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Green Chemistry Approach To Improve Physicochemical Properties Of Glimepiride Through Eutectic Mixture Formation

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Abstract

Glimepiride (GL) is an oral hypoglycaemic agent with compromised solubility. The present work is intended to improve the solubility of this drug by formation of binary eutectic mixtures. The eutectic mixtures were synthesised using gallic acid, ascorbic acid, thiourea and caffeine as coformers as indicated by a significant depression in the melting points in the DSC analysis, accompanied by no apparent change observed in PXRD analysis. The morphological differences were analysed by SEM analysis. A maximum of 14 and 63 times increase in solubility was observed in 0.1 N HCl and phosphate buffer pH 6.8 respectively in GL-GA. Pharmacokinetic studies were performed on all the prepared eutectic formulations and compared with pure GL. GL-GA provided highest plasma blood concentration of GL. As projected, the invitro data very well corroborate with in vivo results and the drug release was found to be maximum when an equimolar concentration of gallic acid was used as conformer, making gallic acid an ideal candidate for the cocrystal formulation. The research work presented a complete preliminary data on improved physicochemical properties of glimepiride.

Keywords: solubility, eutectic, glimepiride, coformers, physicochemical, pharmacokinetic

INTRODUCTION

The oral route is a common, popular, non-invasive, painless and easy-to-handle way for administration of drugs, and thus often associated with improved patient compliance (Lou et al., 2023). Among the oral dosage forms, the solid ones are the most used and have innumerous advantages. Solid pharmaceutical dosage forms do not require sterility during production, can be obtained with relatively simple and cost-effective process, have high physico-chemical stability, are safe and easy to self-administer (Fuhrmann and Fuhrmann, 2017; Zhou et al., 2020).

It is well established that dissolution is the rate-limiting step in the gastrointestinal absorption of a drug from solid dosage forms. Aqueous solubility is a key parameter influencing biological activity, formulation and in vitro and in vivo biopharmaceutical properties (Shekhawat and Pokharkar (2017). However, many drugs currently used and in development have poor water solubility and belong to Classes II and IV of the Biopharmaceutical Classification System (BSC), i.e., have low solubility. Consequently, several approaches like amorphous solid dispersions, co-amorphous systems, micro and nanoparticles, salt formation, nanocrystals, eutectic mixtures, inclusion complexes, liquisolid pellets have been examined to enhance the dissolution rate, solubility and /or oral bioavailabilty of these poorly water-soluble drugs to attain more rapid and more complete absorption performance (Göke et al., 2018; Ha et al., 2019; Leuner, 2000). Each one of these systems have certain advantages and disadvantages which limits their use in drug delivery. A few studies are described in the literature employing simple eutectic formation as an approach that provides better physical, chemical and pharmacological properties in terms of dissolution rates, stability, solubility, hydroscopicity, and bioavailability etc as compared to the mixtures formed by conventional methods (Zhou et al., 2020). It is feasible to contrive and develop eutectic mixtures of pharmaceutical drugs with the help of green chemistry that is an environment friendly approach and can take place easily with minimum use of organic solvents such as solid-state synthesis (Andruch & Varfalvyová et al., 2022).

Glimepiride (GL), 1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl) phenyl] sulfonyl]-3-(trans-4-methylcyclohexyl) urea, is an oral sulfonylurea hypoglycemic agent (**Figure 1**) (Jaafar et al., 2020) that belongs to Class II (low water solubility and high permeability) in the Biopharmaceutics Classification System (BCS) (Amidon et al., 1995). The poor aqueous solubility and slow dissolution leads to an erratic clinical response or, in some cases, therapeutic failure due to sub-therapeutic plasma drug level (Frick et al., 1998; Massi-Benedetti, 2003). Therefore, enhancing the solubility and dissolution of glimepiride is an important and challenging goal in formulation development. Until now, only a few studies have reported innovations that improve solubility, including the formation of a polymorph (Bonfilio et al., 2012; Endo et al., 2003), complexing with cyclodextrin (Ammar et al., 2006a,b), the preparation of a solid dispersion (Gill et al., 2010; Kiran et al., 2009; Mehta et al., 2009; Reven et al., 2010), micronization (Ning et al., 2011), and microencapsulation (Ilić et al., 2009).

