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Studies on Solubility, Bioavailability and Hypoglycemic Activity of Gliclazide β-cyclodextrin Complexes

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Abstract: The effect of beta-cyclodextrin (β-CD) complexation with gliclazide (Gz) on both pharmacokinetic and hypoglycemic parameters of pure drug was evaluated and correlated with its *in vitro* dissolution. The study involves the preparation of solid inclusion complex of Gz with β-CD using different techniques. The prepared binary systems showed significant enhancement for *in vitro* dissolution. The pharmacokinetics of neutralization complex (equivalent to 30 mg of gliclazide) was studied in beagle dogs. Gliclazide plasma levels were measured over a period of 48 h from dosing, using a sensitive HPLC method. Significant changes were observed in the mean values of Gz pharmacokinetic parameters (C_{max}, T_{max} and $AUC_{0-\infty}$) between the control and pre-treated groups. The findings of this study suggest that neutralization complex in 1:1 and 1:2 Gz/β-CD molar ratios was the best in enhancing gliclazide dissolution characteristics and bioavailability. Moreover, the various measures of bioavailability (except AUC) correlated well with both *in vitro* tests and hypoglycemic activity.

Key words: Gliclazide, β-CD, inclusion complex, pharmacokinetics, hypoglycemic activity, beagle dogs

INTRODUCTION

Cyclodextrins (CDs) are cyclic (α-1,4)-linked oligosaccharides α-D glucopyranose containing a relatively hydrophobic central cavity and a hydrophilic outer surface. The most common natural cyclodextrins are α, β and γ, which consist of 6, 7 and 8 glucopyranose units, respectively. Higher CDs containing 9, 10, 11, 12 and 13 glucopyranose units, which are referred to as δ, ε, ζ, θ-cyclodextrin have also been recognized. Of these higher cyclodextrins, only δ-cyclodextrin has been well characterized (Loftsson and Brewster, 1996; Rajewsic and Stella, 1996).

Cyclodextrins are mainly used to increase the aqueous solubility and dissolution rate of drugs (Guyot *et al.*, 1995; Uekama *et al.*, 1984; Chow and Karara, 1986). Thus, higher biological effect or lower drug doses can be reached when complexing drugs with CDs. Cyclodextrins can also be used to prevent drug-drug and drug-additive interactions, convert liquid drugs into microcrystalline powder, decrease volatility, modify gastrointestinal or ocular irritation and mask of objectionable taste or odor of drugs (Loftsson and Brewster, 1996; Rajwski and Stella, 1996).

Gliclazide (Gz) [1-(1-azabicyclo (3, 3, 0) octyl)-3-(p-tolylsulfonylurea)] is an oral hypoglycemic sulphonylurea used for the treatment of non-insulin dependent diabetes mellitus (NIDDM). The drug is characterized by a low solubility in water, leading to poor oral bioavailability with large individual variations (Plainer and Brogden, 1993; Gillman *et al.*, 1990). Therefore, several studies have been reported using β-CD to increase its aqueous solubility, dissolution rate (Moyano *et al.*, 1997; Ozkan *et al.*, 2000) and bioavailability in rats (Aggarwal *et al.*, 2002).

The aim of the present study is to prepare, characterize and determine the effect of different Gz/β-CD binary systems on aqueous solubility, *in vitro* dissolution and *in vivo* bioavailability of Gz in dogs and its correlation with the measured hypoglycemic activity. To achieve these goals, different preparation techniques, such as co-precipitation, neutralization, recrystallization, kneading and physical mixture were evaluated.

MATERIALS AND METHODS

Materials: Gliclazide (Gz) was a gift from Saudi Pharmaceutical Industries and Medical Appliances Corporation (SPIMACO, Saudi Arabia) and glibenclamide (as internal standard in drug assay) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Beta-cyclodextrin (β-CD) was purchased from Fluka Biochemical (Switzerland). All other materials were of analytical reagent grade.

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Phase solubility assay: The stability constant for inclusion complex between Gz and β-CD was determined using the phase solubility method (Higuchi and Connors, 1965). Ten milligrams of Gz were added into glass-stopper flasks containing 20 mL of β-CD solutions of increasing concentrations (0.001-0.02 M). The flasks were sealed and shaken at 25±0.5°C. After equilibration for 72 h, the solutions were filtered through membrane filter (0.22 μm pore size). Aliquots of the filtrates were suitably diluted and the concentration of Gz was determined by HPLC. The apparent stability constant of the complex with β-CD (K_c) was calculated from the phase-solubility diagram using the equation proposed by Higuchi and Connors (1965).

$$K_c = \frac{Slope}{Intercept\ (1 - Slope)}$$

where, the intercept is the apparent solubility of Gz at 25±0.5°C.

