Pharmacodynamic assessment of gliclazide multiparticulate system: single-dose and multiple-dose studies

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Received: 14 May 2022
Revised: 14 July 2022
Accepted: 14 July 2022
Published: xx Month 2022

Egyptian Pharmaceutical Journal 2022, 21:411–423

Background
Conventional dosage forms of oral hypoglycemic drugs, including gliclazide (GLZ), may have a number of limitations, reducing their bioavailability. Thus, efforts are directed to design novel modified-release (MR) dosage forms for these drugs. The possible role of orally administered GLZ-MR multiparticulates in the treatment of hyperglycemia as well as improvement of impaired wound healing associated with type 2 diabetes mellitus was investigated.
Objective
This study aimed to evaluate the pharmacodynamics (PD) of GLZ-MR multiparticulate system against Diamicron MR tablets in nondiabetic (healthy) and streptozotocin-induced diabetic rats, by measuring blood glucose levels. For the first time, the hypothetical wound-healing capabilities of multiple doses of both treatments in diabetic rats were also studied by evaluating the wound diameter and histology.
Materials and methods
Novel cross-linked freeze-dried GLZ-alginate-gelatin beads were prepared. Two GLZ treatments at 4 mg/kg [test (T, MR beads) and reference (R, Diamicron MR 30 mg)] were administered to rats. A single-dose PD study was carried out on both healthy and diabetic rats, whereas the multiple-dose study was evaluated in diabetic rats. A single-dose pharmacokinetics (PK) study was conducted for assessment of the PK-PD relationship in healthy rats.
Results and conclusion
The single-dose study on nondiabetic rats showed that T beads exhibited a greater magnitude of blood glucose level reduction, with 1.5-fold increase in C_{max}, compared with R. A direct linear relationship with high correlation was detected between GLZ glucose-lowering effect and its PK parameters, only for T beads. Multiple dosing of T beads was more efficient than R in managing hyperglycemia of wounded diabetic rats. T beads allowed almost complete wound closure, after multiple dosing for 17 days. The proposed GLZ beads could provide a promising therapeutic prospect for managing hyperglycemia as well as resolving impairment of wound healing associated with diabetes.

Keywords:
antidiabetic activity, gliclazide-alginate-gelatin beads, healthy and diabetic rats, pharmacokinetic-pharmacodynamic relationship, single and multiple dose, wound healing

Egypt Pharmaceut J 21:411–423

1687-4315

Introduction

Type 2 diabetes mellitus (T2DM) is in fact a complex multidimensional metabolic illness associated with dysfunction of pancreatic islet cells (α and β), leading to the progress of insulin resistance [1]. Oral hypoglycemic drugs are widely used to treat T2DM, including sulfonylureas. Nevertheless, conventional dosage forms of such drugs may have limitations, including short half-life, the need for frequent dosing, and low bioavailability [2,3]. Thus, to overcome such problems, efforts are directed to design novel and/or modified-release (MR) dosage forms for oral hypoglycemic drugs as single units and multiparticulates [2].

Multiple-unit solid dosage forms have acquired popularity among oral drug delivery systems as they provide superior technical and clinical benefits compared with single-unit dosage forms. Owing to their minimized size and multiplicity of units per dose, there is less risk of dose dumping and better spreading within the gastrointestinal tract, when administered orally. Besides, multiple-unit dosage forms have a number of biopharmaceutical benefits: their transit

time in various regions of the gastrointestinal tract can be better anticipated and less intrasubject and intersubject variability. Multiparticulate formulations have a larger surface area allowing for a higher drug release, and when spread along the gut, drug absorption can be maximized without local irritation [4–6].

Thereby, combining the merits of both MR and multiparticulate dosage forms for gliclazide (GLZ) would be more beneficial [7,8]. GLZ, a second-generation sulfonylurea oral hypoglycemic agent, has been shown to act directly on the β cells of islets of Langerhans of the pancreas and to stimulate the pancreas to produce and secrete more insulin [9]. Several studies showed that the ability of GLZ to lower blood glucose levels (BGLs) may be mediated, in part, by extrapancreatic effects [10]. In these studies, no increase in insulin levels was observed despite the BGL-lowering effect of GLZ. This suggested an extrapancreatic effect such as increased peripheral sensitivity to insulin and stimulated synthesis of glucose transporters [10]. Target tissues may turn out to be more sensitive to insulin [11]. In-vitro studies in cell cultures proved that sulfonylureas stimulated the synthesis of glucose transporters and enhanced insulin action [12].

A number of in-vitro and in-vivo studies clarified that GLZ acts effectively also as an antioxidant besides its other favorable hemobiological effects [13,14]. Diabetes is commonly associated with endothelial dysfunction. GLZ seems to be able to reverse this dysfunction owing to its antioxidant properties rather than its metabolic actions, as this favorable effect can be imitated by other antioxidants [15].

Few research articles studied the possible effects of some antidiabetic drugs on wound healing. Metformin and acarbose were found to promote the healing process, whereas rosiglitazone has not shown any improvement in nondiabetic rats [16]. The same effect was observed for metformin in T2DM mice [17] and diabetic rats [18]. Yet, no data are available concerning the possible role of orally administered GLZ in improving impaired wound healing in T2DM. The present study hypothesized that GLZ may be able to contribute to wound healing in T2DM rats.

Productive attempts to prepare different cross-linked GLZ-alginate-gelatin (AL-GL) beads have been previously carried out, where the prepared beads have given promising results [7,8]. Recently, successful preparation of MR freeze-dried GLZ-AL-GL beads was developed with improved oral bioavailability [19]. In that study, comparative in-vitro and in-vivo studies, as well as assessment of in-vitro-in-vivo correlation, of the test product (T; GLZ freeze dried beads) were carried out against the reference product (R; Diamicron 30 mg MR tablet) [19].

The aim of this comparative study was to evaluate the pharmacodynamics (PD, glucose-lowering effect) of newly developed GLZ freeze-dried MR AL-GL beads (test; T) against the innovator Diamicron MR 30 mg tablets (reference; R) in nondiabetic (healthy) and streptozotocin (STZ)-induced diabetic rats. To assess the pharmacokinetic-pharmacodynamic (PK-PD) relationship of nondiabetic (healthy) rats, a PKs study was carried out in parallel. Moreover, the study evaluates, for the first time, the hypothetical wound healing capabilities of GLZ in diabetic rats after multiple oral administrations of both T and R products.

