Original Article

Bioavailability and biological activity of liquisolid compact formula of repaglinide and its effect on glucose tolerance in rabbits

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Summary **This study is an extension of the previous enhancement of dissolution properties of repaglinide using liquisolid compacts. The development and validation of a high-performance liquid chromatography (HPLC) assay for the determination of repaglinide concentration in rabbit plasma for pharmacokinetic studies is described. Repaglinide optimizing formula was orally administered to rabbits and blood samples were used to determine the pharmacokinetic parameters of repaglinide, which were compared to pharmacokinetic parameters of marketed tablets (Novonorm 2 mg). Also, to investigate the biological activity of this new formula, in comparison with the commercial product, oral glucose tolerance tests (OGTT), area under the curve and insulin levels were studied. Moreover, we studied the efficacy and safety of this new formula in several potencies (0.5, 1, and 2 mg) and blood glucose, insulin, kidney and liver functions. The relative bioavailability of repaglinide from its liquisolid compact formula was found to be increased significantly in comparison to that of the marketed tablet. In regard to urea and creatinine, no significant change was recorded after the administration of the commercial and the three potencies of the new formulation compared with the control group. Similarly, in liver function tests (serum glutamic pyruvic transaminase, SGPT), there were no changes observed in its level. Regarding insulin levels, the commercial formula increased insulin levels insignificantly (3.52% change) while the new formula increased the insulin level significantly with a percent change of 37.6%. The results of the glucose tolerance test showed that the blood glucose level was decreased significantly after the commercial drug (percent change, 18.1%) while in groups treated with the new formulation the decrease was highly significant ($p < 0.01$) with a percent change of 29.98%. The change in area under the curve for blood glucose was significantly higher in the commercial drug plus glucose load than in the new formulation plus glucose load group ($p < 0.05$) in the periods of 30-45 min and 45-60 min. Furthermore, the new repaglinide formulation significantly decreased blood glucose levels more than the commercial formula.**

Keywords: Repaglinide, glucose tolerance, bioavailability, insulin, pharmacokinetics

1. Introduction

Type 2 diabetes mellitus is a complex heterogeneous metabolic disorder in which peripheral insulin resistance and impaired insulin release are the main pathogenetic factors. The rapid response of pancreatic beta-cells to glucose is already markedly disturbed in the early stages of type 2 diabetes mellitus. The consequence is often postprandial hyperglycemia, which seems to be extremely important in the development of secondary complications, especially macrovascular disease. Meglitinide analogues are a class of oral hypoglycemic agents that increase insulin secretion, in particular, during the early phase of insulin release (*1*,*2*).

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The starting point for type 2 diabetes therapy is a change in lifestyle, especially diet. Unfortunately, dietary and lifestyle measures alone achieve adequate glycemic control in only a minority of patients. Thus, oral hypoglycemic drugs are routinely prescribed for type 2 diabetic patients. Some of these drugs, such as metformin and thiazolidenediones, primarily exert their effects on extra-pancreatic insulin-target tissues while others directly target the pancreatic β-cell and induce insulin secretion (*3*).

Repaglinide is a new carboxymethyl benzoic acid derivative, also known as 2-ethoxy-4-[2-[[3-methyl-1-[2-(1-piperidinyl) phenyl] butyl] amino]-2-exoethyl] benzoic acid. It is a novel post prandial glucose regulator for the treatment of type 2 diabetes mellitus (*4*,*5*). It reduces fasting glucose concentrations in patients with type 2 diabetes mellitus. It helps to control blood sugar by stimulating release of insulin from the pancreatic β-cell by closure of K_{ATP} channels (*6*). Repaglinide is rapidly absorbed from the gastrointestinal tract after oral administration. It differs from other antidiabetic agents in its structure, binding profile, duration of action and mode of excretion (*7*).

Several analytical methods are available for the determination of repaglinide in biological fluids, including an HPLC method and liquid chromatography-tandem mass spectrometry (LC/MS/MS) (*8-10*). However, these methods are costly and require the availability of expensive equipment.