Preparation of the solid complexes: Solid complexes of Gz with β-CD in 1:1 and 1:2 molar ratios were prepared by different techniques described below. The obtained products were powdered and then passed through a 120 μm sieve and stored in a desiccator until used.

Coprecipitation method: The calculated amount of Gz was added in the above-mentioned molar ratios to β-CD dissolved in 50 mL of water. The mixture was shaken for 72 h at 25±0.5°C. The precipitate formed was separated by filtration under vacuum and dried at room temperature.

Neutralization method: Using neutralization method, the specific amounts of Gz and β-CD (1:1 and 1:2 molar ratios) were accurately weighed. Gz was dissolved in 50 mL of 1N sodium hydroxide solution, followed by the addition of β-CD. The mixtures were placed in a magnetic stirrer and stirred until dissolved. Equal amounts of 1N hydrochloric acid were added drop-wise and the solutions were stirred for 2 h with magnetic stirrer. The precipitated products were filtered off under vacuum and dried at room temperature till constant weight (Ozkan *et al.*, 2000).

Recrystallization method: Similarly, solid complexes of Gz with β-CD in the same aforementioned molar ratios were prepared using recrystallization method. Gliclazide was dissolved in 50 mL of methylene chloride. To the solutions of gliclazide, β-CD was added. The solutions were stirred for 2 h and then evaporated to dryness in vacuum at 40°C (Ozkan *et al.*, 2000).

Kneading method: A solid complex of Gz with β-CD at similar aforementioned ratio was prepared by kneading method. Cyclodextrin was triturated in a mortar with purified water to obtain a paste before gliclazide was added. The resulting mixtures were mixed for 30 min and then dried in an oven at 40°C for 6 h.

Physical mixture: The physical mixtures of Gz with β-CD (1:1 and 1:2 molar ratios) were prepared by light mixing the two components in a mortar using the geometric dilution technique.

Characterization of the mixtures and the inclusion complexes: The complexation of Gz with β-CD in solid state was characterized by X-ray diffraction (XRD) and Infrared Spectroscopy (IR) techniques. The X-ray powder diffraction pattern of Gz and various β-CD systems were obtained with a Siemens X-ray diffractometer (Model D-5000, Germany), equipped with a single crystal monochrometer and using a Ni filtered copper Kα radiation. The patterns were obtained for all samples at scanning speed of 2 deg min⁻¹ on the basis of scattering angles (2θ), interplaner d-spacing (Å) and relative intensities (I/I_{max}) of the three most intense lines based on the strongest line as 100.

The infrared absorption spectra of Gz and its complexes were obtained using a Perkin-Elmer FTIR spectrophotometer (USA). Each sample was analyzed using KBr disk method in the range of 600 to 4000 cm⁻¹ wave number and compared with corresponding physical mixture. The number of scans was adjusted automatically as a function of sample concentrate in the disk.

Dissolution studies: The dissolution studies of pure Gz and its various β-CD systems were performed using USP XXIV apparatus (Calva Ltd., Model 85T). In each flask, 500 mL of 0.1 N hydrochloric acid was used as a dissolution medium. The rotation speed was 50 rpm and the temperature was adjusted at 37±0.5°C. An accurately weighed amount of the prepared system equivalent to 10 mg of the drug was added to each flask. Sampling read times were at 0.00, 0.05, 0.15, 0.15, 0.30, 0.45 and 1 h. The concentration of the drug was calculated using HPLC. For each system, dissolution was run in triplicate and the average percentage of the drug dissolved was determined.

***In vivo* studies:** Three healthy male beagle dogs weighing 12 kg on average were used in this study. The animals were fasted overnight prior to drug administration. During all experiments water was allowed ad libitum and the dogs were fed 4 h after dosing. Gelatin capsules containing 30 mg of Gz or its equivalent weight of inclusion

complexes were administered followed by 20 mL of water. The oral capsules were administered in a randomized cross design to minimize any residual or commutative effect of the preceding dose with, at least, 2-week washout period between doses.

Blood samples (3 mL) for the measurement of Gz were withdrawn just before and 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 24, 30 and 48 h after oral administration of one capsule of pure drug and its inclusion complexes. The blood samples were then immediately centrifuged at 3000 rpm for 5 min and aliquots of 1 mL of plasma samples were stored at -70°C pending analysis.