To the best of our knowledge, there have been no attempts to study eutectic mixtures of glimepiride with gallic acid, (GL), ascorbic acid (AA), thiourea (TH) and caffeine (CA) (**Figure 1**). However, one cocrystal of glimepiride with caffeine has been reported by (Chhajed, et al., 2020). The present study aims to investigate the change in the physicochemical properties when binary mixture with a focus on improved dissolution rate and biological efficacy of the poorly water-soluble glimepiride are investigated. The solid-state characteristics of GA binary mixtures by differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD) along with the estimation of solubility and dissolution studies were included in this study. Furthermore, in-vivo studies were also designed for the prepared eutectic mixtures to validate the improvement in activity of the eutectic mixture.

MATERIALS AND METHODS

Materials

Glimiperide was obtained as a gift sample from Dr Edwin Labs Medilabs Pvt Ltd. (99% purity). Gallic acid, ascorbic acid, thiourea and caffeine were obtained from Sigma Aldrich, USA and all other chemicals and solvents used were of analytical grade.

Preparation of eutectic mixture

For the preparation of the binary mixtures the solvent drop assisted solid-state grinding method was used. Glimepiride and coformers such as gallic acid, ascorbic acid, thiourea and caffeine, were triturated in a glass pestle mortar for 90 min with periodic addition of drops of catalytic (trace) amounts of ethanol at molar ratio 1:1. The dry product; glimepiride-gallic acid (GL-GA), glimepiride-ascorbic acid (GL-AA), glimperide-thiourea (GL-TH) and glimepiride-caffeine (GL-CA) eutectic mixture obtained after grinding were stored under dry and airtight conditions at a temperature not exceeding 25 °C for further analysis. The experiment was conducted in a room where the temperature and humidity were controlled at 25 ± 2 °C and 40 ± 5% relative humidity (RH), respectively.

Differential Scanning Calorimetry (DSC):

The DSC graphs were obtained on DSC Q20 (TA Instruments DE) using accurately weighed sample (1-2 mg) which was heated from 50 °C to 350 °C in an aluminum pan at a rate of 5 °C/min under a constant stream of dry nitrogen (50 mL/min). The Tm are defined as the temperature at the apex of each endothermic melting peak.

Powder X- ray Diffraction (PXRD) technique:

Powder X-ray diffraction spectra were collected over the range of 4° to 50° (2q) at a step rate of 0.017° with time of 25 s/step on X'Pert PRO diffractometer system (PANalytical, Almelo, the Netherlands) using Cu Ka radiation of 1.54060° A. The tube voltage was set at 45 kV, power at 40 mA, and the divergence and anti-scattering slit were set at 0.48.

Scanning Electron Microscopy (SEM):

The SEM figures were taken on scanning electron microscope (SEM) model JSM6100 (Jeol) with image analyzer. About 10-20 mg of the powder samples were dispersed on an aluminum stub fixed with double-sided tape. The powder was made electrically conductive by coating the powder with gold-palladium (60%:40%) under argon atmosphere in a "Sputter Coater" instrument. The stubs with the coated powder were then mounted in the microscope, and the images were observed and recorded at magnification range of 100X to 5000X.

Solubility analysis

For determination of solubility of glimepiride in various solvents (phosphate buffer pH 6.8 & 0.1 N hydrochloric acid), excess amount of sample was suspended in 10 ml of aqueous 0.1 N HCl and phosphate buffer pH 6.8 contained in a stoppered flask was shaken at 37 °C on a water bath shaker at 100 rpm. The analysis was performed in duplicate. Aliquots of 0.2 mL were withdrawn with replacement by fresh solvent at time intervals of 15, 30, 45, 60, 90, 120, 150, 180, and 240 min with the final aliquot taken at 24 h. The samples were diluted up to 1 mL with solvent and filtered through 0.2-mm nylon filter before being quantified for lafutidine content by UV analysis. The fixed concentrations of glimepiride at 20, 40, 60, 80, and 100 mg/mL in 0.1 N HCl provided the calibration curve for glimepiride.