The present study aimed *first* to develop a simple, rapid, easy to handle, accurate and inexpensive method for the quantification of repaglinide in plasma with a low limit of quantification (< 10 ng/mL). Application of a modified HPLC method for investigation of its pharmacokinetic and a bioequivalence study of the commercially available product versus the new repaglinide liquisolid compact formula as representative of the most promising formula according to the previous study was investigated (*11*). *Second*, the study aimed to investigate the biological activity of this new repaglinide liquisoild compact formula in comparison, with the commercial ones. This included their effect on oral glucose tolerance tests (OGTT), area under the curve and insulin levels. The study also illustrated the efficacy and safety of this new formulation by estimating the effect of several potencies of this new formulation (0.5, 1, and 2 mg) on blood glucose, insulin, kidney, and liver function.

2. Materials and methods

2.1. *Reagents*

Repaglinide was provided by Amoun Pharma, Egypt. Indomethacin was purchased from Sigma-Aldrich, St. Louis, Mo, USA. Coarse granular microcrystalline cellulose (Avicel PH101) was from FMC, Philadelphia, PA, USA; polysorbate 80 and sodium starch glycolate (explotab) were purchased from Sigma Chemical Co., USA; calcium silicate was from BDH Chemicals, Ltd., Poole, UK; acetonitrile HPLC grade was from Ramil Chemicals, Liece, UK; ammonium acetate, potassium dihydrogen phosphate, sodium hydroxide, magnesium stearate, and sodium cirtate were from El-Nasr Pharmaceutical Co., Egypt; ethyl acetate, ethanol, and isoamyl alcohol were from Merck, Darmstadt, Germany.

Table 1. Composition of repaglinide liquisolid compact formulation per 100 tablets

Ingredients	Dose (mg)		
	0.5	1	2
Drug (mg)	50	100	200
Liquid (mg)	100	200	400
Avicel PH101 (g)	0.526	1.05	2.11
Calcium silicate (g)	0.105	0.211	0.421
Explolab (mg)	39.1	78.2	156.3
Magnesium stearate (mg)	7.82	15.63	31.26
100 tablet (mg)	828	1657	3314

2.2. *Equipment*

The rotary tablet machine M 912-512L using a 14-standard round flat-face punch was from Stokes, USA and the compression force was adjusted to obtain tablets with hardness up to 7 kg. Vortex-mixer was Heidolph Reax Top; Heidolph Instruments, Germany, and centrifuge was Universal 16, Hettich, Germany.

2.3. *Preparation and mixing of the powders of repaglinide liquisolid compact* (*11*)

The liquisolid compact of repaglinide containing polysorbate 80 as liquid medication, Avicel PH_{101} as a carrier and calcium silicate as a coat at excipient ratio 5, showing the most promising liquisolid compact formula for enhancement of repaglinide dissolution rate, was prepared according to the previous study (*11*). Table 1 represents the composition of repaglinide liquisolid compact formulation per 100 tablets. The concentration of repaglinide in polysorbate 80 used as liquid medication was 50% (*12-14*).

The repaglinide dose (0.5, 1, or 2 mg) was manually mixed with polysorbate 80 in the required ratio using a porcelain mortar until a homogenous mixture was obtained. The carrier (Avicel PH_{101}) was then added to absorb the liquid. The excess fluid was absorbed by the coating material (calcium silicate), that was added later. This order of mixing was proved by Sheth *et al*. (*15*) to produce the most optimal release rate. Explotab (disintigrant) and magnesium stearate (lubricant) were added to tablet preparations in concentrations of 5% and 1% w/w, respectively.

2.4. *Oral absorption profile of the prepared repaglinide liquisolid compact tablet*

2.4.1. *Study design*

Studies were carried out to compare the oral absorption of repaglinide (2 mg) from tablets containing liquisolid compact formula (treatment A) to commercial tablets (treatment B) following administration of a single dose each using a randomized crossover design.

