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INFLUENCE OF PIPERINE ON PHARMACODYNAMICS AND PHARMACOKINETICS OF REPAGLINIDE IN RABBITS

PAMU S[1,2], THAKKALAPALLY L[3], PATYAR RR[4] AND PATYAR S[2*]

1: School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab-144411

2: Anand Pharmacy College, Anand, Gujarat, India-388001

3: Orbicular Pharmaceutical Technologies Pvt Ltd, R & D Center, ALEAP Industrial Estate, Behind Pragathinagar, Kukatpally, Hyderabad, Telangana, India – 500 090

4: Department of Technical Education and Industrial Training, Government of Punjab (India)

***Corresponding Author: Dr. Sazal Patyar: E Mail: sazal.17050@lpu.co.in**

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ABSTRACT

Background: Dietary alkaloids such as piperine can cause significant pharmacodynamic and pharmacokinetic food-drug interactions which can affect drug metabolism and elimination. Piperine's impact on the pharmacodynamics and pharmacokinetics of repaglinide is investigated in this study.

Method: The study was conducted in normal and diabetic rabbits to estimate the single and multi-dose interactions. In normal and diabetic rabbits, repaglinide alone and in combination with piperine were administered. Rabbits were treated for 21 days and blood samples were collected on day 1, 3, 7, 14 & day 21. For investigating the pharmacodynamic-interactions, blood glucose levels were estimated while pharmacokinetic parameters were determined to investigate the pharmacokinetic interactions.

Results: The study results found that combination of piperine with repaglinide significantly decreased blood glucose levels. Additionally, the combination elicited more percent blood

glucose reduction than repaglinide alone treatment in normal as well as diabetic rabbits. In pharmacokinetic studies, repaglinide with piperine significantly increased the $T_{1/2}$, AUC_{0-t}, $AUC_{0-\infty}$, $AUMC_{0-t}$, $AUMC_{0-\infty}$, MRT_{0-t} & $MRT_{0-\infty}$ and decreased the CL & K_e of repaglinide as compared with alone repaglinide in normal and diabetic rabbits on day 1 and day 21. The study results suggested that combination of piperine with repaglinide showed the synergistic effect on blood glucose levels and piperine substantially improved the bioavailability of repaglinide in both normal and diabetic rabbits.

Conclusion: On the basis of findings obtained from pharmacodynamic and pharmacokinetic studies, it may be concluded that piperine shows significant pharmacodynamic and pharmacokinetic interactions with repaglinide thereby altering its pharmacodynamic and pharmacokinetic parameters. Thus it is recommended that diabetic individuals should be monitored on a regular basis to prevent hypoglycemic effects when repaglinide and piperine are taken together.

Keywords: Piperine, repaglinide, pharmacokinetics drug interaction, diabetes, rabbits

INTRODUCTION

The study of food-drug interactions and their underlying mechanisms is very important so as to provide rational drug therapy **[1]**. Some dietary products elicit significant food-drug interactions, which can affect drug metabolism and elimination **[2]**. One such dietary alkaloid is piperine, which is an active constituent of black pepper and is used as household spice **[3]**. Piperine shows some beneficial pharmacological effects such as anti-hyperglycemic effect, anti-hyperlipidemic effect and antioxidant activity etc. **[4]**.

Piperine is a thermonutraceutical which has been reported to exhibit β-agonistic action on adrenergic receptors **[5]**. Piperine activates β3-receptors and increases the proliferation of β-cells which produce more insulin thereby lowering blood glucose levels **[6]**. Piperine also acts as a metabolic inhibitor in several cytochrome P-450 (such as CYP3A4, CYP1A2, CYP2D6 and CYP2C9) mediated pathways **[7-9]**. Thus piperine has a potential to affect the pharmacodynamics and pharmacokinetics of different drugs.

Repaglinide acts on sulfonylurea receptors on pancreatic β cells to promote insulin secretion and suppresses postprandial elevation of plasma glucose. Repaglinide is used to decrease postprandial hyperglycemia and raise glycated hemoglobin levels related to Type-2 diabetes **[10]**. It is also a novel,

short acting hypoglycemic agent for Type-2 diabetes **[11]**. So, patients taking repaglinide should be monitored regularly. Studies have shown that repaglinide is metabolized by oxidation and de-alkylation by CYP3A4 & CYP2C8 enzymes **[12]**. Since there is a possibility of concomitant use of repaglinide with piperine in diabetes, there is also a possibility of piperine-repaglinide drug interaction as piperine may inhibit CYP3A4 metabolic enzyme of repaglinide. Hence this study aimed to explore the effect of piperine on the pharmacodynamics and pharmacokinetics of repaglinide.