Materials and methods

Materials

GLZ powder (of particle size <15 μm) and glibenclamide (internal standard) were gift samples from Sigma Pharmaceutical Industries (Menoufia, Egypt). STZ was obtained from Sigma Chemical (USA). Gelatin (Bovine-B) and sodium AL (high viscosity) were obtained from Sigma Aldrich (Germany). Glutaraldehyde (50% w/w) and anhydrous calcium chloride were obtained from ADWIC (Al Qalyubiyah, Egypt). All were used for the formulation of GLZ-MR beads. The reference product used in this study was Diamicron MR 30 mg tablets (Batch No. 25298; Servier, Cairo, Egypt). Methanol and acetonitrile were of chromatographic HPLC grade and were bought from Merck (Germany). All other chemicals and solvents used were of analytical grade.

Methods

Formulation of gliclazide modified-release beads

MR freeze-dried GLZ-loaded AL-GL beads (GLZ : AL : GL; 1 : 1 : 5) were formulated as described previously [19] (test preparation; T). Initially, AL and GL powders were dissolved in 200 ml of distilled water at a temperature of 65±0.5°C, employing a temperature-controlled circulator water bath 'Julabo model F10-VC (Germany).' Then, quantitative transfer of GLZ powder into AL-GL solution (100 mg GLZ for each 600 mg of total polymers; GLZ : AL : GL; 1 : 1 : 5) was done with constant stirring. The formed GLZ-AL-GL suspension was dropped onto the curing solution for cross-linking

(0.1% w/w glutaraldehyde in 0.2 M calcium chloride maintained at a temperature of 5.0±0.5°C) employing a peristaltic pump at a fixed rate [7,19]. Falling distance of the drops of GLZ-AL-GL suspension above the curing solution surface was kept at 7.5 cm. After 30-min curing time, GLZ-AL-GL beads were washed with distilled water and finally freeze dried using 'Alpha 1-4 LSC-plus freeze drier (Christ, Germany).'

These beads were prepared to have a diameter of 1.5 mm, which was small enough to be easily swallowed by rats in a multiparticulate form. Blank (GLZ-free) beads (AL-GL beads) were prepared as a control.

In-vivo assessment of gliclazide modified-release beads

Several in-vivo studies were conducted for comparison of two GLZ treatments at 4 mg/kg [8,19]: test (T, beads) and reference (R, Diamicron MR 30 mg/tablet). Single-dose studies were performed including PD evaluation of BGLs for nondiabetic and STZ-induced diabetic rats, as well as evaluation of PK parameters for proper estimation of the PK-PD relationship (for healthy rats). Additionally, GLZ ability to promote wound healing in diabetic rats in a manner comparable to the normal healing process in nondiabetic rats was assessed for the first time, where multiple doses of the two treatments (T and R) were administered in diabetic rats.

Experimental animals and housing requirements

All in-vivo experiments conducted on rats were in agreement with the 'National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.' This study protocol was accepted by the 'Medical Ethical Committee of the National Research Centre' (protocol approval number: 16–058). Reporting of all experimental procedures complied with recommendations in ARRIVE guidelines for the reporting of animal experiments.

A total of 72 male albino Wistar rats (age: 6–7 weeks) having a body mass of about 200–250 g were obtained from the 'animal house' of the National Research Center, Cairo, Egypt, and were selected for the in-vivo studies. All rats were housed in a well-ventilated holding room within a central care facility for experimental animals, under standard conditions (temperature: 25°C, relative humidity: 30–70%, and 12/12 h day-night cycle). Unrestricted access to water and standard laboratory rodent chow was permitted. After adaptation for 1 week, rats were randomly allocated into the different experimental groups.

Induction of diabetes

For experimental groups employing diabetic rats, T2DM was induced using STZ. A single STZ dose (55 mg/kg body weight), dissolved in a sterile sodium citrate buffer solution (pH 4.5), was administered to the rats intraperitoneally [20]. Nondiabetic rats received citrate buffer alone. BGL estimation of rats on day 3 after STZ administration served as the criterion of induction of the required level of hyperglycemia [21]. A glucometer-strip method (AccuCheck Active glucometer, Germany) was used to measure BGLs via a blood drop from the tail vein [22]. BGLs within 200–600 mg/dl confirmed the T2DM model [23].

Drug administration

Rats received a single GLZ dose (4 mg/kg) as either the test formula (T) or the reference (R) using an oral feeding tube [8,24]. The administered dose was selected based on different studies that have safely used a wide range of GLZ doses for healthy and diabetic rats [25,26]. A calculated weight of T beads, equivalent to the specified dose, was administered to each rat. For reference product R, Diamicron MR 30-mg tablet (R) was divided to give single units, each equivalent to the specified dose, with a dissolution profile similar to the entire tablet (f_2>50) as confirmed previously [19].

Single-dose pharmacodynamics study

A total number of 48 rats were included in the single-dose PD study. Rats were given a standardized type of food and allowed to drink water throughout the study. Rats were randomly allocated into six groups (number of rats/group=8) in a parallel design.

Group 1 (nondiabetic control group): blank beads (GLZ-free).
Group 2 (nondiabetic reference group): GLZ 'R' tablets (Diamicron MR 30 mg).
Group 3 (nondiabetic test group): GLZ 'T' beads (GLZ-AL-GL beads).
Group 4 (STZ-induced diabetic control group): blank beads (GLZ-free).
Group 5 (STZ-induced diabetic reference group): GLZ 'R' tablets (Diamicron MR 30 mg).
Group 6 (STZ-induced diabetic test group): GLZ 'T' beads (GLZ-AL-GL beads).

BGLs were measured at different time intervals after administration of R, T, and blank beads. All nondiabetic rats were given glucose overload (2 g/kg per-oral) 30 min after drug dose administration [27].

Single-dose pharmacokinetics study

Rats allocated in groups 2 and 3 were subjected to PK evaluation. Blood samples were collected into heparinized vacutainer tubes at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 7, 12, 24, 48, and 72 h. Collected blood samples were centrifuged at 4000 rpm for 10 min, and the obtained plasma was then separated promptly and frozen at −20°C until assayed.