Drug analysis: A reverse phase High Pressure Liquid Chromatography (HPLC) assay method was used to determine the Gz concentrations in the phase solubility, dissolution and *in vivo* studies. The HPLC consisted of a Waters (M-600E) multisolvent delivery pump, an in-line degasser and an auto-sampler (M717 plus) programmable UV dual wavelength absorbance detector (M-2487), coupled to a Personal Computer (PC). Data and system management was handled by millennium 32-chromatography manager software. Chromatographic conditions were as follows: the separation was performed at 20°C using a 5 μm symmetry C_{18} stainless steel column, 150×3.9 mm (i.d.), coupled with a sentry guard column (20×3.9 mm) of the same material (all from Water Associates Co, Milford, MA, USA). Flow rate was isocratic at 1.5 mL min^{-1}. Mobile phase consisted of 45% v/v acetonitrile in 0.05 M ammonium dihydrogen phosphate buffer (pH of the buffer adjusted with phosphoric acid to 3.8 before mixing with acetonitrile). The final pH of the mobile phase was 4.6. UV detector set at wavelength 229 nm. Glibenclamide (100 μg mL^{-1} in ethanol) was used as internal standard. Twenty-five microliters of standards and samples were injected onto the column for analysis.

Gliclazide content in the inclusion complexes: Gliclazide content in the various freshly prepared solid complexes was determined before being subjected to any *in vitro* testing. An amount of the prepared system equivalent to 10 mg of the drug was dissolved in 100 mL of ethanol. Five milliliters of the obtained solution was centrifuged for 5 min at 4000 rpm, then 100 μL of the clear supernatant was pipetted in 1.5 mL eppendorf tube containing 50 μL of the internal standard and the volume completed to 1 mL with methanol. Twenty-five microliters were injected into the HPLC system. The concentration of the drug was calculated from standard curve constructed simultaneously. The experiment was carried out in triplicate and the average value was determined.

Gliclazide content *in vivo* studies: Gliclazide working standard (100 μg mL^{-1}) was added in appropriate volumes to ten volumetric flasks (5 mL capacity) and volume completed to 5 mL with drug free beagle-dog plasma to provide calibration standards of 0 (no gliclazide added), 0.25, 0.5, 0.75, 1, 2.5, 5, 7.5, 10, 15 and 20 μg mL. Each of these standard solutions was distributed in disposable polypropylene micro-centrifuge tubes (1.0 mL, Eppendrof) in volumes of 0.6 mL and kept at -70°C until analysis.

For preparation of samples for injection onto HPLC system, a 75-μL aliquot of the internal standard solution (Glibenclamide 100 μg mL^{-1}) was added to 0.5 mL plasma sample in a 10 mL glass stoppered tube and vortex-mixed for 10 seconds. Then 0.5 mL of 0.2 M dipotassium hydrogen phosphate buffer of pH 7.0 (adjusted with phosphoric acid) was added to the tube and vortex-mixed for 10 sec. Chloroform (5 mL) was added to the tube and the mixture was rotamixed for 15 min at 20 rpm, then centrifuged at 4000 rpm for 10 min. The organic phase was transferred to a 10 mL glass centrifuge tube and evaporated to dryness at 40°C under gentle stream of purified nitrogen. The dried residue was redissolved in 200 μL of mobile phase by shaking for 30 sec on vortex-mixer. Aliquots were loaded in auto-sampler tray (in glass tubes) and volumes of 50 μL were injected into the HPLC system.

Measurement of plasma glucose levels: Ten microlitres of the plasma sample (A_{sample}) or standard ($A_{standard}$) were pipetted into a 1.5 mL microcentrifuge tube containing 1000 μL of the glucose reagent (Randox), mixed well and incubated for 10 min at 20±5°C. The slightly pink mixture was then transferred to 1 cm path length cuvette and the absorbance of the standard and sample were measured at $\lambda_{500\ nm}$ against the 1000 μL reagent blank within 30 min. Glucose concentrations were determined according to the following equation:

$$\text{Glucose concentration (mmol L}^{-1}) = \frac{A_{sample}}{A_{standard}} \times 5.55$$

Pharmacokinetics and statistical analysis: Pharmacokinetics parameters for Gz/β-CD complex were calculated. These include maximum plasma concentration (C_{max}) and the time to achieve the maximum concentrations (T_{max}) which were directly determined from the resulting concentration-time profiles. The elimination rate constant (K_{el}) was calculated from log-linear least-squares regression of the terminal phase data points.