MATERIALS AND METHODS

Animals

Rabbits weighing 1.8-2.5 kg of either sex were purchased from Muppandal Genomics and Immunologicals Pvt Ltd, Nacharam, Hyderabad. The rabbits were provided access to uncooked vegetable diet with water *ad libitum*. The animal experimentation was carried out as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and the protocol design was approved by the CPCSEA-Institutional Animal Ethics Committee (IAEC), Guru Nanak Institute of Pharmacy, Ranga Reddy District, Telangana (Ref 04/GNIP/CPCSEA/IAEC/2019). Rabbits were subjected to a constant daily cycle of 12 hours of light and 12 hours of darkness (06.00-18.00), constant temperature (21 ± 3^{o}C) and relative humidity of 55 ± 15%.

Drugs and chemicals

Piperine and repaglinide were received as gift samples from Herbochem (Balanagar, Hyderabad, India) and Dr. Reddy's Laboratories Ltd. (Patancheru, Hyderabad, India) respectively. Streptozotocin was bought from Sisco Research Laboratories Pvt. Ltd. (Hyderabad, India). For High Performance Liquid Chromatography (HPLC) sampling analysis, diazepam was used as an internal standard (RL Fine Chem, Bengaluru, India) and chemicals like dichloromethane (Balaji Formulations Pvt Ltd, Hyderabad, India) and diethyl ether (Standard Reagents Pvt Ltd, Hyderabad, India) were used as mobile phase.

Preparation of drug solutions

Piperine solution (20mg/ml): Piperine (was dissolved in 2% gum acacia for oral administration.

Repaglinide solution (0.3mg/ml): Repaglinide was dissolved in a few drops of 0.1N NaOH then made up to the volume with distilled water for oral administration.

Streptozotocin solution (65mg/ml): Streptozotocin was dissolved in citrate buffer (pH 4.5) for intraperitoneal administration.

Experimental design

Four groups of rabbits were included in the study and each group comprised of 4 rabbits. The study was performed in normal and diabetic rabbits to estimate the single and multiple dose piperine-repaglinide interactions. Diabetes was induced by streptozotocin-nicotinamide to the overnight fasted rabbits. The doses for piperine and repaglinide were selected as 20 mg/kg/po and 0.3 mg/kg/po respectively. The study design was as follows:

Group 1: Normal rabbits treated with Repaglinide (0.3mg/kg/po)

Group 2: Normal rabbits treated with Piperine (20mg/kg/po) and Repaglinide (0.3mg/kg/po)

Group 3: Diabetic rabbits treated with Repaglinide (0.3mg/kg/po)

Group 4: Diabetic rabbits treated with Piperine (20mg/kg/po) and Repaglinide (0.3mg/kg/po)

These groups were treated for 21 days and blood samples were collected from each rabbit on day 1, 3, 7, 14 & 21 for blood glucose levels estimation and on day 1 & day 21 at predetermined time intervals of 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8 hr for pharmacokinetic parameters estimation.

Collection of blood samples

Blood samples (≈2 mL) were collected from the marginal ear vein of each rabbit at specified time intervals and 10% ethylene-diamine-tetra-acetic acid (EDTA) solution (10µl EDTA in 1ml of blood) was added and vortexed. Blood samples were then centrifuged at 2000-3000 rpm for 15 mins at 2-8^{o}C. Plasma was collected (translucent upper layer) and saved for further studies. Collected plasma samples were transferred into previously labeled tubes and stored at 1-6^{o}C.

Pharmacodynamic studies

The study evaluated the blood glucose levels by GOD-POD method and calculated the percent blood glucose reduction. The blood glucose levels and percent blood glucose reduction comparisons were done for treated groups of repaglinide in absence and presence of piperine on day 1 (for single dose study) and on day 3, 7, 14 & 21 (for multiple dose study) in normal and diabetic rabbits.

