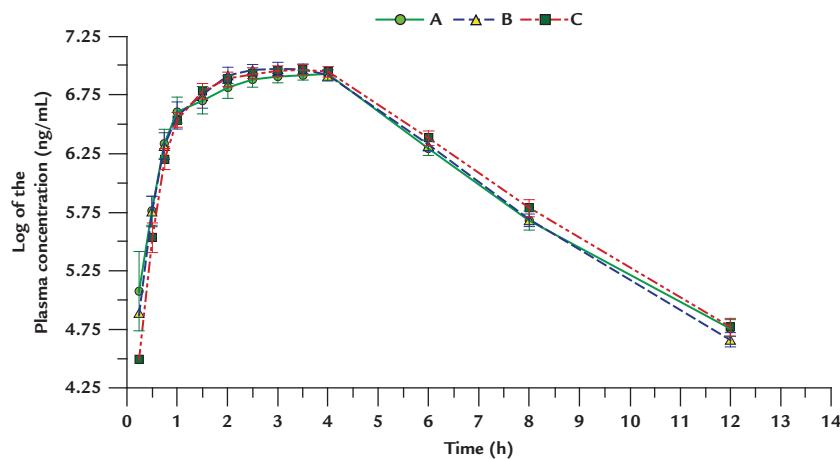


Figure 3. Log of the plasma concentration versus time. Treatment A (reference), treatment B (test), and treatment C (test).

the lower limit of quantification for the analyte. No interference from endogenous compounds, anticoagulants, or possible drugs concomitantly administered (eg, diclofenac, aspirin, acetaminophen, naproxen, loratadine, and caffeine) was observed. Comparison of Limit of quantification versus blank plasma are shown in **Figure 1**.

Plasma pharmacokinetic analysis

The mean plasma concentration for all volunteers is shown in **Table I**.

The plot of mean plasma concentration ($N = 12$) versus time for all volunteers is shown in **Figure 2 and 3**. There were no significant between-group differences for any of the pharmacokinetic parameters.

Metformin was absorbed rapidly, with a mean (SD) T_{max} of 3.0 (1.0) hours for treatment A (reference), 2.8 (0.78) hours for treatment B (test), and 3.0 (0.87) hours for treatment C (test).

The mean (SD) C_{max} was 1163.5 (295.2 ng/mL) for treatment A (reference), 1184.6 (215.0) ng/mL for treatment B (test), and 1167.8 (176.8) ng/mL for treatment C (test).

The mean (SD) AUC_{0-t} was 6240.7 (1629.4) ng/mL/h for treatment A (reference), 6433.7 (1249.8) ng/mL/h for treatment B (test), and 6567.1 (1145.5) ng/mL/h for treatment C (test).

The mean (SD) $AUC_{0-\infty}$ was 6837.3 (1618.5) ng/mL/h for treatment A (reference), 6911.8 (1178.4) ng/mL/h for treatment B (test), and 7178.6 (1086.8) ng/mL/h for treatment C (test).

The mean (SD) $t_{1/2}$ (h) 2.34 (0.34) hours for treatment A (reference), 2.38 (0.21) hours for treatment B (test), and 2.42 (0.31) hours for treatment C (test).

Table II

Pharmacokinetic parameters of metformin for treatment A (reference) versus treatment B (test).

Pharmacokinetic parameter	Treatment B: treatment A (%)	95% CI	P value	Power
$\ln(C_{max})$	102.86	95.12–111.24	0.00017	0.99772
$\ln(AUC_{0-t})$	104.46	95.98–113.68	0.00076	0.99476
$\ln(AUC_{0-\infty})$	103.48	95.50–112.13	0.00033	0.99685

Ln = natural logarithm.

Table III

Pharmacokinetic parameters of metformin for treatment A (reference) versus treatment C (test).

Pharmacokinetic parameter	Treatment C: treatment A (%)	90% CI	P value	Power
$\ln(C_{max})$	101.94	94.27–110.24	0.00010	0.99772
$\ln(AUC_{0-t})$	106.94	98.26–116.38	0.00234	0.99476
$\ln(AUC_{0-\infty})$	106.78	98.24–116.06	0.00207	0.99533

Ln = natural logarithm.

Table IV

Pharmacokinetic parameters of metformin for treatment B (test) versus treatment C (test).