The elimination half-life ($t_{½}$) was calculated as ln(2) divided by the elimination rate constant. Non-compartmental model was applied to calculate the area

under the plasma concentration-time curve (AUC), which was determined by the linear trapezoidal rule. The area from last point to infinity was determined by dividing the last plasma concentration by the elimination rate constant. The program used for determination of Gz pharmacokinetic parameters was Kinetica software 2.0.1, 1996-2006 (MicroPharm International USA). Data are presented as mean±standard deviations (SD). Statistical analysis was performed with the STAT program (Graphpad InStat-2 software program). Groups were compared using one-way analysis of variance (ANOVA) at p<0.05 level.

RESULTS AND DISCUSSION

Phase-solubility studies: Figure 1 represents the solubility of Gz/β-CD complexes. It shows a B_S type solubility curve (Higuchi and Connors, 1965). The first portion of the plot shows that the aqueous solubility of the drug linearly increases as a function of β-CD concentrations. Then a short plateau region is reached which is followed by a decrease in total gliclazide and precipitation of crystalline complex. Because of the ascending linear portion of the diagram, it was assumed that the solubility increase was due to the formation of 1:1 complex. The apparent solubility constant (K_c) was estimated by linear regression analysis method from initial straight portion of the solubility diagram with equation (1) and was found to be 323 M^{-1}. This K_C value is higher than that obtained in previous study (Ozkan *et al.*, 2000).

Evidence of complex formation in solid state: To confirm the complexation of gliclazide with β-CD in the solid state, XRD and IR were employed and compared with pure drug and the corresponding physical mixtures in 1:1 and 1:2 molar ratios.
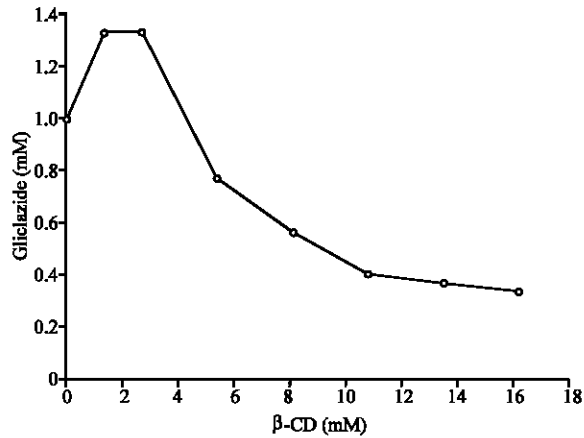

Fig. 1: Phase solubility diagram of gliclazide-beta-CD complex

To examine crystallinity and to provide evidence of complex formation in solid state, the diffraction analyses were performed. The three highest values for relative intensities, their corresponding scattering angles (2θ) and interplaner d-spacing values were utilized to compare drug, β-CD and their corresponding systems. A difference of ±0.03 for the (d) values implies a change in the crystal lattice of the drug and β-CD.

The X-ray diffractograms of Gz, β-CD and its binary complexes in comparison with the physical mixtures in the molar ratios 1:1 and 1:2 are shown in Fig. 2. From the above, it may be evidenced that differences were identified among diffraction patterns of each complex when compared with patterns of the pure gliclazide β-CD and their physical mixtures (Table 1 and 2).

The functional groups of gliclazide involved in the complexation were also involved in IR. Spectra of pure gliclazide, its inclusion complexes and physical mixtures with β-CD are shown in Fig. 3. The infrared spectrum of pure gliclazide (Fig. 3a) showed principal peaks at 1596 cm^{-1} (NH deformation band), 1710 cm^{-1} (C=O stretching band) (C=0 deformation), 3272 cm^{-1} (NH stretching band),
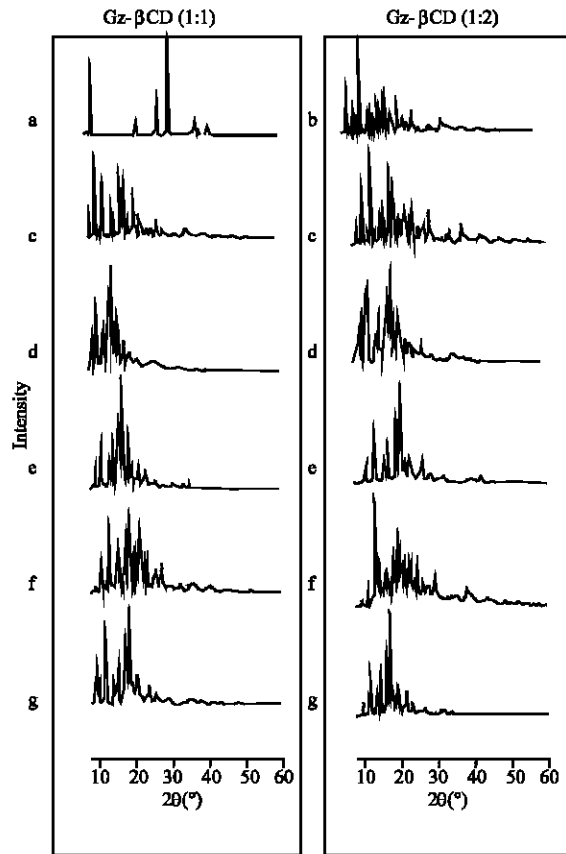