Plasma sample preparation for HPLC analysis

The sample preparation involved the liquid-liquid extraction technique. An aliquot of human plasma sample (1 mL) was taken in 15 mL stoppered test tubes followed by addition of 50 mL of diazepam (10mg/mL) working solution (internal standard) and was vortex-mixed for 1 min. To this, added the 5 ml of dichloromethane:diethyl ether (4:6,

v/v) and mixed on a reciprocating shaker at 100 strokes/min for 30 min. For effect phase separation, mixed solutions were centrifuged for 10 mins at 3000 rpm. After centrifugation, 4 ml of supernatant organic layer was transferred to other tube and vaporized to dry. The residue was reconstituted in mobile phase (250 ml) and exposed to HPLC analysis.

Pharmacokinetic analysis

The study estimated the repaglinide plasma concentrations and evaluated the non-compartmental pharmacokinetic parameters such as peak plasma concentrations (C_{max}), time taken to reach C_{max} (T_{max}), terminal half-life ($T_{1/2}$), area under curve (AUC_{0-t} & $AUC_{0-\infty}$), area under first moment curve ($AUMC_{0-t}$ & $AUMC_{0-\infty}$), elimination rate constant (K_e), mean residence time (MRT_{0-t} & $MRT_{0-\infty}$), clearance (CL) and volume of distribution (V_d) by using the pharmacokinetic software WinNonlin Standard version 4.1. The pharmacokinetic significant comparisons of repaglinide were done in absence and presence of piperine in normal and diabetic rabbit's groups separately for day 1 (single dose of repaglinide and in combination with piperine) and day 21 (multi dose of repaglinide and in combination with piperine).

RESULTS

Influence of piperine on the pharmacodynamics of repaglinide in normal rabbits

Figure 1 shows the mean percent blood glucose reduction by repaglinide in absence and presence of piperine on day 1, 3, 7, 14, 21 in normal rabbits. Repaglinide along with piperine resulted in significant decrease in blood glucose levels **(Table 1)** in normal rabbits and significantly increased percent blood glucose reduction **(Table 2)** as compared with repaglinide alone therapy for both single and multiple dose treatment. However, the study results showed multidose treatment was more effective than single dose treatment in normal rabbits **(Table 1, 2)**. This study indicated that piperine alters the pharmacodynamics of repaglinide by showing hypoglycemic activity in normal rabbits for single dose and multi dose treatment.

Influence of piperine on the pharmacodynamics of repaglinide in diabetic rabbits

Figure 2 shows the mean percent blood glucose reduction by repaglinide in absence and presence of piperine on day 1, 3, 7, 14, 21 in diabetic rabbits. Repaglinide along with piperine resulted in significant decreased blood glucose levels in diabetic rabbits

(Table 3) and significantly increased percent blood glucose reduction **(Table 4)** as compared with repaglinide alone therapy for both single and multiple dose treatment. However, the study results showed multidose treatment was more effective than single dose therapy in diabetic rabbits **(Table 3 and Table 4)**. This study found that piperine alters the pharmacodynamics of repaglinide by showing anti-hyperglycemic activity in diabetic rabbits for single dose and multi dose therapy.

Influence of piperine on the pharmacokinetics of repaglinide in normal rabbits

Figure 3 shows the mean plasma concentration-time curve of repaglinide in the absence and presence of piperine in normal rabbits on days 1 and 21. The mean plasma concentration of repaglinide with piperine in normal rabbits significantly increased by 5.44% and 7.11% in single dose and multi-dose study respectively as compared with alone repaglinide. In normal rabbits, on day 1 and day 21, it found that repaglinide along with piperine administration significantly increased the $T_{1/2}$, AUC_{0-t}, $AUC_{0-\infty}$, $AUMC_{0-t}$, $AUMC_{0-\infty}$, MRT_{0-t} & $MRT_{0-\infty}$, and significantly decreased the K_e & CL of repaglinide when compared with alone repaglinide. T_{max} of repaglinide showed no change and the V_d was non-significantly altered on concomitant administration of piperine in normal rabbits. **Table 5** shows the pharmacokinetics of repaglinide in normal rabbits in the absence and presence of piperine for single dose and multi-dose studies. This study found that piperine alters the pharmacokinetics of repaglinide in normal rabbits also.