A total number of twelve white male rabbits (Boskat breed) weighing from 1.5-2 kg were used as a model animal for determining the bioavailability of repaglinide. They were divided into two groups of six rabbits each. Animals were obtained from the animal house of the National Organization for Drug Control and Research (NODCAR). Animals were kept individually in cages with wire-mesh bases constructed of galvanized steel for two weeks before treatment for acclimatization in a room with controlled lighting (14 h/day), constant temperature (16-20°C) and relative humidity (55-65%). Sick, injured and abnormal animals were eliminated. Rabbits were fed a commercial pellet diet and water *ad libitum*.

Ten tablets from each treatment (A and B) were well pulverized and suspended in 50 mL distilled water. Five mL from each treatment were given orally to each rabbit with oral cannula. Blood samples (2 mL) were collected prior to administration and after 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 5, and 6 h of drug administration. Blood was collected in heparinized tubes. Blood samples withdrawn as above were transferred to a series of graduated centrifuge tubes containing 0.4 mL of 2.5% w/v sodium citrate solution. Samples were centrifuged at 2,500 rpm for 5 min. Plasma was transferred into another set of sample tubes and frozen until assay. One undosed plasma sample was kept as a blank. Samples were filtered through a 0.25 μm membrane filter (Millipore). The repaglinide concentration in blood samples was analyzed using the modified HPLC method (*8,16*).

2.4.2. *Chromatographic conditions*

The mobile phase composed of acetonitrile: ammonium acetate buffer, 10 mM (pH 4.0) in a ratio of 50:50 by volume was degassed for 20 min in an ultrasonic bath (Bransonic Cleaning Equipment Co., USA) prior to use. HPLC system (Shimadzu, Japan) was equipped with a C_{18} reversed-phase column (Cosmosil Nacalai Tesque, 5 μm particle diameter, 4.6 × 150 mm, Bondapak, USA).

Chromatography was performed at a flow rate of 1 mL/min at 30°C (*16*). All solutions were filtered through a 0.45 μm membrane (Sartorious, Germany) prior to use. The channel on the diode-array detector was configurated to acquire data at 244 nm. The injection volume was 30 μL. The column was equilibrated for a minimum of 20 min with the mobile phase flowing through the system before injection of the drug standards. The run time was set at 10 min with the system operating at an air-conditioned temperature of 20°C.

2.4.3. *Preparation of stock solutions and standard working solutions*

Stock solutions of repaglinide (100 μg/mL) and indomethacin (100 μg/mL) were prepared by dissolving 1 mg of each drug in 10 mL ethanol. The standard solutions were stored at 4°C in a clear glass volumetric flask and light protected with aluminum foil. Repaglinide concentrations in the working solutions used for the calibration curve were 20, 30, 50, 75, 100, 150, and 200 ng/mL. Quality control (QC) samples (of low, medium, and high concentrations) at 40, 80, and 150 ng/mL were prepared in the same way as the calibration standards. These working solutions were prepared fresh daily by making dilutions of the stock solution in ethanol. Working standard solutions of indomethacin were prepared by diluting the stock solution with ethanol at a concentration of 500 ng/mL. Fifty microlitres of indomethacin solution was used for every analysis (*8*).

2.4.4. *Calibration curve*

Blank plasma samples were spiked with working standard solutions to obtain final concentrations in the range of 20-200 ng/mL.

2.4.5. *Extraction procedure (8)*

The internal standard, indomethacin, and repaglinide solutions were added to blank plasma samples in round bottom glass tubes. One milliliter extraction buffer 0.1 M potassium dihydrogen orthophosphate (KH_2PO_4, pH 5.9) was added to the tubes. After the mixture was vortexed, 5 mL of ethyl acetate and 50 μL of isoamyl alcohol were added and the pH was adjusted to 7.4 with 2 M NaOH. The tubes were shaken on a rotator for 10 min followed by centrifugation at 3,000 rpm for 30 min. After centrifugation, the ethyl acetate phase was transferred into V-tubes and evaporated to dryness under a stream of nitrogen at 45°C. The dried extract was reconstituted with 70 μL of mobile phase, vortex-mixed and transferred to a clean autosampler vial. Thirty μL of this solution was injected into the HPLC system.