Fig. 2: X-ray diffraction patterns for a) Gz; b) β-CD; c) physical mixture; d) kneaded mixture; e) coprecipitated; f) recrystallized and g) neutralized

Table 1: XRD patterns of pure Gz, β-CD and their binary systems in the molar ratio (1:1)

Product	Peak 1			Peak 2			Peak 3		
	2θ	d-spacing	I/I_{max}(%)	2θ	d-spacing	I/I_{max}(%)	2θ	d-spacing	I/I_{max} (%)
a) Pure Gz	18.182	4.8752	100	10.513	8.4077	85.5	17.095	5.1825	46.7
b) β-CD	12.657	6.9878	100	9.053	9.7600	50.9	18.925	4.6854	34.1
c) Physical mixture	10.539	8.3871	100	17.091	5.1839	82.4	18.175	4.8771	73.5
d) Kneaded complex	18.230	4.8624	100	17.932	4.9424	84.8	17.155	5.1645	70.7
e) Coprecipitated complex	18.662	4.7508	100	17.810	4.9760	60.5	17.578	5.0412	59.6
f) Recrystallized complex	18.751	4.7283	100	17.739	4.9957	5.901	10.086	8.7624	55.7
g) Neutralized complex	18.664	4.7502	100	12.030	7.3507	76.8	17.584	5.0396	69.1

Table 2: XRD patterns of pure Gz, B-CD and their binary systems in the molar ratio (1:2)

| Product	Peak I			Peak 2			Peak 3		
	2θ	d-spacing	I/I_{max}(%)	2θ	d-spacing	I/I_{max}(%)	2θ	d-spacing	I/I_{max} (%)
a) Pure GZ	18.182	4.8752	100	10.513	8.4077	85.5	17.095	5.1825	46.7
b) B-CD	12.657	6.9878	100	9.053	9.7600	50.9	18.925	4.6854	34.1
c) Physical mixture	12.654	8.9896	100	17.212	5.1476	91.2	18.297	4.8446	72.4
d) Kneaded complex	18.849	4.7040	100	17.752	4.9922	65.7	12.210	7.2429	56.6
e) Coprecipitated complex	12.496	7.0770	100	17.918	4.9464	54.9	18.546	8.3816	49.5
0 Recrystallized complex	18.658	4.7517	100	17.025	7.3537	96.5	10.577	5.0415	66.9
g) Neutralized complex	18.42	4.7307	100	17.636	5.0246	61.9	17.646	5.6594	43.4
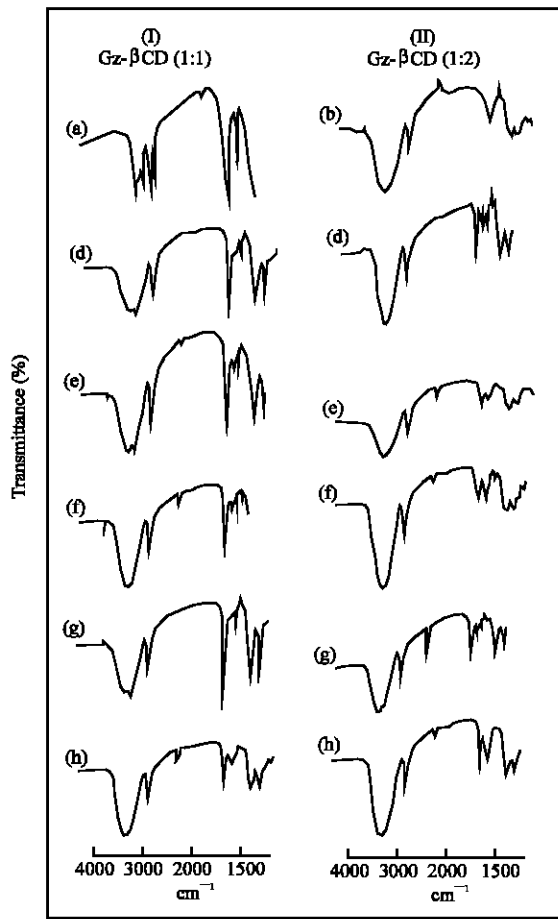
Fig. 3: IR spectra profiles of a) pure Gz; b) pure β-CD and
their Gz/CD systems, where: c) IR spectrum for
physical mixture; d) kneading; e) coprecipitation;
f) recrystallization and g) neutralization complexes

1347.5 cm^{-1} and 1164.3 cm^{-1} (S = 0 asymmetrical and symmetrical vibration bands), respectively.