Influence of piperine on the pharmacokinetics of repaglinide in diabetic rabbits

Figure 4 shows the mean plasma concentration-time curve of repaglinide in the absence and presence of piperine in diabetic rabbits on days 1 and 21. The mean plasma concentration of repaglinide with piperine in diabetic rabbits significantly increased by 6.68% and 6.74% in single dose and multi-dose study respectively as compared with alone repaglinide. In diabetic rabbits, on day 1 and day 21, it was found that repaglinide with piperine significantly increased the $T_{1/2}$, AUC_{0-t}, $AUC_{0-\infty}$, $AUMC_{0-t}$, $AUMC_{0-\infty}$, MRT_{0-t} & $MRT_{0-\infty}$ of repaglinide as compared with alone repaglinide. The K_e of repaglinide significantly decreased on day 1 and non-significantly altered on day 21, whereas CL of repaglinide significantly decreased on day 1 and day 21 on concomitant administration of piperine. T_{max}

of repaglinide found with no change and the V_d was non-significantly altered on concomitant administration of piperine in diabetic rabbits. Table 6 shows the pharmacokinetics of repaglinide in diabetic rabbits in the absence and presence of piperine for single dose and multi-dose studies. The study found that piperine can alter the pharmacokinetics of repaglinide in diabetic rabbits on both day 1 and day 21.

Table 1: Mean blood glucose levels in normal rabbits

Type of Treatment	Mean ± standard deviation of blood glucose levels (mg/dl)				
	Day	Control	Piperine	Repaglinide	Piperine + Repaglinide
SDT	0	106.62 ± 1.44	107.92 ± 3.21	106.84 ± 2.73	107.32 ± 2.65 ns
	1	106.22 ± 1.48	105.76 ± 3.48	77.11 ± 0.26	70.79 ± 2.18 **
MDT	3	107.79 ± 1.67	104.70 ± 3.20	75.93 ± 0.54	69.18 ± 2.74 **
	7	106.93 ± 0.67	102.92 ± 2.43	70.56 ± 0.60	66.19 ± 1.57 **
	14	105.71 ± 0.91	102.26 ± 2.31	68.51 ± 0.63	64.53 ± 1.22 **
	21	105.38 ± 0.84	101.52 ± 2.39	65.39 ± 0.71	61.12 ± 1.57 **

SDT- Single dose treatment, MDT-Multi dose treatment; ns-nonsignificant, **P<0.01 when compared with repaglinide alone significance comparison was done by one-way anova followed by Tukey's multiple comparison

Table 2: Mean percent blood glucose reduction in normal rabbits

Type of Treatment	Mean ± standard deviation of percent blood glucose reductions (%)				
	Day	Control	Piperine	Repaglinide	Piperine + Repaglinide
SDT	1	0.36 ± 1.58	2.00 ± 0.61	27.79 ± 1.96	34.01 ± 2.67 **
MDT	3	-1.12 ± 2.39	2.98 ± 0.54	28.89 ± 2.18	35.51 ± 3.22 **
	7	-0.30 ± 1.45	4.62 ± 0.64	33.93 ± 1.49	38.31 ± 1.99 **
	14	0.84 ± 0.55	5.23 ± 0.76	35.85 ± 1.67	39.86 ± 1.51 **
	21	1.15 ± 1.75	5.91 ± 0.67	38.78 ± 1.22	43.03 ± 1.99 **

SDT- Single dose treatment, MDT-Multi dose treatment
(% Blood Glucose Reduction = 0^{th} day sample blood glucose levels/Day of collected sample blood glucose levels after drug administration's X 100)
ns-nonsignificant, **P<0.01 when compared with repaglinide alone significance comparison was done by one-way anova followed by Tukey's multiple comparison