Evaluation of GLZ concentrations in plasma was carried out by an ultra-high-performance liquid chromatography/UV spectrophotometry (UHPLC/UV), as previously discussed in details [28]. Revalidation of the UHPLC/UV method has been carried out [19,28], 2 weeks before the start of the PK study. Glibenclamide was used as the internal standard. The UHPLC apparatus consisted of Waters Acquity Arc, with Quaternary Solvent Manager-R, Sample Manager (FTN-R), connected to a 2489 UV/Vis detector set at a wavelength of 230 nm and Empower 3 computer software. A Symmetry column was used [C18, 5 µm (3.9 cm×150 mm)], kept at room temperature, with a packed preguard column and Symmetry C18 inserts (5 µm). The mobile phase consisted of acetonitrile : deionized H_2O (pH adjusted to 3.8) 55 : 45 (v/v). The lower and higher limits of quantification were estimated.

The PK parameters were calculated from the plasma concentration versus time data using a non-compartmental model employing the WinNonLin, professional 2.1 computer program (Pharsight, Sunnyvale, California, USA). The following PK parameters were evaluated: C_{max}, T_{max}, AUC_{0-72}, $AUC_{0-\infty}$, and $T_{1/2}$.

Pharmacokinetic-pharmacodynamic relationship

Regression analysis was carried out to assess the relation between PK and PD performance of the two treatments. PK-PD relationship was evaluated by comparing the PK parameters (C_{max} and AUC_{0-72}) versus the PD parameters [G_{max} (the maximum blood glucose concentration) and AUG_{0-8} (area under the BGL-time curve)] in healthy rats.

The reduction in AUG_{0-8} reflected the PDs effect studied (lowering of BGLs), whereas the total bioavailable amount of the drug is represented by AUC_{0-72}. Therefore, computing the relationship between AUC_{0-72} and AUG_{0-8} could reflect the possible PK-PD relationship of GLZ for the applied animal model. Comparison of AUC_{0-72} to AUG_{0-8} was adopted from previous studies performed on an antidiabetic drug, metformin [29,30].

Multiple-dose pharmacodynamics study

Wounding and treatment: All rats were anesthetized by isoflurane inhalation. After removing the hair on the back, the skin was sterilized with 75% alcohol. Two full-thickness round wounds were created on both sides of the dorsum with a sterilized 6-mm biopsy punch [31]. Wounds were then protected with one layer of sterilized dressing and four layers of gauze and fastened with a surgical adhesive tape. The wound creation day was considered as day 0. Wounded rats were divided into three groups of eight rats each: group 1: nondiabetic control group, receiving blank beads, and groups 2 and 3: STZ-induced diabetic rats, treated with oral administration of 4 mg/kg GLZ in the form of T and R, respectively. This multiple dose study lasted for 17 consecutive days.

Body weight, blood glucose, and wound diameter measurements: Measurements of body weight and BGLs were done at successive days along the study. Wound diameter was measured by a 'digital caliper' [31] on days 0, 3, 7, 9, 11, and 14, following wound creation. The wounds were then reobserved on day 17. Wounds were considered closed if moist granulation tissue was not obvious anymore, and the wounds seemed to be covered with new epithelium [32].

Histological analysis: wound sites were harvested from rats on day 17 (time of euthanasia), after wound closure. After anesthesia, rats were euthanized, and a sample was taken. Samples were fixed in 10% neutral buffered formalin. Skin tissues were then embedded in paraffin. Tissues were sectioned at 4–5 µm, stained with hematoxylin and eosin (H&E) for morphological observations, and subjectively assessed for histological characteristics [18,31].

Statistical analysis

Student *t* test was selected for statistical analysis where *P* value less than or equal to 0.05 was considered being statistically significant [32,33]. Data were analyzed using SPSS Statistical Package, version 22 (SPSS Inc., Chicago, Illinois, USA).

Results and discussions

In a previous study [19], a successful MR multiparticulate system of GLZ-loaded freeze-dried AL-GL beads enhanced GLZ release rate in-vitro by 1.5 folds, as compared with the reference product (Diamicron 30 mg MR tablets), with minimal secondary absorption peak, in-vivo. Therefore, in the current study, a follow-up PD study was conducted to compare the performance of T and R, including

antihyperglycemic evaluation of single and multiple doses of the two treatments as well as their wound-healing capabilities after multiple oral administrations. Assessment of PK-PD relationship was conducted by comparing PK versus PD parameters.

Single-dose pharmacodynamics study

Previous studies were conducted to evaluate the antidiabetic activity of oral single dose of GLZ-MR microcapsules [34,35], beads [36], or nanoformulations [22,23,37,38] in diabetic animal model. Their results showed higher and prolonged hypoglycemic activity compared with pure GLZ [22,23,34,35,37,38] or to the reference conventional 80 mg GLZ tablet (Gliclazide) [36]. However, the current study was the first one in which comparative antidiabetic activity of a MR-GLZ formulation was done against a MR reference market product after oral administration in nondiabetic and diabetic rats.

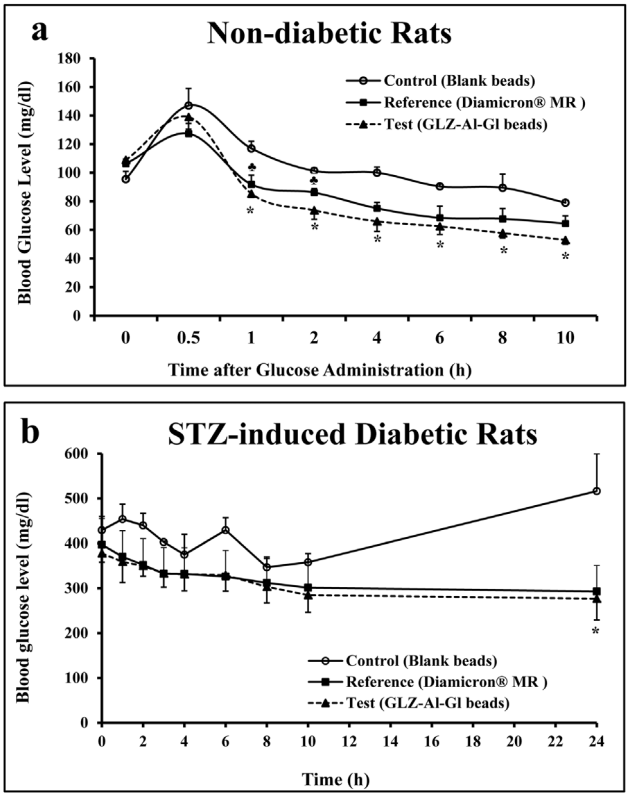
Nondiabetic rats

The mean BGLs of nondiabetic rats after administration of blank AL-GL beads (control), R, and T are presented in Fig. 1a. Experimental induction of hyperglycemia was done by glucose overload (2 g/kg per-oral), 30 min after GLZ dose administration. After 30 min of glucose administration, the BGLs of rats were elevated by 54, 20, and 28% for control, R, and T groups, respectively (Fig. 1a).