The IR spectrum of β-CD (Fig. 3b) showed intense bands at 3300-3500 cm^{-1} associated with the absorption of the hydrogen bonded-OH groups of β-CD. The vibration of -CH and -CH$_2$ groups appear in 2800-3000 cm^{-1} region.

The functional groups of Gz involved in the complexation were also involved in IR. The changes in the IR spectra of the mixtures and inclusion complexes (in the ratios 1:1 and 1:2) may be explained by different preparation methods. As can be observed in the spectral pattern of the physical mixtures (Fig. 3c) it corresponds simply to superposition of the IR spectra of the two components. However, the spectra of the neutralized complexes in the molar ratios (1:1) and (1:2) (Fig. 3g) showed the most noticeable complexation compared to other inclusion complexes, as there was considerable differences when compared with those of the pure drug. They showed broadening in the IR signal corresponding to the N-H stretching band at 3272 cm^{-1} and pronounced reduction peak intensity at 1596 cm^{-1} (N-H deformation), in addition to shifting of the peak to 1640 cm^{-1}. The carbonyl stretching band (1710 cm^{-1}) also exhibited marked reduction in the peak intensity. Moreover, the neutralized complexes showed shifting towards shorter frequency of S = 0 symmetrical vibration (for 1164 to 1158 cm^{-1}). The coprecipitation mixtures (Fig. 3e) showed noticeable changes in the IR spectra compared with that of the pure drug. They exhibited lower intensities in the 1596, 1710 and 1348 cm^{-1} peak and broader peaks at 3381 cm^{-1}. The peak at 1164 cm^{-1} shifted towards lower frequency (1162 cm^{-1}) with lesser intensities. Kneaded (Fig. 3d) and recrystallized (Fig. 3f) inclusion complexes exhibited minor changes in their IR spectra compared with that of pure drug, indicating weak interaction of Gz with β-CD.

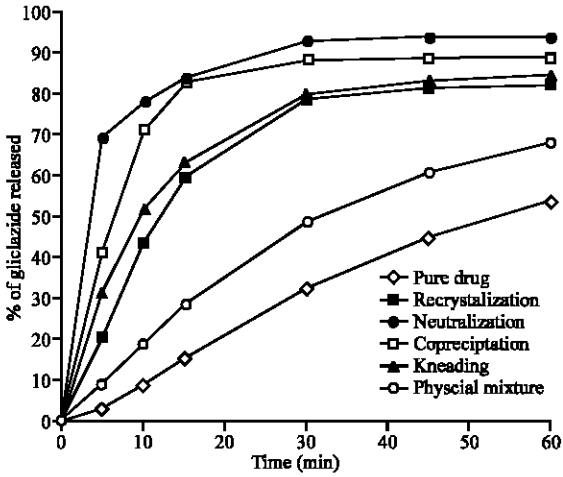Fig. 6: Time courses of mean gliclazide plasma concentrations in beagle dogs following 30 mg dose of pure gliclazide and its complex systems

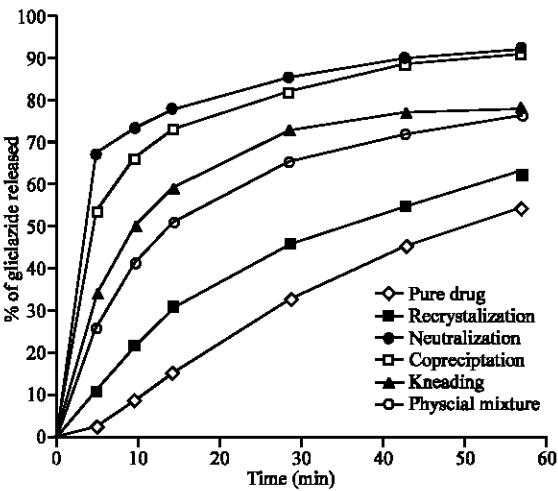Fig. 4: Dissolution rate profile of gliclazide and its β-CD system (1:1) ratio