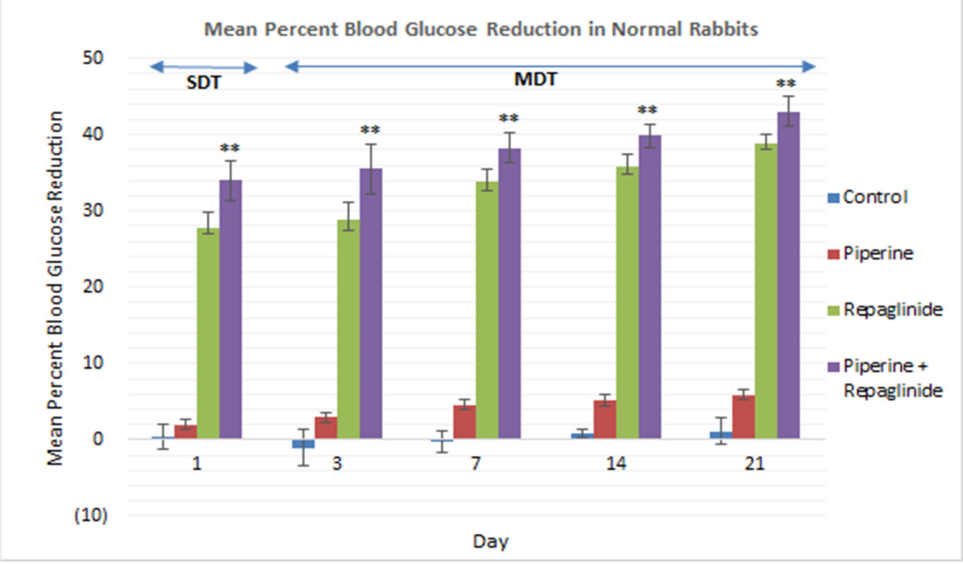

Figure 1: Mean percent blood glucose reduction in normal rabbits
SDT- Single dose treatment, MDT-Multi dose treatment; ns-nonsignificant, **P<0.01 when compared with repaglinide alone significance comparison was done by one-way anova followed by Tukey's multiple comparison

Table 3: Mean blood glucose levels in diabetic rabbits

Type of Treatment	Mean ± standard deviation of blood glucose levels (mg/dl)				
	Day	Control	Piperine	Repaglinide	Piperine + Repaglinide
	0	284.11 ± 1.94	284.85 ± 1.32	285.59 ± 3.93	284.56 ± 3.48 ns
SDT	1	303.00 ± 3.55	207.57 ± 1.47	201.30 ± 1.64	194.42 ± 2.44 **
MDT	3	324.12 ± 2.81	199.78 ± 1.11	193.51 ± 1.03	186.73 ± 3.16 **
	7	358.60 ± 5.71	193.6 ± 1.26	182.58 ± 1.35	172.32 ± 2.26 **
	14	371.03 ± 3.49	188.53 ± 0.93	174.43 ± 3.13	164.60 ± 2.44 **
	21	392.06 ± 4.68	182.28 ± 0.65	169.99 ± 3.26	158.88 ± 2.27 **

SDT- Single dose treatment, MDT-Multi dose treatment; ns-nonsignificant, **P<0.01 when compared with repaglinide alone significance comparison was done by one-way anova followed by Tukey's multiple comparison.

Table 4: Mean percent blood glucose reduction in diabetic rabbits

Type of Treatment	Mean ± standard deviation of percent blood glucose reductions (%)				
	Day	Control	Piperine	Repaglinide	Piperine + Repaglinide
SDT	1	-6.65 ± 0.63	27.13 ± 0.57	29.50 ± 1.39	31.68 ± 0.62 *
MDT	3	-14.08 ± 0.82	29.87 ± 0.43	32.24 ± 0.87	34.38 ± 1.07 *
	7	-26.23 ± 2.45	32.03 ± 0.31	36.06 ± 1.30	39.44 ± 0.76 *
	14	-30.59 ± 1.05	33.81 ± 0.41	38.90 ± 1.91	42.15 ± 1.11 *
	21	-37.99 ± 0.94	36.01 ± 0.30	40.46 ± 1.94	44.16 ± 0.77 *

SDT- Single dose treatment, MDT-Multi dose treatment
ns-nonsignificant, *P<0.05 when compared with repaglinide alone
significance comparison was done by one-way anova followed by Tukey's multiple comparison.