Figure 1a shows that both R and T had lower BGL compared with control along the whole experiment duration, with greater magnitude in case of T. Figure 1a also revealed that T had significant difference in BGL values than the control group (six time points: 1 h–10 h), whereas the R group showed significance at only two time points (2 h and 4 h). Moreover, Fig. 1a shows that the T group had lower BGLs than the R groups, starting from the 1-h sample till the end of experiment; however, the difference between groups T and R was not significant.

The glucose-induced hyperglycemia was reduced until 10 h by 46.3, 61.9, and 49.4% for control, T, and R, respectively. The more efficient reduction in BGL of T group could be owing to enhanced GLZ release and favorable bioavailability from AL-GL beads (multiparticulate dosage form) [19].

Figure 1



Effect of GLZ formulations (4 mg/kg) on blood glucose levels of nondiabetic rats (given glucose; 2 g/kg per-oral) (a) STZ-induced diabetic rats (b) (mean±SD, n=8 rats for each group). *Statistical significance at P value less than or equal to 0.05 (for T compared to control group). ♣Statistical significance at P value less than or equal to 0.05 (for R compared with the control group). GLZ, gliclazide; STZ, streptozotocin.

Streptozotocin induced-diabetic rats

Figure 1b shows the mean BGL for diabetic rats after administration of blank AL-GL beads (control), R, and T. STZ-induced diabetic rats showed remarkable variations in BGL values compared with the nondiabetic rats as indicated by the elevated error bars (c.f. Fig. 1a and b). Figure 1b shows that BGL values were reduced from 397.2 to 293 mg/dl and from 378 to 276.8 mg/dl after 24 h for R and T groups, respectively. Both R and T maintained a steady low BGL, compared with varied elevated BGL exhibited by the control diabetic group along the 24-h study period. The 24-h BGL value of T was significantly lower than that of control group, whereas no significant difference was observed between T and R groups at any time point (Fig. 1b). Meanwhile, undesired excessive hypoglycemia was not observed within all groups of diabetic rats.

Noticeably, the proposed GLZ beads were able to decrease BGL in both nondiabetic and diabetic rats, compared with the corresponding control groups. On the contrary, a previous study by Varshosaz *et al.* [4] discussed BG-lowering effect of GLZ controlled-release chitosan beads for nondiabetic and STZ-induced diabetic rat models. Tested GLZ chitosan beads were able to decrease BGL in nondiabetic rats for 24 h compared with the control group, yet it was not

able to significantly reduce the BGLs of diabetic rats compared with the corresponding control group [4].

Single-dose pharmacokinetics study

GLZ concentration in the collected plasma samples at different time intervals was determined by a previous UHPLC/UV assay [28]. The retention times for GLZ and glibenclamide were equal to 4.2 and 5.4 min, respectively. The determination coefficient (R^2) values were equal to 0.9892 and 0.9735 for the low (0.1–2 μg/ml) and high (1–40 μg/ml) calibration curves, respectively. The lower limit of quantification was 0.1 μg/ml, whereas the higher limit of quantification was 40 μg/ml. The accuracy was measured as the mean percentage recovery, which ranged from 94.54 to 102.78%. The analytical precision of the method was determined by the percentage relative standard deviation of the peak area ratios, which ranged from 3.03 to 7.89%.

Table 1 The pharmacokinetic parameters of two treatments (R and T) following oral administration of gliclazide single dose (4 mg/kg) in healthy rats (mean±SE, *n*=8 rats in each)

PK parameters	R[a]	T[b]
T_{max} (h)	2.00±0.47	3.38±0.60
C_{max} (μg/ml)	4.82±1.82	8.05±2.61
AUC_{0-72} (μg.h/ml)	42.92±13.39	77.40±17.08
$AUC_{0-\infty}$ (μg.h/ml)	49.14±15.01	81.23±16.86
$t_{½}$ (h)	19.31±1.78	21.83±4.03

PK, pharmacokinetic. [a]R: Diamicron 30 mg modified-release tablets (Servier). [b]T: freeze dried GLZ-AL-GL beads.

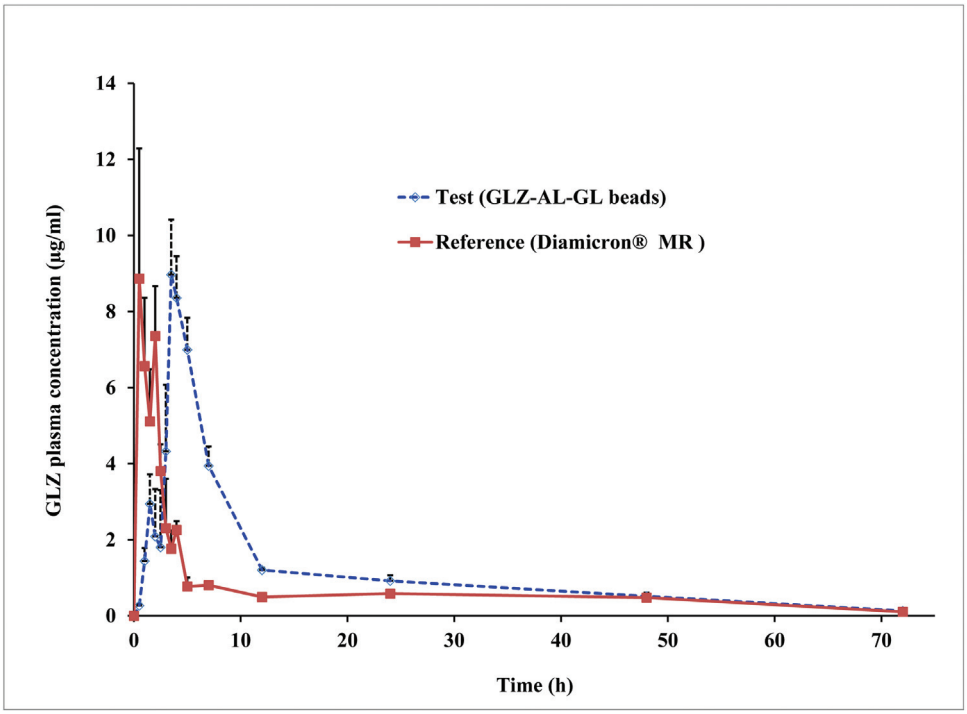
GLZ is known to exhibit high intersubject variation in the oral absorption in both type II diabetic patients and healthy volunteers [10,39]. After a single 80-mg oral dose, the peak concentration ranged from 2 to 8 μg/ml within 2–8 h [40–42]. Previously, El-Ashmawy *et al.* [19] confirmed GLZ intersubject variation and secondary absorption peak phenomena in a healthy rat model.