2.4.6. *Method validation*

Plasma calibration curves were prepared and assayed in triplicate on three different days to evaluate linearity, precision, accuracy, recovery, limit of quantification (LOQ), limit of detection (LOD), and stability.

2.4.7. *Pharmacokinetic analysis*

The maximum plasma concentration C_{max} was obtained

directly from the experimental data; T_{max} was defined as the first occurrence of C_{max}. The terminal elimination rate constant (K_{el}) was estimated using least squares regression analysis of plasma concentration time data obtained during the terminal log-linear elimination phase. Individual half-lives ($t_{½}$) were calculated as $0.693/K_{el}$ and mean half-lives were defined as 0.693/ mean K_{el}. The area under the plasma concentration-time curve (AUC) from 0 – time t ($AUC_{0\text{-}t}$) was estimated by linear trapezoidal approximation (*17*,*18*). The AUC from t to infinity ($AUC_{t\text{-}\infty}$) were estimated as ct, est K_{el}, where ct, est represents the estimated concentration at time t based on the regression analysis. The total area under the curve ($AUC_{0\text{-}\infty}$) was estimated as the sum of (AUC_t) plus ($AUC_{t\text{-}\infty}$).

2.4.8. *Statistical analysis*

The pharmacokinetic parameters for both treatments A and B were analyzed using one way analysis of variance using the SPSS computer program.

2.5. *Biological study design*

The study was carried out to determine the hypoglycemic effect of repaglinide (2 mg) from tablets containing the liquisolid compact formula in comparison to the commercial tablet as well as its effect on the blood glucose tolerance test (OGTT) and insulin levels. The study also evaluate the safety and efficacy of the new formula by studying the effect of several potencies of the liquisolid formula (0.5, 1.0, and 2.0 mg) on blood glucose and insulin level as well as the hepatic and renal toxicity of this newly prepared formula.

2.5.1. *Experimental animals*

Thirty white male rabbits (Boskat breed) weighing from 1.5-2 kg was used as model animals for determining the biological activity of repaglinide. The animals were housed in a standard temperature and humidity controlled room with a 12 h light/dark cycle. The animal had free access to water and a standard diet.

2.5.2. *Experimental plan*

Rabbits were divided into five groups (six animals each): GP1-Control group, animals received a single oral dose of glucose D (+) (2 g/kg BW) (glucose load) using the special oral syringe; GP2-Commercial drug group, animals received the glucose load (2 g/kg BW) + the oral dose of commercial drug (2 mg); GP3-Liquisoild compact group (0.5 mg), animals received the glucose load (2 g/kg BW) + the oral dose of the liquisoild compact formula (0.5 mg); GP4-Liquisoild compact group (1.0 mg), animals received the glucose load (2 g/kg BW) + the oral dose of the liquisoild compact formula (1.0 mg); GP5-Liquisoild compact group (2.0 mg), animals received the glucose load (2 g/kg BW) + the oral dose of the liquisolid compact formula (2.0 mg).

2.5.3. *Blood sampling*

Blood samples (2 mL) were withdrawn from the animals after 0, 15, 30, 45, 60, 90, and 120 min from retro-orbital plexuses according to the method of Shermer (*19*) and left to coagulate.

2.5.4. *Blood glucose and glucose tolerance test (OGTT)*

After overnight food privation, fasting blood glucose was determined in all groups. Simple, direct, colorimetric and automation-ready procedures for measuring glucose were performed using the BioAssay Systems' glucose assay kit, (QuantiChrom™ Glucose Assay Kit), according to Yoon and Mekalanos (*20*).

Glucose tolerance tests were carried out after glucose load in group 1, after glucose load with commercial drug in group 2, and after glucose load and the new formula drug in other groups 3, 4, and 5. The blood samples were withdrawn for determination of blood sugar after 15, 30, 45, 60, 90, and 120 min. The blood sugar levels were then plotted against time as blood glucose tolerance curves. The first point (zero) of these curves represents the fasting blood sugar level. Insulin levels were also determined at each time interval.