Fig. 5: Dissolution rate profile of gliclazide and its β-CD system (1:2) ratio

Dissolution studies: The dissolution profiles of gliclazide and its β-CD 1:1 and 1:2 molar ratios are shown in Fig. 4 and 5, respectively. From the Figures it is evident that the dissolution rate of the physical mixtures and inclusion complexes showed faster rates than that of the pure drug. It is also observed that the neutralization and coprecipitation methods in both molar ratios with drug showed the most remarkable increase in dissolution rate compared with other inclusion complexes and physical mixtures. The percentage of Gz dissolved after 60 min was 54% for pure drug compared with 93.7 and 91.0% for the drug in neutralized complexes in the 1:1 and 1:2 molar ratios, respectively and 88.7 and 89.9% in coprecipitated complexes 1:1 and 1:2 molar ratios, respectively.
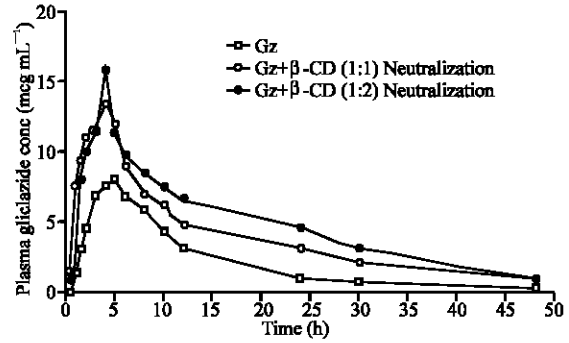
The extent of enhancement of the dissolution rate was found to be dependent on the method of complex preparation. However, the increase in dissolution rate for the kneaded complex may be attributed to improved wetability due to the presence of highly hydrophilic cyclodextrins as well as the possibility of in-situ formation of relevant inclusion complex.

Bioavailability of Gz/β-CD complex: An *in vivo* absorption study was undertaken using the best Gz/β-CD complex (i.e., neutralization complexes in both molar ratios) to determine whether the enhanced *in vitro* dissolution of Gz increases its absorption compared with that of Gz alone. The mean plasma concentration-time profiles of Gz after oral administration (30 mg or equivalent) of drug alone or in complex with β-CD to beagle dogs are shown in Fig. 6. Pharmacokinetic parameters are summarized in Table 3. The mean peak concentrations after oral administration of pure Gz, neutralization complexes in 1:1 and 1:2 molar ratios, were 8.05 ± 1.08, 15.84 ± 1.99 and 13.51 ± 3.34 μg mL^{-1}, occurring at 4.7, 3.67 and 4.00 h, respectively. The ratios of areas under the plasma concentration-time curve (AUC) of neutralization complexes in 1:1 and 1:2 complexes were found to be 1.50 and 1.82 fold, respectively, greater than that of Gz alone. On the other hand, both complexes prepared by neutralization in both ratios reached maximum plasma concentration (T_{max}) faster than that of pure Gz (Table 3). This is probably due to rapid dissociation of the complex following the dissolution in the GI fluids. The mean residence times (MRT) of pure Gz and its neutralized complex (1:2) were 19.00 ± 5.68 and 18.48 ± 2.38 h, respectively. This indicates that the pure drug and its complex remained in the body for the same period. However, the concentration (C_{max}) of the complex was significantly higher than that of the pure drug.

Table 3: Pharmacokinetic parameters of pure Gz and its inclusion complexes with β-CD after 30 mg Gz dose administered to Beagle dogs

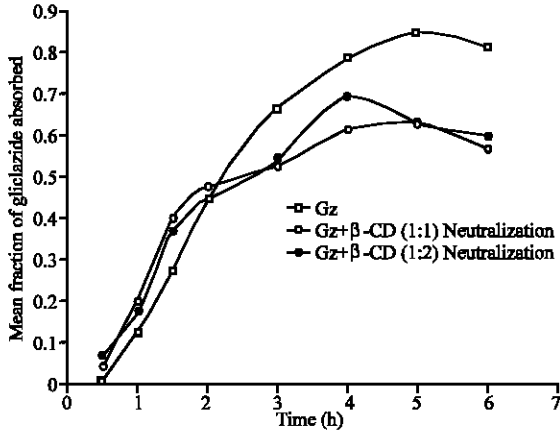| Description/sample	Parameter (mean±SD) $AUC_{0-\infty}$ (μg h mL^{-1})	C_{max} (μg mL^{-1})	T_{max} (h)	K_{el} (h^{-1})	$t_{1/2}$ (h)	MRT(h)	p-value*
Pure drug	175.00±33.15	8.05±1.08	4.7±0.5	0.075±03	12.48±5.07	19.00±5.68	
Neutralization with β-CD (1:1)	255.20±45.39	15.84±1.99	3.67±0.0	0.110±0.03	6.614±1.37	16.13±1.32	<0.05
Neutralization with β-CD (1:2)	318.25±60.73	13.51±3.34	4.0±0.0	0.072±0.017	10.083±2.16	18.48±2.38	<0.05