The PK parameters and plasma concentration-time profiles of GLZ after oral administration of reference tablet product (R: group 2) and test beads (T: group 3) in healthy rats are shown in Table 1 and Fig. 2, respectively. T beads were characterized by longer T_{max} as compared with R. The obtained C_{max} value for T was about 1.5-fold greater than R tablets, with relative bioavailability (T/R) in terms of $AUC_{0-\infty}$ of 160%. This in turn indicated a marked increase in the amount of drug absorption after oral administration of GLZ beads.

Pharmacokinetic-pharmacodynamic relationship in healthy rats

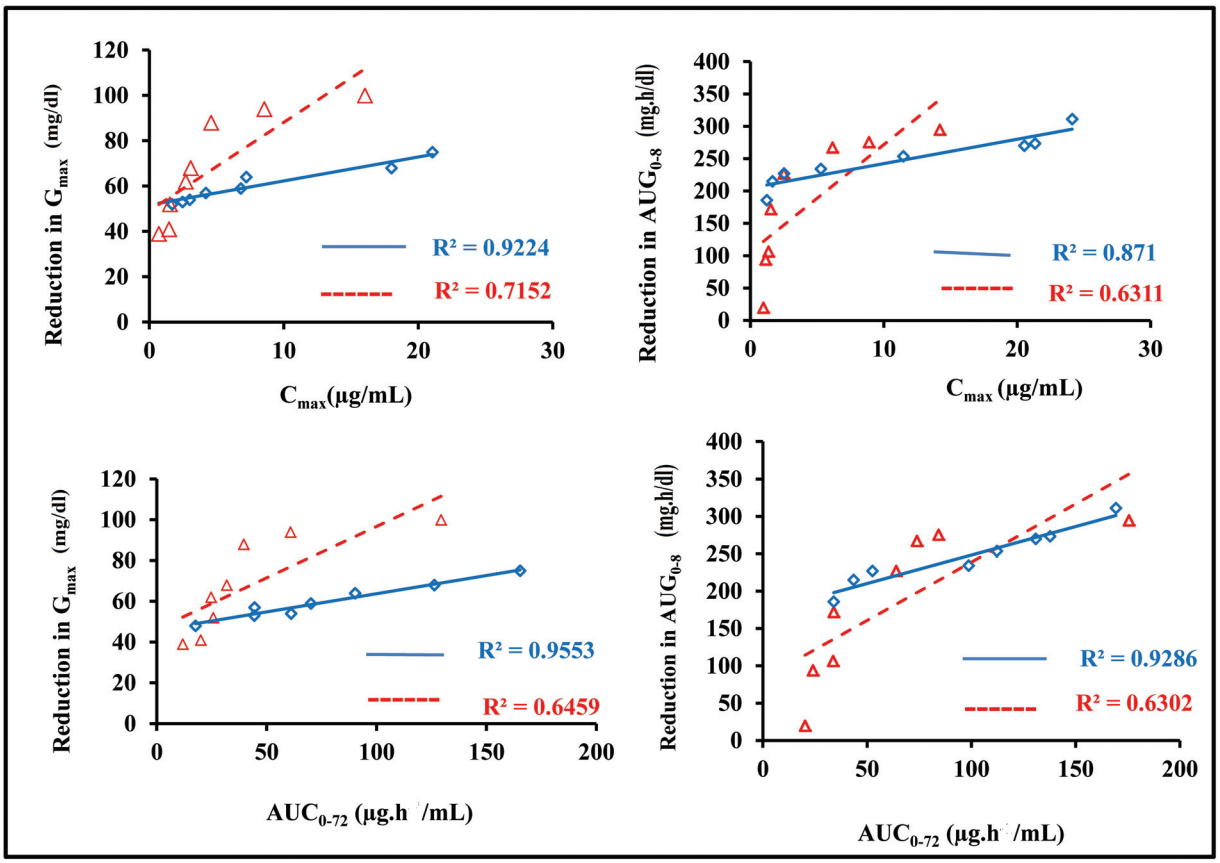
The relationships between PK parameters represented by C_{max} and AUC_{0-72} against PD parameters represented by reduction in G_{max} and AUG_{0-8} of

Figure 2



Mean GLZ plasma concentration-time profiles following oral administration of 4 mg/kg of reference and test in healthy rats (mean±SE, *n*=8 rats for each group). GLZ, gliclazide.

Figure 3



Relationship between pharmacokinetic and pharmacodynamic (PK-PD) parameters of two treatments: test (blue squares) and reference (red triangles) following oral administration of GLZ single dose (4 mg/kg) in healthy rats (n=8 rats for each group) (the solid lines and dashed lines represent the linear relationship for T and R, respectively). GLZ, gliclazide.

the two treatments (R and T) are displayed in Fig. 3. The criterion for selection of PK-PD parameters was adopted based on previously published studies by Chung *et al.* [29], who plotted reduction in AUG_{0-3h} against AUC_{0-12}, and Kim *et al.* [30], who plotted AUG_{0-3h} against AUC_{0-12}, to estimate the possible PK-PD relationship.

A direct linear relationship with high correlation was established for T (GLZ-AL-GL beads) regarding C_{max} and AUC_{0-72} against reduction in G_{max} with regression coefficient (R^2) values of 0.9224 and 0.9553, respectively. Similarly, a high correlation was observed for T when C_{max} and AUC_{0-72} were plotted against reduction in AUG_{0-8} with R^2 values of 0.871 and 0.9286, respectively. On the contrary, R (Diamicron 30 mg MR tablets) showed a linear PK-PD relationship, with low correlation for C_{max} and AUC_{0-72} against reduction in G_{max} (R^2 values of 0.7152 and 0.6459, respectively). Moreover, a low correlation was observed for R regarding C_{max} and AUC_{0-72} against reduction in AUG_{0-8} with R^2 values of 0.6311 and 0.6302, respectively (Fig. 3). The applied linear PK-PD relationship established for T was supported by the PK and PD results presented in Figs 1a and 2. The greater magnitude of BGL reduction, reflecting better hypoglycemic effect exhibited by T beads was mirrored by its 1.5-fold increase in C_{max} compared with R.