The area under the curve (AUC) of change in blood glucose was determined using the following equation:

$$AUC = \Sigma\{[(C_n - C_o) + (C_{n+1} - C_0)] \times (t_{n+1} - t_0)]\}$$

2.5.5. *Biochemical parameters*

Insulin levels were also determined at each time interval using Calbiotech Insulin ELISA kit based on direct sandwich technique according to the method of Beischer (*21*). Urea was determined using a colorimetric urea kit (Dam method) according to the method of Wybenga (*22*). Creatinine was colorimetrically quantified using the QuantiChrom™ Creatinine Assay Kit according to Wang *et al*. (*23*). SGPT was determined using a UV kinetic method kit according to the method of Herny (*24*).

2.6. *Statistical analysis*

Two types of statistical tests were performed in this study, Student "*t*" test and the analysis of variance "Two-way ANOVA" according to the methods of Campbell (*25*) and Bailey (*26*). The data are expressed as mean ± SD and the values of $p < 0.05$ were considered statistically significant.

3. Results and Discussion

3.1. *Method validation for bioavailability*

3.1.1. *Calibration curves, precision, accuracy, and linearity*

The calibration curve for repaglinide was linear in the concentration range of 20-200 ng/mL in rabbit plasma. The correlation coefficient of the line was 0.9930. The precision and accuracy of the assay were determined from the low (40 ng/mL), medium (80 ng/mL), and high (150 ng/mL) QC plasma samples. Inter-day assays were determined by analyzing QC samples in triplicate and were analyzed on three different days. Intra-day assays were determined for each QC sample in plasma, each in triplicate on one day. Intra and inter-day precision in this study was expressed as percent of coefficient of variation (CV). Accuracy was expressed as the mean percentage of analyte recovered in the assay (*27*). According to center for Drug Evaluation Research (CDER) (*28*), the precision determined at each concentration should not exceed 15% of CV except for LOQ, where it should not exceed 20% of the CV. The results of the precision and accuracy determined by the intra- and inter-day method are shown in Table 2.

3.1.2. *Recovery*

The recovery assay was assessed by comparing the peak area ratio (repaglinide/indomethacin) obtained from spiked plasma samples of different repaglinide concentrations (20-200 ng/mL) to the peak area ratios for the samples containing the equivalent amounts of the drug and internal standard directly dissolved in the mobile phase. Three replicate samples were measured at each drug concentration and the % average recovery study for repaglinide in plasma was 107.6%.

3.1.3. *Stability*

Repaglinide was found to be stable in rabbit plasma (< 5% loss) after three freeze/thaw cycles.

3.2. *Pharmacokinetic study*

Mean plasma concentration-time profiles in treatments A and B are presented in Table 3 and Figure 1. The mean peak time (T_{max}) in treatments A and B is 1 hour and the average peak plasma concentration values (C_{max}) were 125 and 70 ng/mL, respectively. The mean area under the plasma concentration-time curve ($AUC_{0\text{-}6}$) values were 373.5 and 190.38 ng·hr/mL and $AUC_{0\text{-}\infty}$ values were 470.78 and 226.69 ng·hr/mL, respectively.

Statistical analysis of the pharmacokinetic data revealed that there were significant differences between treatments A and B in C_{max}, K_{el}, t_{el}, *TCR*, $AUC_{0\text{-}6}$, $AUC_{0\text{-}\infty}$, $AUMC_{0\text{-}6}$, $AUMC_{0\text{-}\infty}$, and *MRT*. Also, there was an insignificant difference in T_{max}, while there was a significant difference in $C_{max}/AUC_{0\text{-}6}$.