In vitro Drug Release studies

The in vitro release of binary mixtures of glimepiride were tested in a USP Type II dissolution apparatus. An equivalent weight of binary mixtures (GA-AA, GA-GA, GA-TH, GA-CA) containing 4

mg of glimepiride was placed separately in 900 ml of 0.1N HCl (pH 1.2) maintained at 37 ± 0.5 °C and stirred at 100 rpm. About 5 ml aliquots of sample were collected at regular time intervals, and the same amount of fresh dissolution medium was replaced into a dissolution vessel to maintain the sink condition throughout the experiment. The aliquots were filtered, further diluted suitably, and estimated spectrophotometrically at 220 nm.

Animal studies

The present experimental study was conducted using Wistar rats weighing about 220-250 g procured from the "Central Animal House" of ISF College of Pharmacy, Moga, Punjab (India). All the animals were kept in clean polyacrylic cages and maintained in an air-conditioned animal house under standard laboratory conditions (room temperature 25 ± 3 ℃ and relative humidity of 55-60 %) with 12 h light/dark cycle. The food and water were made available *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) (ISFCP/IAEC/CPCSE/2020/P 17) and experiments were carried out in accordance with the Committee for the Control and Supervision of Experiments on Animals (CCSEA) for the use and care of experimental animals and "Animal Research: Reporting of In Vivo Experiments (ARRIVE)" guidelines. All experiments were performed using age-matched animals in an attempt to avoid variability between experimental groups. Animals were acclimatized for 2 weeks to the new environment before experimentation.

Pharmacokinetic Studies

Pharmacokinetic evaluation of a single oral dose of glimepiride and prepared eutectic formulations was conducted on Wistar rats of both sexes (n = 7), with body weights ranging from 200 to 250 g, maintained under standard laboratory conditions. The compounds were administered orally to fasted rats (food deprived for 18 h, with water available *ad libitum*) at a dose of 1 mg/kg proportional to their body weight, using a 0.5% w/v aqueous sodium carboxymethyl cellulose (CMC) suspension as the vehicle. Blood samples (0.1 - 0.3 ml) were collected from tail vein method at intervals of 15, 30, 60, 120, 240, 480, and 720 min post-dosing, into heparinized glass tubes. These samples were centrifuged at 12,000 rpm for 15 min at 4 ℃ to obtain plasma. Plasma samples were treated with 50 µL of acetonitrile and centrifuged under the same conditions, repeated three times. Quantitative analysis of glimepiride in the plasma was performed using HPLC. Pharmacokinetic parameters, including C_{max}, T_{max}, and AUC, were calculated using PKSolver (an add-in for Microsoft Excel).

Experimental Groups and Induction of Type 2 Diabetes

Rats were fasted for 12 h prior to the experiments, with water available *ad libitum*. 49 Wistar rats were distributed randomly into 2 different groups in a single-blind fashion: one group (n = 7) served as the vehicle control and the other cluster (n = 42) received a single intraperitoneal injection of streptozotocin (STZ) after nicotinamide. Afterwards, experimental diabetes was induced on day 1 by intraperitoneal (i.p.) injection of streptozotocin (STZ) at a dose of 60 mg/kg, dissolved in freshly prepared cold citrate buffer (0.1 M, pH 4.5) after nicotinamide (110 mg/kg in normal saline, i.p.). Following STZ administration, the animals were provided with normal food and water, and monitored every 2 h for a 12-h period to observe for signs of marked hypoactivity, unresponsiveness, or convulsions. Diabetes was confirmed three days post-STZ injection (day 3) by measuring fasting blood glucose levels using the glucometer ACCU-CHEK (Roche, Basel, Switzerland). Rats with fasting blood glucose levels exceeding 250 mg/dL were classified as diabetic and included in the study. The blood sample was taken from the tail vein.