Previous studies have also explored the PK-PD relationship of GLZ after a single oral dose but applying different models than discussed in the current study. The applied PK-PD correlation (through plotting BGL and GLZ plasma concentration versus time) by Panda *et al.* [23] showed good point-to-point correlation of GLZ plasma concentration with BGL in the T2DM-induced rat model. Jović *et al.* [43] displayed the PD-PK correlation by plotting blood glucose concentration versus GLZ plasma concentration followed by linear regression, which showed that the maximum GLZ absorption was mirrored by the most profound GLZ hypoglycemic effect after being orally administered to healthy rats. On the contrary, Kim *et al.* [44] found that the maximum hypoglycemic effect

of GLZ was achieved 1.5 h after administration in healthy volunteers, and afterward, the effect decreased, possibly because of the homeostasis mechanism. Štětinová *et al.* [21] also studied the GLZ PK-PD relationship and found that although the bioavailability of GLZ was similar in both diabetic and nondiabetic rats, its hypoglycemic effect was not constant until 60 min after dosing, and the decrease of glycemia was smaller in diabetic compared with nondiabetic rats. Later, the strength of GLZ hypoglycemic effect persisted in diabetic rats, whereas in nondiabetic rats, a reversal of GLZ effect occurred.

Multiple-dose pharmacodynamics study

GLZ, besides its hypoglycemic effect, has been demonstrated for its antioxidative potentials as well as the prevention of the vascular complications of diabetes [45,46]. In addition to its free-radical scavenging property [10], GLZ inhibits TNF-α production, prostanoid release, and platelet aggregation [47,48].

Despite all of these reported favorable PD properties of GLZ, still no published studies have yet investigated how such merits would be reflected on improving troublesome wound healing associated with diabetes. Accordingly, there is a need to investigate the hypothesis that impaired wound healing in diabetic rat model might be redirected to simulate normal healing process in nondiabetic rats by the consecutive oral administration of GLZ (4 mg/kg). The wound healing study explored the ability of the two GLZ treatments administered to diabetic rats for 17 consecutive days, to normalize the wound healing process in a manner comparable to nondiabetic (normal) rats.

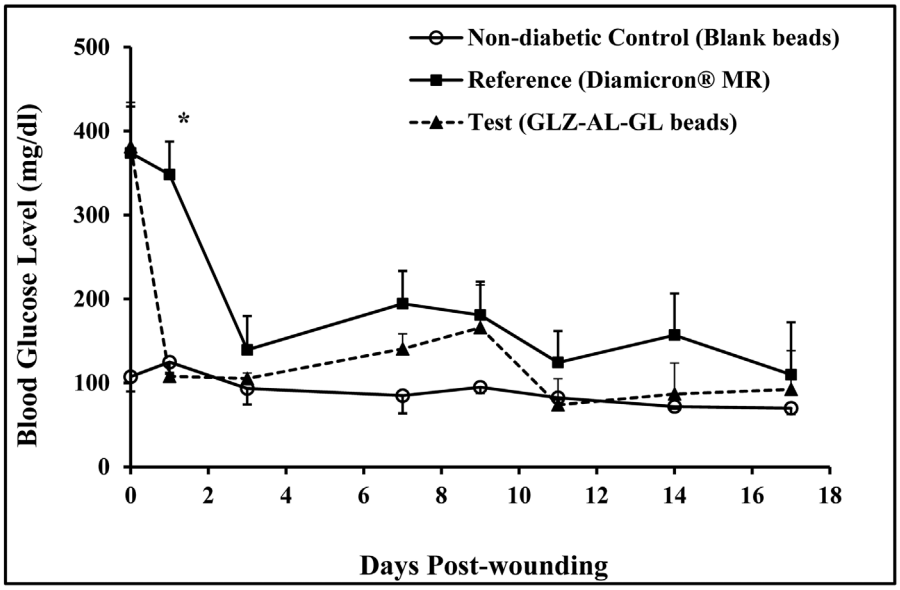
The wounding procedure was well tolerated by all rats, and they showed no noticeable weight loss after wounding. Initially, the mean body weight of rats was 226.9±23.4 g. No significant difference in body weight was found between any two studied groups at any experimental time point.

Antidiabetic activity

Changes of BGL encountered by wounded rats from the three groups (nondiabetic control, R, and T) throughout the whole wound healing experiment are demonstrated in Fig. 4. After GLZ administration to the wounded rats, T showed earlier normalization of BGL (day 1) compared with R (day 3) (Fig. 4). At day 1, mean BGL values were 122.5, 348.3, and 89.3 mg/dl for nondiabetic control, R, and T, respectively. The pattern of reduction of BGL for T was pronounced throughout the treatment phase (1st–17th day) compared with the R group (Fig. 4).

The areas under the BGL-time curve (AUG) of wounded rats after multiple oral administrations of R and T were calculated and statistically compared (Fig. 4). No significant difference was observed between the AUG of T and AUG of nondiabetic control group. On the contrary, the AUG of R was

Figure 4



Blood glucose levels of wounded rats: nondiabetic control and treatment diabetic groups after multiple oral administration of GLZ (4 mg/kg) (mean±SD, *n*=8 rats for each group). *Statistical significance between two groups at *P* value less than or equal to 0.05. GLZ, gliclazide.