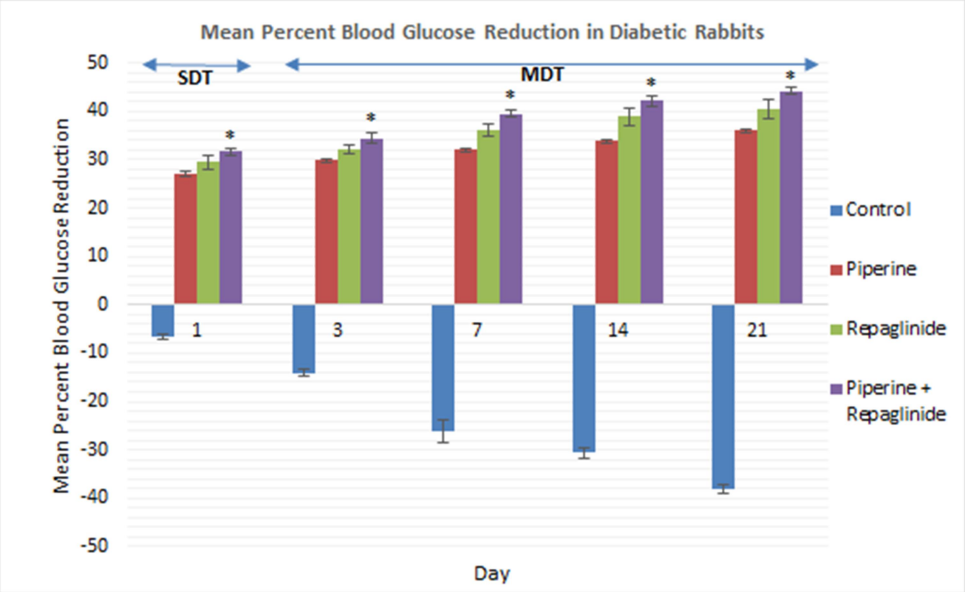

Figure 2: Mean percent blood glucose reduction in diabetic rabbits
SDT- Single dose treatment, MDT-Multi dose treatment; ns-nonsignificant, *P<0.05 when compared with repaglinide alone; significance comparison was done by one-way anova followed by Tukey's multiple comparison

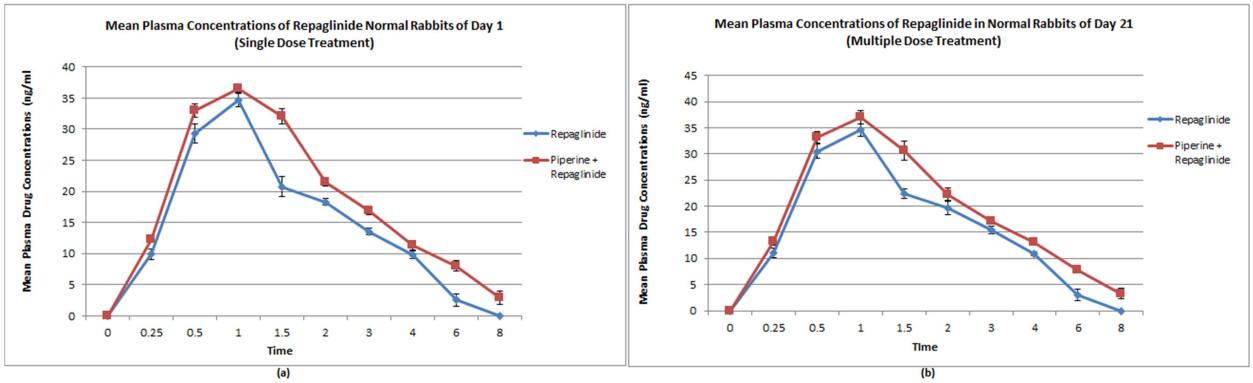

Figure 3: Mean plasma drug concentrations of repaglinide in absence and presence of piperine in normal rabbits of day 1 (a) and day 21 (b)

Table 5: Mean pharmacokinetics of repaglinide in absence and presence of piperine in normal rabbits on day 1 (single dose treatment) and day 21 (multi dose treatment)

Mean pharmacokinetics of repaglinide (ng/ml) in normal rabbits				
PK Parameters	Day 1 (Single dose interaction study)		Day 21 (Multi dose interaction study)	
	Repaglinide	Piperine + Repaglinide	Repaglinide	Piperine + Repaglinide
C_{max} (ng/ml)	34.68 ± 1.10	36.57 ± 0.59 *	34.56 ± 1.22	37.02 ± 1.23 *
T_{max} (h)	1 ± 0	1 ± 0.00 ns	1 ± 0.00	1 ± 0.00 ns
AUC_{0-t} (ng/ml*h)	85.75 ± 1.35	118.78 ± 4.04 ****	92.15 ± 0.35	121.83 ± 2.78 ****
$AUC_{0-\infty}$ (ng/ml*h)	91.07 ± 4.39	128.13 ± 8.06 ***	98.15 ± 3.75	132.12 ± 5.85 ****
$AUMC_{0-t}$ (ng/ml*h^2)	181.52 ± 7.52	320.1 ± 22.40 ****	199.45 ± 6.49	331.22 ± 13.02 ****
$AUMC_{0-\infty}$ (ng/ml*h^2)	224.99 ± 37.51	425.38 ± 76.71 **	248 ± 36.64	446.89 ± 62.99 **
MRT_{0-t} (h)	2.12 ± 0.06	2.69 ± 0.10 ****	2.16 ± 0.06	2.72 ± 0.06 ****
$MRT_{0-\infty}$ (h)	2.46 ± 0.30	3.3 ± 0.40 *	2.53 ± 0.28	3.37 ± 0.35 **
K_e (1/h)	0.57 ± 0.18	0.34 ± 0.06 *	0.56 ± 0.14	0.33 ± 0.05 *
$T_{1/2}$ (h)	1.31 ± 0.41	2.11 ± 0.39 *	1.3 ± 0.35	2.15 ± 0.32 *
CL (mg/kg)/(ng/ml)/h	0.0033 ± 0.0002	0.0023 ± 0.0002 ***	0.0031 ± 0.0001	0.0023 ± 0.0001 ****
V_d (L)	0.0062 ± 0.0016	0.0071 ± 0.0009 ns	0.0057 ± 0.0013	0.0070 ± 0.0008 ns