The group of non-diabetic rats served as the vehicle control group (Group I, $n = 7$). Diabetic rats were randomly assigned into 6 groups ($n = 7$ per group) for treatment as follows: Group II (STZ) received 0.5% w/v aqueous sodium CMC vehicle; Group III (STZ+GL) was treated with glimepiride (GL, 1 mg/kg, orally); Group IV (STZ+GL+GA) was treated with a glimepiride and gallic acid mixture (GL+GA, 1 mg/kg, orally); Group V (STZ+GL+AA) received (GL+AA, 1 mg/kg, orally); Group VI (STZ+GL+CA) was administered (GL+CA, 1 mg/kg, orally); and Group VII (STZ+GL+TH) received (GL+TH, 1 mg/kg, orally). All treatments were administered once daily via the oral route for 28 consecutive days, from day 3 to day 30. Glimepiride was taken as standard drug in this study. Body weight and serum glucose was analyzed weekly. Plasma insulin (ELISA kit ThermoFisher Scientific), homeostatic model assessment for insulin resistance (HOMA-IR = Fasting insulin level (μU/ml) × Fasting blood glucose (mg/dl) / 405), homeostatic model assessment for β cell function (HOMA-β = 20 × Fasting insulin level (μU/ml) / Fasting blood glucose (mmol/l) – 3.5), levels were evaluated on day 3 and day 30 of the study.

Assessment of Oxidative Stress

Estimation of catalase activity

Catalase activity was assayed by the method of Claiborne (1985). Briefly, the assay mixture consisted of 1.95 ml phosphate buffer (0.05 M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M) and 0.05 ml post mitochondrial supernatant (10%) in a final volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of k min^{-1} and expressed as mean ± SEM.

Estimation of Lipid Peroxidation

The malondialdehyde (MDA) content, a measure of lipid peroxidation (LPO), was assayed in the form of thiobarbituric acid-reactive substances (TBARS) by the method of Wills (1965). Briefly, 0.5 ml of post mitochondrial supernatant and 0.5 ml of Tris HCl were incubated at 37 °C for 2h. After incubation 1 ml of 10% trichloroacetic acid was added and centrifuged at 1000 rpm for 10 min. To 1 ml of supernatant, 1 ml of 0.67% thiobarbituric acid was added and the tubes were kept in boiling water for 10 min. After cooling 1 ml double distilled water was added and absorbance was measured at 532 nm. Thiobarbituric acid-reactive substances were quantified using an extinction coefficient of $1.56 \times 105\ M^{-1}\ cm^{-1}$ and expressed as nmol of malondialdehyde per ml.

Estimation of Reduced Glutathione

Reduced glutathione was assayed by the method of Jollow et al. (1974). Briefly, 1.0 ml of post mitochondrial supernatant (10%) was precipitated with 1.0 ml of sulphosalicylic acid (4%). The samples were kept at 4 °C for at least 1 h and then subjected to centrifugation at 12000 rpm for 15 min at 4 °C. The assay mixture contained 0.1 ml supernatant, 2.7 ml phosphate buffer (0.1 M, pH 7.4) and 0.2 ml 5,5, dithiobis (2-nitro benzoic acid) (Ellman's reagent, 0.1 mM, pH 8.0) in a total volume of 3.0 ml. The yellow colour developed was read immediately at 412 nm.

Total Nitrites

Nitrite was estimated in the plasma regions using the Greiss reagent and served as an indicator of nitric oxide production. 500 μl of Greiss reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% naphthylamine diamine dihydrochloric acid in water) was added to 100 μl of post mitochondrial supernatant and absorbance was measured at 546 nm (Green et al., 1982). Nitrite concentration was calculated using a standard curve for sodium nitrite. Nitrite levels were expressed as nmol/ml.

Determination of inflammatory makers (TNF-α, IL-6, and IL-1β)

The quantitative estimation of inflammatory cytokines, specifically tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), in plasma was performed using a rat enzyme-linked immunoassay kit obtained from KRISHGEN BioSystem (Mumbai, India). The quantification was carried out using a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) specifically designed for rat IL-1β and IL-6 detection. The assay employed a microtiter plate reader (iMARK, Bio-Rad). Concentrations of TNF-α, IL-1β, and IL-6 were determined from standard curves generated during the assay, with results expressed in picograms per milliliter (pg/ml).