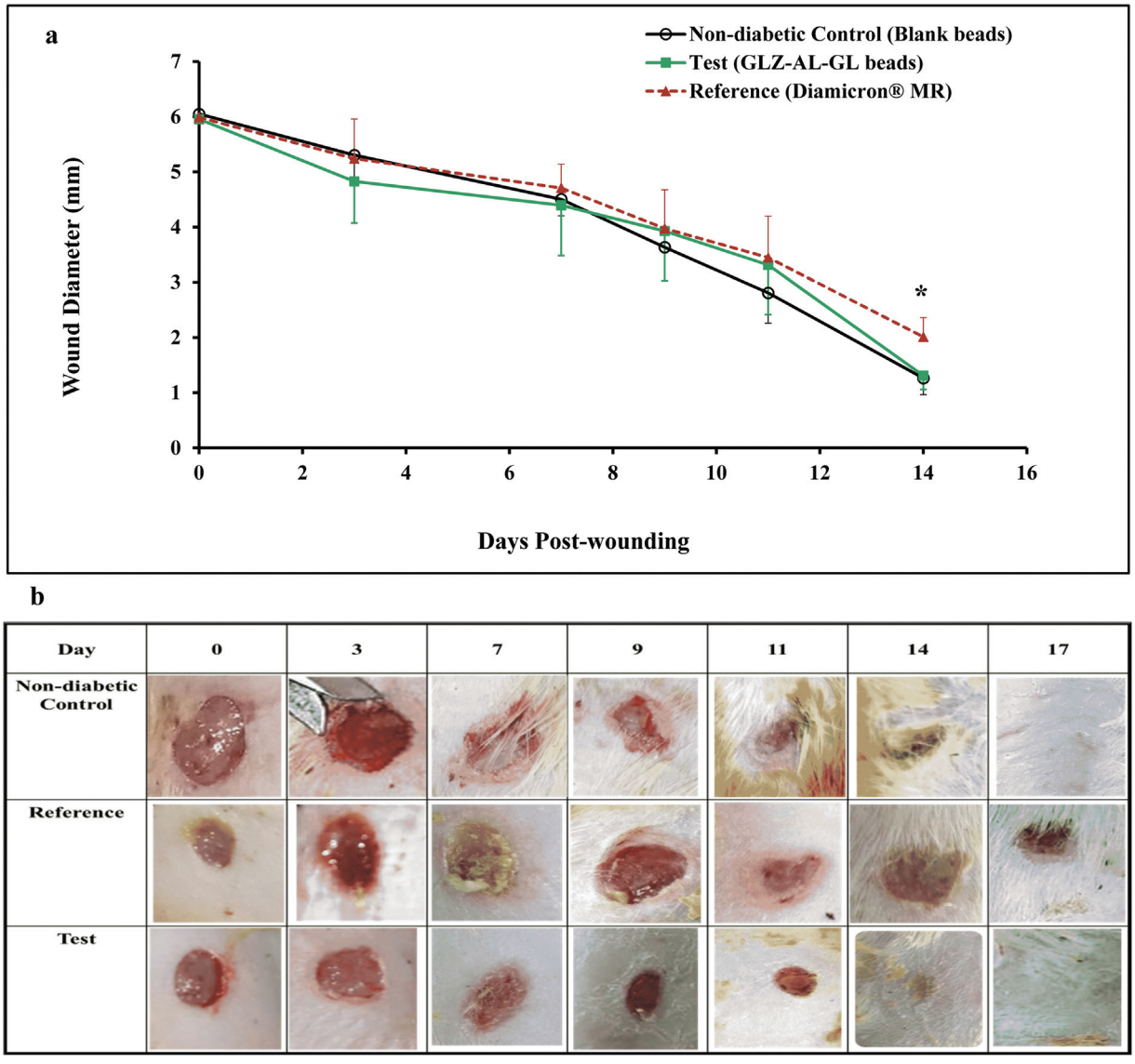
significantly greater than the AUG of control and T groups by 2 and 1.5 folds, respectively. This highlighted that multiple dosing of T beads was more efficient than R in managing the hyperglycemia of the STZ-induced diabetic rats.

A previous study reported a multiple daily dose (4 mg/kg) of GLZ-Aerosil solid dispersions for 15 days in alloxan-induced diabetic rats [49]. The authors found that by day 15 (last day of their study), the studied formulation decreased the BGL by 64% (from 391.5 to 138.6 mg/dl) against a 37% reduction in BGL by conventional GLZ (from 319.5 to 198 mg/dl). However, by only day 1, our test formula was able to decrease BGL by 72% (from 381.4 to 108 mg/dl) in STZ-induced diabetic rats.

Wound diameter

Impairment of wound healing remains one of the major complications of diabetes [50]. To examine the effects of GLZ multiple treatment on wound closure in diabetic rats, 6-mm punch biopsy wounds were created; afterward, the wound diameter was measured at specified times until day 17 (complete wound closure). Figure 5a illustrates the wound diameter progression along the period of the study, whereas Fig. 5b displays representative images of wound closure from the three studied groups. Figure 5a revealed that for all treated rats (R and T), the wound diameter progressively decreased, with gradual closure throughout the study duration. Statistically, treatment with T beads significantly decreased the wound diameter in diabetic rats,

Figure 5



Wound diameter progression over the course of the study (mean±SD, n=8 rats for each group). *Statistical significance between two groups at P value less than or equal to 0.05 (a) and photographic representation of the wound healing progression on different days for nondiabetic control, reference and test groups (b).

Table 2 Clinical observations throughout the wound healing study (total number of rats/group=8)

Observation	Days postwounding	Number of rats		
		Control	Reference	Test
Abscess	9	0	3	2
Scab formation*	11	6	3	5
	14	2	5	3
Complete wound closure**	14	5	3	5
	17	3	2	2
Wound closure with scab***	17	0	3	1

*Scab formation: defined as development of a crust of dried blood, serum, and exudate [51]. **Complete wound closure: defined as full re-epithelialization of the wound surface (scab-free) with no discernible exudate [32]. ***Wound closure with scab: where no obvious open wounds can be seen, instead partial re-epithelialization occurred and a remnant scab covering the rest of the original wound area.

compared with the R group, at day 14 after wounding. Moreover, images presented in Fig. 5b revealed almost complete wound closure, with intact skin exhibited by the T group, with development of granulation tissue and re-epithelialization by day 17, comparable with the control group. On the contrary, observed wound closure with remnant of scab was still more detected for the R group (Fig. 5b).

Table 2 depicts the most important clinical observations detected throughout the wound healing study. There was almost no sign of infection in any of the wounds at any time point until day 9, where three rats in case of R and two rats in case of T developed abscess. Complete wound closure was observed on day 14 for five rats in both control and T groups versus three rats in the R group. By day 17 after wounding, almost all rats in the T group showed no more open wounds, with closure of all remaining wounds (seven out of eight), whereas three rats of the R group showed remaining small scab (Table 2).