Pharmacokinetic parameter	Treatment C: treatment B (%)	90% CI	P value	Power
Ln(C _{max})	99.104	91.65–107.17	0.000039	0.99772
Ln(AUC _{0-t})	102.376	94.07–111.41	0.000266	0.99476
Ln(AUC _{0-∞})	103.186	95.23–111.81	0.000285	0.99685

Ln = natural logarithm.

The test/reference ratios for the pharmacokinetic parameter AUC_{0-t}, AUC_{0-∞}, and C_{max} are shown on **Tables II, III, IV, and V**. A 90% CI was applied.

ANOVA for the crossover design was used to assess the formulation, period, and sequence effects on the plasma pharmacokinetic parameters (**Table VI**). The parameters were not significantly different in terms of variation in formulation.

Discussion

The pharmacokinetic parameters obtained in this study with the test and reference formulations were not significantly different, which reflects the comparable pharmacokinetic characteristics of the 3 formulations. These results demonstrate that the test formulations are bioequivalent to the reference product, as well as to each other.

Metformin is not metabolized and is excreted unchanged in the urine; active tubular secretion in the kidneys is the principal route of metformin elimination. The drug is widely distributed into body tissues by organic cation transporters. Genetic

polymorphisms in these transporter genes are also likely to have a direct influence on metformin pharmacokinetics and variability in drug responses.²⁰

The pharmacokinetic parameters; that is, C_{max} (1171.96 [± 227.68] ng/mL) and AUC_{0-t} (6413 [± 1325.17] ng/mL/h), are not different from data reported by Santos et al⁶ in healthy Mexican volunteers (C_{max} 1390 [± 440] ng/mL and AUC_{0-t} 7590 [± 3170] ng/mL/h).

A study of 500 mg metformin in Chinese volunteers,²¹ C_{max} 1128 [± 160] ng/mL, AUC_{0-t} 8626 [± 1800 ng/mL/h], and t_{1/2} of 3.5 [± 1.3] h were different from this study. The difference in t_{1/2} could be a reason for a bigger AUC.

The use of pharmacokinetic bioequivalence to demonstrate that generic formulations are bioequivalent is currently a matter of discussion. Several studies have shown that generic formulations are not bioequivalent to the reference product in the postmarketing setting; however, 1 generic formulation had not been compared against another generic formulation.

Some studies¹⁰ are limited by small sample size, but our study had an adequate number of healthy volunteers, and the power of

Table V

Pharmacokinetic parameters of metformin for treatment C (test) versus treatment B (test).

Pharmacokinetic parameter	Treatment B: treatment C (%)	90% CI	P value	Power
Ln(C _{max})	100.90	93.31–109.12	0.000039	0.99772
Ln(AUC _{0-t})	97.68	89.76–106.30	0.000266	0.99476
Ln(AUC _{0-∞})	96.91	89.44–105.01	0.000285	0.99685

Ln = natural logarithm.

Table VI

P values for variations between test formulations and reference metformin in healthy adult Mexican volunteers, based on formulation, period, and sequence using ANOVA.

Source of variation	Ln(C _{max})	Ln(AUC _{0-t})	Ln(AUC _{0-∞})
Formulation	0.8185	0.3987	0.4445
Sequence	0.6278	0.5878	0.6226
Period	0.4332	0.2413	0.2324

Ln = natural logarithm.

the statistical analysis is appropriate to establish bioequivalence among the three formulations of metformin.

Conclusions

The HPLC-MS/MS detection used for metformin quantification provided the appropriate sensitivity, specificity, and high sample throughput required for pharmacokinetic studies.

Our study established that the test formulation 500-mg metformin tablets were bioequivalent to the reference formulation and to each other, according to the general laws of health care in Mexico in this population of healthy adult Mexican volunteers; therefore, all treatments can be safely substituted.

Acknowledgments

The authors thank the staff of the Departamento de Farmacología y Toxicología, Universidad Autónoma de Nuevo León. Lourdes Garza Ocaña is responsible for study design, data interpretation, writing. Dr. Sandra Montoya Eguía is responsible for literature search, study design, data collection, data interpretation, writing. Dr. Christian T. Badillo Castañeda is responsible for data collection, data interpretation, writing. Dr Eduardo Taméz de la O and Dr. Teresa Zanatta Calderón are responsible for data collection. Marco Vinicio Gómez Mesa is responsible for statistic analysis, figure creation, data interpretation. MC Humberto J. Garza-Ulloa is responsible for study design.

Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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