RESULTS AND DISCUSSION

In the present study the solvent-drop grinding method was utilized to prepare the binary mixtures of glimepiride with various coformers to improve the physicochemical properties of solubility compromised glimepiride. The solvent acts as a catalyst in this method of synthesis of binary mixtures providing a medium for the drug and coformer to interact through certain kind of forces such as hydrogen bond, vander Waal's forces or hydrophobic interactions. A number of coformers were screened from the GRAS (generally regarded as safe) list before ascorbic acid, gallic acid thiourea and caffeine were selected for the formation of binary mixtures. The grinding method can result in formation of either cocrystal or eutectic mixture depending upon the duration of grinding, amount of solvent used and intensity of force which is applied during manual grinding. The grinding was optimized by monitoring the melting point of the product after every 30 min of grinding. After 90 min and upto 180 min, no change in the melting was observed in the product. So, the duration of grinding of all the final products (GA-AA, GA-GA, GA-TH and GA-CA) during the scale up process was kept 90 min. The amount of ethanol used during the grinding process was 4 ml.

Differential Scanning Calorimetry (DSC):

DSC is one essential technique to characterize the solid phase behaviour of a material and mixtures. DSC curve of the pure glimiperide showed melting endotherm peak at 214.59 °C as shown in **figure 2**. It is crucial to detect the melting point to verify the system as typically the melting point of the cocrystal is inbetween both the components while the melting point of eutectic mixture is lower than both of the parent components. The thermogram of the GL-AA mixture exhibited sharp endothermic peak at 166.17 ℃. When compared with the melting points of AA (which appears at 193.19 °C) and that of glimiperide (at 214.59 °C), the melting endotherm of GL-GA mixture was found to be lower than both individual components suggesting it to be a eutectic mixture. The melting endotherm of GL-CA was found to be at 189.40 ℃, GL-TH at 190.78 ℃ and GL-GA at 186.09 ℃. The thermogram of caffeine exhibited only a single endothermic peak at 236.08 ℃, gallic acid at 262.10 ℃ and thiourea at 190.78 ℃ (**Table 1**). The melting endothermic peaks for all prepared binary mixtures are also significantly lower than the individual components suggesting that the binary mixtures are eutectic in nature. The single endothermic peak for 1:1 stoichiometric ratio observed in all the mixtures suggests the pure nature of products formed. In case of GL-TH, a second endothermic peak after the melting point indicates the conversion of mixture to a stable form which melts at 187.89 ℃. Further confirmation of eutectic nature of binary mixtures are performed by PXRD and SEM.

Powder X-ray diffraction study (PXRD): PXRD analysis is an important tool for assessing the degree of crystallinity and formation of new phases. Each crystalline phase can be identified by its unique

diffraction pattern. The purity and crystallinity of glimepiride is determined by sharp and intense peaks at 2θ position 6.4°, 13.59°, 16.86°, 18.31°, 19.34°, and 21.39° owing to the crystalline nature of the drug. Similarly, when analysed the PXRD pattern of GA, major peaks at 2θ position 14.68°, 15.12°, 18.45° and 19.79° were observed, however, the GL-GA mixture showed the peaks of both individual components at 6.4°, 13.4°, 13.8°, 14.66°, 16.47°, 18.18° and 19.18° as shown in **figure 3** suggesting that although the crystallanity of the mixture was retained there was no new crystalline phase was formed. This suggests that the GL-AA mixture is not a cocrystal but a eutectic mixture which is formed. In the case of GL-TH, GL-CA, and GL-GA, as can be seen in **table 2 and figure 3**, a similar overlapping and mixing of the peaks of both the components were observed again suggesting that the eutectic mixture are formed instead of cocrystals.

Scanning Electron Microscopy (SEM)

The micrographs of glimepiride and its binary mixtures are given in figure. The comparison of micrographs show that the GL-TH and GL-CA appear to be more crystalline in morphology as compared to GL-GA and GL-AA where a reduction in particle size is observed at the same magnification (**Figure 4**).