Table 2. Intra-and inter-day accuracy and precision of the assay method

	Nominal concentrations (ng/mL)	Mean concentrations observed (ng/mL)	Accuracy (%)	Precision (%)
Inter-day	40	37	92.5	6.7
	80	73.53	92.29	4.73
	150	151	100.66	5
Intra-day	40	36.9	92	8.3
	80	72.15	90.187	4.85
	150	135	90	12

Table 3. *t*-Test independent for comparison between liquisolid formula and commercial product of ripaglinide

Items	Liquisolid formula			Commercial product			*p*
	Mean	SD	SE	Mean	SD	SE	
C_{max} (ng/mL)	125	2.915	1.304	70	4.743	2.121	< 0.0001
T_{max} (h)	1	0.07211	0.03225	1	0.04743	0.02121	1
K_{ab} (h^{-1})	1.76663	0.00152	0.0006796	1.768	0.005856	0.002619	0.103
$t_{(1/2)ab}$ (h)	0.3931	0.0003384	0.0001513	0.3919	0.001294	0.0005789	0.1024
K_{el} (h^{-1})	0.2776	0.002842	0.001271	0.3306	0.007362	0.003293	< 0.0001
$t_{(1/2)el}$ (h)	2.497	0.02557	0.01144	2.097	0.04678	0.02092	< 0.0001
TCR (mL/min)	0.07388	0.393	0.753	0.1558	0.001187	0.000531	< 0.0001
$AUC_{0\text{-}6}$ (ng•hr/mL)	373.5	2.069	0.9253	190.38	3.321	1.485	< 0.0001
$AUC_{0\text{-}\infty}$ (ng•h/mL)	470.78	1.086	0.4855	226.69	2.513	1.124	< 0.0001
$AUMC_{0\text{-}6}$ (ng•h^2/mL)	936.56	2.126	0.9507	447.1	12.539	5.608	< 0.0001
$AUMC_{0\text{-}\infty}$ (ng•h^2/mL)	1520.2	3.869	1.73	670.36	1.792	0.6673	< 0.0001
MRT (h)	3.229	0.01551	0.006938	2.958	0.03929	0.01757	< 0.0001
$C_{max}/AUC_{0\text{-}6}$ (h^{-1})	0.3346	0.005965	0.002668	0.3674	0.01851	0.008279	0.0055

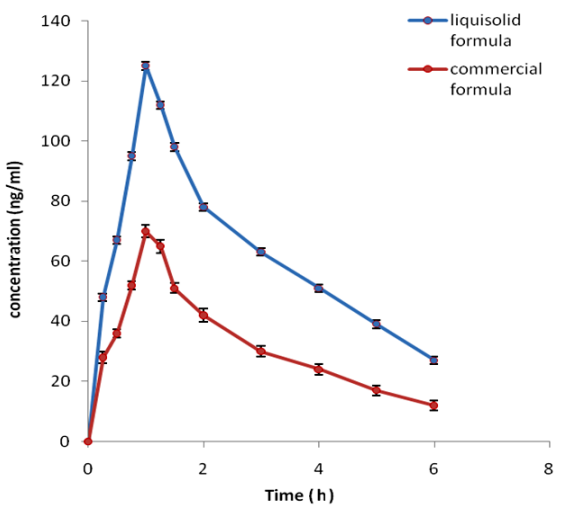

Figure 1. Mean plasma level of repaglinide in rabbits following oral administration of liquisolid formula and commercial product. Each value represents the mean ± SD (n = 6).

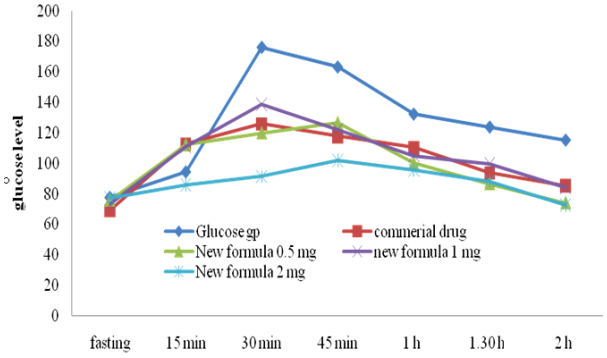

Figure 2. Time course changes of blood glucose level (mg/dL) in commerial drug group as well as new drug formula groups after adminatration of glucose load.