* compared with pure drug

Fig. 7: Plot of mean fraction of gliclazide dose absorbed as a function of time from pure gliclazide and its complex systems
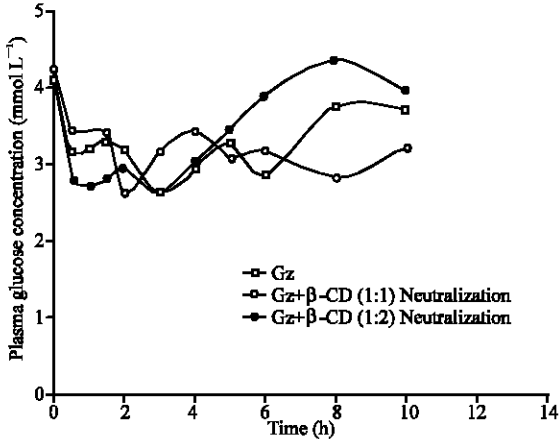

Fig. 8: Time courses of mean plasma glucose concentration following the administration of 30-mg dose of gliclazide and its complex systems

Absorption of Gz from pure drug and β-CD complexes was assessed by Wagner-Nelson method. The result is shown graphically in Fig. 7, from which it may be seen that absorption began earlier from neutralized complexes followed by the pure drug. However, in order of earlier release, they could be ranked as follows:

Neutralized complex (1:1) > Neutralized complex (1:2)>Pure Gz

Table 4: Mean plasma glucose concentrations (mean±SD) of pure Gz and its inclusion complex and time (mean±SD) needed to achieve these concentrations

Method Parameter	β-CD (1:1) Neutralization	β-C (1:2) Neutralization	Pure drug
C_{max} (μg mL^{-1})	2.63±0.12	2.64 ±0.55	2.65±0.85
T_{max} (h)	2.00±0.05	3.00 ±0.11	3.00±0.21

Table 5: Ranking of various methods of assessing *in vitro*, *in vivo* and hypoglycemic potency Ranking

Method	Ranking		
	β-CD (1:1) Neutralization	β-CD (1:1) Neutralization	Pure drug
Dissolution/Bioavailability	1	2	3
C_{max}	1	2	3
T_{max}	1	2	3
$AUC_{0-\infty}$	2	1	3
Absorption (Wagner-Nelson)	1	2	3
Plasma glucose reduction	1	2	3

The mean time courses of plasma glucose concentration changes following Gz administration from the pure drug and its β-CD complexes are shown in Fig. 8. The mean minimum plasma glucose concentrations and the mean times to reach these concentrations are shown in Table 4. However, when the earlier glucose plasma concentration time curves were compared, it would appear that neutralized complex in 1:1 ratio resulted in early onset of hypoglycemic activity in comparison with neutralization complex in 1:2 ratio followed by pure Gz (Fig. 8). This result indicates the existence of correlation between Gz concentrations and fasting blood glucose level. The order of effectiveness of pure drug and its β-CD complexes in reducing plasma glucose concentrations could be ranked as follows:

Neutralization 1: 1>Neutralization 1:2 > Pure Gz

The rankings of the pure drug and its neutralized complexes (in the ratios 1:1 and 1:2) on the basis of the *in vitro* and *in vivo* studies for predicting therapeutic effects are compared with their measured effectiveness in producing hypoglycemia and the obtained data are shown in Table 5. The results revealed that various measures of in vivo bioavailability as well as *in vitro* tests correlated well with *in vitro* studies (except for AUC).

The objective of this study was to prepare, characterize and determine the effect of different Gz/β-CD binary systems on aqueous solubility, *in vitro* dissolution and *in vivo* bioavailability of Gz in dogs and its correlation with the measured hypoglycemic activity. The results of

the study indicate that Gz/β-CD neutralized complexation (in 1:1 and 1:2 molar ratios) were the best in achieving both *in vitro* and *in vivo* enhancement of gliclazide dissolution characteristics and bioavailability, therefore, showing good correlation between the two studies. These findings have the potential of reducing inter-patient variability and decreasing the daily dose of Gz with little side effects.

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