Data represents Mean ± Standard deviation; ns-nonsignificant, *significant at P<0.05, **significant at P<0.01, ***significant at P<0.001, ****significant at P<0.0001 when compared with alone Repaglinide significance comparison was done by students t-test

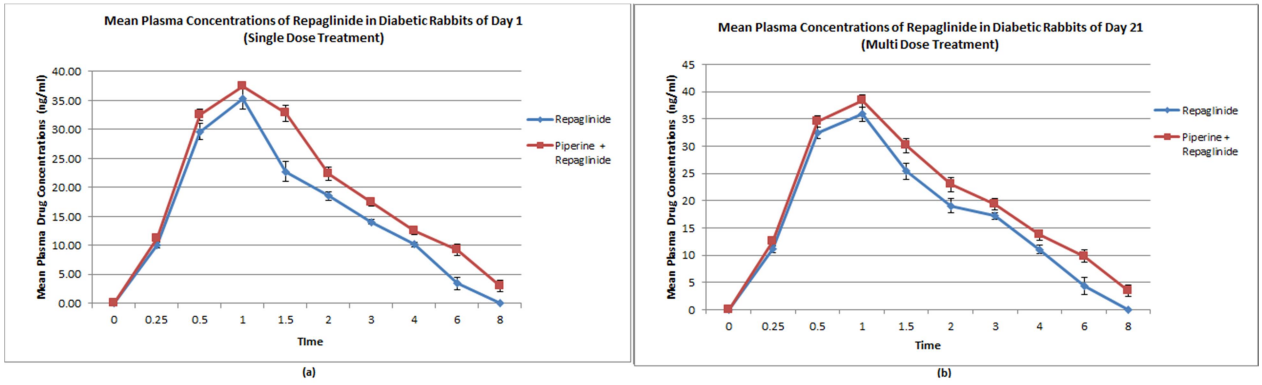

Figure 4: Mean plasma drug concentrations of repaglinide in absence and presence of piperine in diabetic rabbits of day 1 (a) and day 21 (b)

Table 6: Mean pharmacokinetics of repaglinide in absence and presence of piperine in diabetic rabbits on day 1 (single dose treatment) and day 21 (multi dose treatment)

Mean pharmacokinetics of repaglinide (ng/ml) in diabetic rabbits				
PK Parameters	Day 1 (Single dose interaction study)		Day 21 (Multi dose interaction study)	
	Repaglinide	Piperine + Repaglinide	Repaglinide	Piperine + Repaglinide
C_{max} (ng/ml)	35.28 ± 1.75	37.64 ± 0.49 *	35.87 ± 1.27	38.29 ± 1.15 *
T_{max} (h)	1 ± 0.00	1 ± 0.00 ns	1 ± 0.00	1 ± 0.00 ns
AUC_{0-t} (ng/ml*h)	89.22 ± 1.31	117.65 ± 3.55 ****	97.91 ± 3.15	130.43 ± 4.49 ****
$AUC_{0-\infty}$ (ng/ml*h)	97.5 ± 3.98	129.1 ± 3.99 ****	108.14 ± 8.58	142.00 ± 8.82 **
$AUMC_{0-t}$ (ng/ml*h^2)	192.25 ± 6.38	324.17 ± 14.80 ****	216.1 ± 14.08	369.06 ± 24.81 ****
$AUMC_{0-\infty}$ (ng/ml*h^2)	262.09 ± 37.75	456.8 ± 69.27 **	302.63 ± 67.90	500.09 ± 82.02 **
MRT_{0-t} (h)	2.15 ± 0.07	2.75 ± 0.07 **	2.21 ± 0.08	2.83 ± 0.10 ****
$MRT_{0-\infty}$ (h)	2.68 ± 0.28	3.53 ± 0.43 *	2.77 ± 0.41	3.51 ± 0.36 *
K_e (1/h)	0.43 ± 0.06	0.3 ± 0.05 *	0.48 ± 0.13	0.31 ± 0.04 ns
$T_{1/2}$ (h)	1.62 ± 0.22	2.33 ± 0.42 *	1.53 ± 0.41	2.23 ± 0.29 *
CL (mg/kg)/(ng/ml)/h	0.0031 ± 0.0001	0.0023 ± 0.0002 ****	0.0028 ± 0.0002	0.0021 ± 0.0001 **
V_d (L)	0.0072 ± 0.0007	0.0078 ± 0.0012 ns	0.006 ± 0.0012	0.0068 ± 0.0005 ns