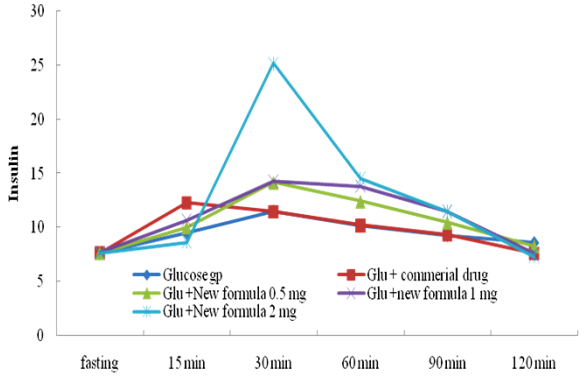

Figure 3. Time course changes of insulin level (ng/mL) in commerial drug group as well as new drug formula groups after adminatration of glucose load.

3.3. *Biological study*

3.3.1. *Blood glucose tolerance*

As shown in Table 4 and Figure 2, the blood glucose time course change data revealed that the new liquiosolid compact formula exerted a significant hypoglycemic effect compared to the repaglinide commercial product. The percent change glucose level, as compared with that in the glucose group, 30.8% and 19.1% in liquisolid compact (2 mg) and commercial product, respectively. Similarly, the other two potencies of the new compact formula exhibited a percent change glucose level of 21.3% and 16.9% at 0.5 mg and 1 mg, respectively.

3.3.2. *Insulin*

The time change of insulin, as shown in Table 4 and Figure 3, showed a significant increase in insulin levels in groups treated with the new liquisolid compact formula compared with the commercial product. The percent changes were 3.2, 11.2, 15.8, and 32.2% in repaglinide commercial product (2 mg), liquiosolid compact formula (0.5, 1, and 2 mg), respectively, compared with that in the glucose group.

3.3.3. *Area under the curve*

Table 5 shows the AUC of blood glucose in the rabbit groups treated with glucose only and glucose + commercial product (2 mg), and glucose + liquisolid compact formula (2 mg), respectively. The statistical study revealed that the main two effects (liquisolid compact formula and time course) were significant (p < 0.05) for AUC. For the glucose new formula rabbit group, the AUC was significantly lower than that in the

Tables 4. Time course change of blood glucose and insulin levels in all groups after a glucose load (group 1) or glucose load-repaglinide commercial product, 2 mg (group 2) or glucose load-liquisolid compact formula, 0.5, 1, and 2 mg (groups 3, 4, and 5)

	Group 1	Group 2	Group 3	Group 4	Group 5
Glucose levels (mg/dL)					
Mean	126.1	101.9	99.21	104.80	87.29
SD	35.0	20.1	21.12	22.28	10.17
SE	13.2	7.6	7.98	8.42	3.84
% of change	–	19.1%	21.3%	16.9%	30.8%
Insulin levels (ng/mL)					
Mean	9.41	9.72	10.48	10.90	12.45
SD	1.32	1.95	2.47	2.91	6.81
SE	0.54	0.80	1.01	1.19	2.78
% of change	–	3.2%	11.3%	15.8%	32.2%

Table 5. Blood glucose calculated from area under the curves of blood concentration at specific time periods

Time (min)	Glucose group			Commercial group			Liquiosolid compact formula group		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
0-15	1,294	27	11	1,378	53	21	1,197	39	16
15-30	2,045	29	12	1,779	28	11	1,330	39	16
30-45	2,595	49	20	1,817	38	16	1,462	35	14
45-60	2,243	54	22	1,669	55	22	1,478	21	8
60-90	3,785	63	26	2,977	107	44	2,703	44	18
90-120	3,500	47	19	2,640	72	29	2,357	45	18

Table 6. Biochemical parameters in treated groups in comparison with control group