Wound histology

Samples of wound tissues were taken on day 17 (time of euthanasia), after wound closure, processed and inspected for histological changes. Representative photomicrographs of wound sites from rats belonging to the control and treatment groups at day 17 are shown in Fig. 6 at different magnifications (×100, ×200, and ×400) stained with H&E. Histological examination of wound sites from the three studied groups revealed a normal healing process with different degrees of re-epithelialization and mixed inflammatory response (infiltration of epidermis and/or dermis with polymorphonuclear cells and lymphocytes) (Fig. 6a–c).

Figure 6a and c represents skin sections from control and test groups showing clear wound borders and almost intact epidermis of two to three cell layers, with the re-epithelialization process extended to the surface and abundant connective tissues. Almost healed skin was observed, with mild infiltration of the dermis with lymphocytes and polymorphonuclear cells, some hair follicles together with collagen deposition.

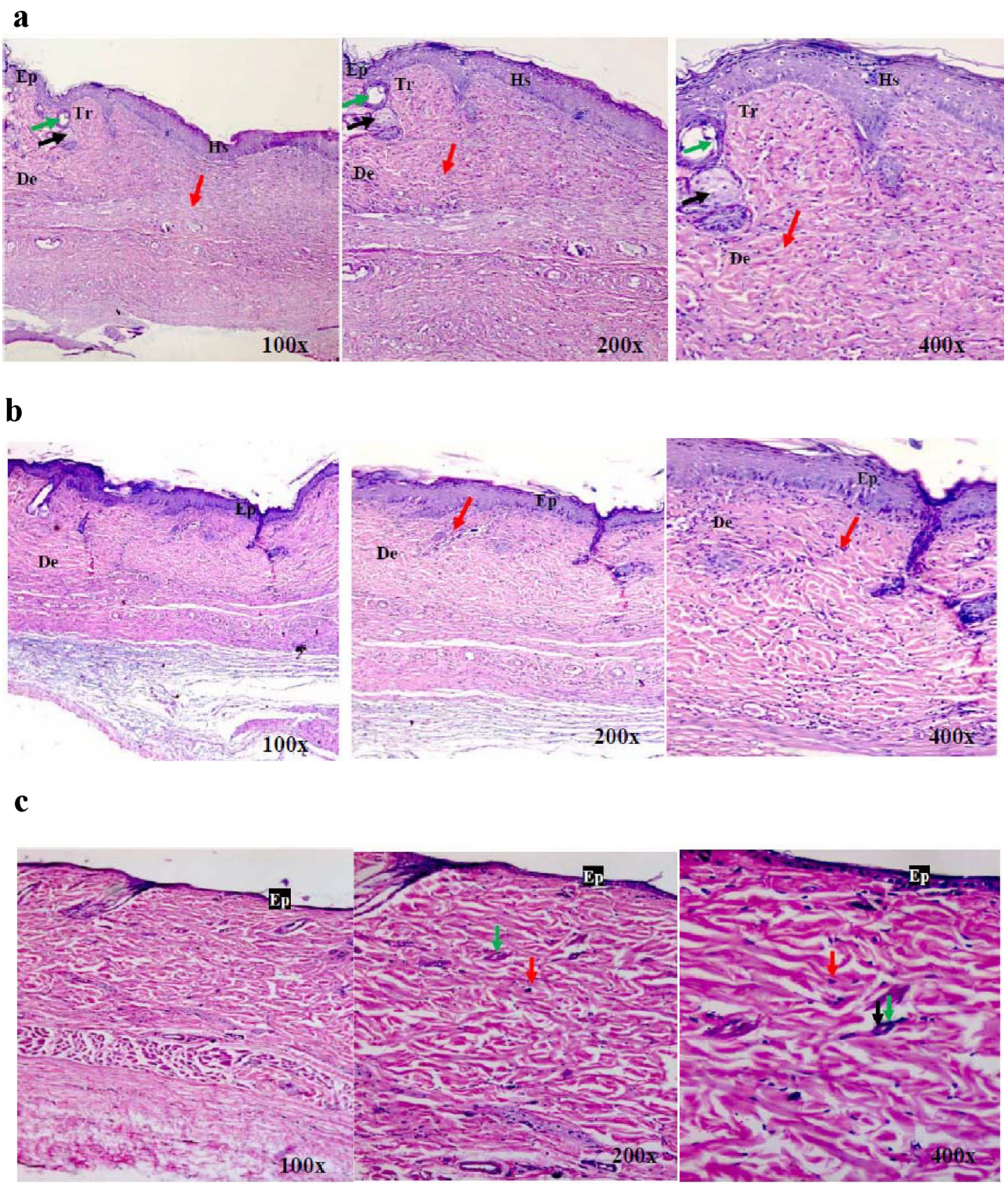
On the contrary, Fig. 6b demonstrates a skin section from the reference group showing almost healed skin, with slight delay in the re-epithelialization process, but inward epithelial growth was sustained.

Hence, an overall conclusion confirmed that oral administration of GLZ-AL-GL beads to diabetic wounded rats, for 17 consecutive days, was able to promote wound healing in such a way to go parallel with the normal healing process of nondiabetic normal rats.

Conclusion

The proposed MR freeze-dried GLZ-AL-GL beads gave encouraging PD results. A combined antihyperglycemic and wound healing activity of this new GLZ multiparticulate system was presented. In case of a single oral dose, the proposed T beads succeeded more significantly in lowering the BGL of both nondiabetic and diabetic rats compared with the market product (Diamicron MR). A direct linear PK-PD relationship with high correlation was found between the PD (glucose-lowering effect) and PK parameters, for T only, with simultaneous favorable bioavailability compared with R. In case of multiple GLZ oral doses to wounded rats, T showed faster ability to normalize the rats' hyperglycemia than R. For the first time, the effect of GLZ treatment on improving wound healing of diabetic rats was explored where T beads exhibited almost complete wound closure after multiple dosing for 17 days,

Figure 6



Photomicrographs of wound sites on day 17 from rats belonging to control (a), reference (b), and test (c) (magnifications: ×100, ×200, and ×400 stained with H&E). Hs: healed skin; Ep: epidermis; De: dermis; Tr: transition area between normal and healed skin; red arrows: infiltration of the dermis with PMNLs and lymphocytes; green arrows: sebaceous glands; black arrows: hair follicles. PMNL, polymorphonuclear cell.

comparable with the control healthy rats. The proposed GLZ-MR AL-GL beads seem to be an interesting therapeutic prospect for the treatment of hyperglycemia as well as resolving impairment of wound healing associated with diabetes.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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