Data represents Mean ± Standard deviation; ns-nonsignificant, *significant at P<0.05, **significant at P<0.01, ***significant at P<0.001, ****significant at P<0.0001 when compared with alone Repaglinide significance comparison was done by students t test

DISCUSSION

Diabetes mellitus is a severe metabolic disorder characterized by absolute or relative insufficiency in insulin secretion and/or its action **[13]**. Repaglinide (meglitinide) is the preferred drug of choice in the treatment of type-2 diabetes mellitus as anti-hyperglycemic agent, but it also acts as short acting hypoglycemic agent **[14]**. Repaglinide enhances insulin secretion by acting on pancreatic β-cells and it is primarily metabolized by CYP3A4 & CYP2C8 enzymes **[12]**. Piperine is thermonutraceutical which also proliferates β-cells and increases insulin secretion **[6]**. Piperine has been reported to possess antidiabetic action per se **[15]** and it also suppresses CYP3A4 metabolic enzyme **[7]**.

In the current study, pharmacodynamic results showed blood glucose levels were significantly decreased on co-administration of repaglinide and piperine as compared with repaglinide alone treatment in normal and diabetic rabbits. This decreased blood glucose levels were reported in single dose and multi-dose studies. Based on decreased blood glucose levels it was found that there was significant increase in percent blood reduction in normal and diabetic rabbits for single and multi-dose study. Sama *et al*., reported that piperine and nateglinide synergistically impacted the β-cells to release more insulin **[16]**. Based on findings of this study, it is suggested that piperine and repaglinide have a synergistic effect on β-cells, causing them to produce more insulin and lowering blood glucose levels, as seen by enhanced antihyperglycemic activity in diabetic rabbits or enhanced hypoglycemic activity in normal rabbits. However, multi-dose treatment is more effective than single dose treatment and demonstrates that

piperine boosts the action of repaglinide at its usual dosage.

The study results showed that piperine significantly increased the C_{max}, reduced clearance and increased $T_{1/2}$ of repaglinide contributed to a clear increase in AUC of repaglinide in normal and diabetic rabbits in single dose and multi-dose study. Piperine seemed to increase plasma drug concentrations of antidiabetic drugs such as glimepiride and gliclazide, possibly by the inhibition of CYP2C9 metabolic pathway **[17, 18]**. Piperine also increased the plasma drug concentrations of other drugs, such as pioglitazone and docetaxel, by CYP3A4 and P-gp inhibition **[19, 20]**. Based on the literatures and study results, it is suggested that piperine induces time-dependent suppression of repaglinide metabolism, which leads in slower clearance and, as a result, higher repaglinide plasma concentrations, as reflected by pharmacokinetic findings such as decreased CL/K_{el} and increased $MRT/T_{1/2}$. Above discussed results provide the evidence that piperine significantly improves the bioavailability of repaglinide in presence of piperine in normal and diabetic rabbits.

CONCLUSION

This study concludes that piperine showed a pharmacodynamic interaction with repaglinide in diabetic rabbits. Piperine also showed the pharmacokinetic interaction by showing an increased bioavailability of repaglinide. Because the interaction was shown in rabbits, it is likely to occur in humans as well, resulting in significantly increased repaglinide activity, which may necessitate dose adjustments especially for hypoglycemic condition. As a result, when this combination is recommended for therapeutic benefit, caution should be taken.

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