	Control	Group 1	Group 2	Group 3	Group 4	Group 5
Urea (mg/dL)	31.31	29.92 ± 2.8	33.62 ± 4.3	30.09 ± 3.1	30.14 ± 5.4	32.19 ± 2.7
SGPT (U/L)	28.11 ± 3.5	25.91 ± 2.1	26.79 ± 2.3	27.09 ±2.9	27.79 ± 2.3	27.32 ± 3.1
Creatinine (mg/dL)	0.15 ± 0.019	0.13 ± 0.008	0.21 ± 0.1	0.14 ± 0.008	0.14 ± 0.01	0.14 ± 0.01

Table 7. ANOVA analysis of the tested parameters

		SS	DF	MSS	F calculated
Glucose	Between groups	5,390	2	2,695	4.67
	Within groups	10,390	18	577	
	Total	15,780	20		
Insulin	Between groups	2,017	2	1,009	58.3
	Within groups	259.3	18	17.3	
	Total	2,277	20		
Urea	Between groups	65.2	5	32.6064625	2.17
	Within groups	451.5	30	15.049145	
	Total	516.7	35		
Creatinine	Between groups	0.0249	5	0.012452611	2.46
	Within groups	0.1516	30	0.005054133	
	Total	0.1765	35		
SGPT	Between groups	18.1	5	9.062	1.18
	Within groups	230.9	30	7.697	
	Total	249.0	35		

Abbreviations: SS, sums of squares; DF, degrees of freedom; MSS, mean sums of squares.

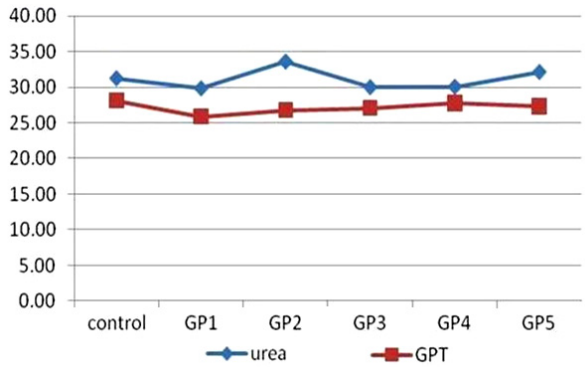

Figure 4. Biochemical parametrs changes in treated groups as copmared with control group.

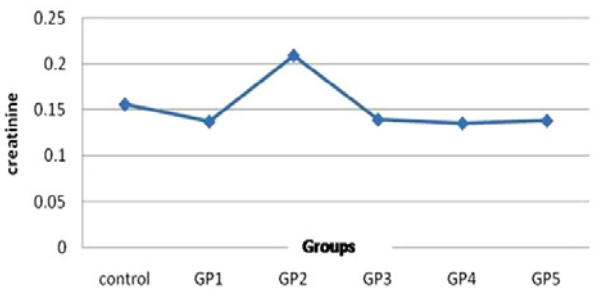

Figure 5. Ceratinine level (mg/dL) changes in treated groups as compared with control group.

G group ($p < 0.05$) in the periods 15-30, 30-45 min, and 45-90 min. The total AUC was also significantly lower in the glucose + liquisolid compact formula (2 mg) group than that in the G group ($p < 0.05$).

3.3.4. *Biochemical parameters*

As shown in Tables 6 and 7, and Figures 4 and 5, the new liquisolid compact formula did not exert any significant changes in the levels of urea, creatinine, and SGPT in all studied groups.

4. Conclusion

On the basis of the previous findings, it was concluded that the modified HPLC method used was rapid, simple, and sensitive for quantification of repaglinide in plasma samples. The method has good linearity, accuracy, precision, selectivity, and stability over the relevant therapeutic range. Also, the bioavailability of repaglinide was improved significantly when administered orally as the liqiusolid compact preparation. In turn, the new liquisolid formula controls blood sugar more efficiently than the commercial product by stimulating the release of insulin from the β-cell of the pancreas.

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