Solubility Studies:

According to FDA, solubility should be estimated at physiological pH. The solubility experiments of glimepiride eutectic mixtures were performed in phosphate buffer pH 6.8 and 0.1 N HCl. The results showed in (**Table 3 and Figure 4**) indicate that the eutectic mixtures improved the solubility of glimepiride as compared to the pure drug when dissolved in the individual solvent. The maximum increase in solubility was found with GL-GA eutectic formulation while minimum increase was seen in GL-CA eutectic mixture indicating the greater ability of gallic acid to increase the aqueous solubility of poorly water-soluble drug as compared to other conformers. The solubility was found to be in the range of 1.756±0.027 to 13.157±0.108 in the case of 0.1N HCl and 3.747±0.108 to 31.027±0.108 in the case of phosphate buffer pH 6.8. Also, the fold solubility increase was found to be ~14 and 63 times in the case of 0.1 N HCl and phosphate buffer pH 6.8 respectively.

Thermodynamic parameters such as the heat of fusion (ΔH_{fus}) can also related to the overall solubility of prepared forms. It can be seen in **Tables 1 and 3** that in the GL-GA mixture, there is a considerable decrease in ΔH_{fus} and it shows higher solubility. However, higher ΔH_{fus} was observed for GL-CA and GL-TH and thus the resulting solubility is less. The experimental solubility results are well coordinated with the heat of fusion data of all the forms.

In vitro Drug Release of eutectic formulations of glimepiride

The in vitro release of glimepiride from the eutectic mixture was tested in a USP Type II dissolution apparatus. An equivalent weight of eutectic mixtures containing 4 mg of glimepiride was placed separately in 900 ml of 0.1N HCl (pH 1.2) maintained at 37 ± 0.5 °C and stirred at 100 rpm. About 5 ml aliquots were collected at regular time intervals, and the same amount of fresh dissolution medium was replaced into a dissolution vessel to maintain the sink condition throughout the experiment. The aliquots were filtered, further diluted suitably, and estimated spectrophotometrically at 220 nm. All the formulations of the prepared eutectics of glimepiride were subjected to in vitro release studies. As can be seen in **table 4** and **figure 6**, the drug release was found to be maximum when an equimolar concentration of gallic acid was used as coformer, making gallic acid an ideal candidate for the cocrystal formulation

Pharmacokinetic studies

The plasma concentration-time graph comparing GL and its eutectic formulations is displayed in **Figure 6**, with pharmacokinetic parameters summarized in **Table 5**. The peak plasma concentration of GL in its eutectic form with gallic acid was observed to be 99.36 µg/ml approximately four hours after oral administration. This value is 1.39 times higher, respectively than that of pure GL. This increase in concentration suggests enhanced in vivo absorption of GL due to binary mixture formation.

Effect on body weight, blood glucose levels, insulin, IR, and *β*-cell function in diabetic rats

The *i.p.* administration of a single dose of STZ on day 1 resulted in a significant elevation in plasma glucose levels in rats measured on day 3 and caused a sustained elevation in blood glucose levels. This increase in blood glucose levels in the STZ group was statistically significant when compared to the vehicle control group. Furthermore, the STZ-treated rats experienced a notable reduction in body weight relative to their age-matched vehicle controls **(Figure 8)**. Importantly, oral treatment with glimepiride (standard orally active anti-diabetic drug, dose 1 mg/kg) alone or glimepiride eutectic mixtures (dose 1 mg/kg) for 28 days daily led to a significant decrease in blood glucose levels and improvement in body weight in STZ-treated rats in comparison to the rats that received STZ and vehicle only. These results underscore the potential anti-hyperglycemia efficacy of this therapeutic approach. However, the GL+GA eutectic mixture declined the blood glucose levels and ameliorated the STZ-induced decrease in body weights of rats more pronouncedly relative to other eutectic mixtures and showed anti-hyperglycemia efficacy comparable to the standard drug used in this study, glimepiride.

Assessment of HOMA-IR and HOMA-β on day 3 (before treatment) after a single STZ injection on day 1 indicated the development of IR (insulin resistance) and loss of pancreatic-β cell function. The data showed a significant increase in HOMA-IR and a decrease in HOMA-β in STZ-treated groups of rats relative to the vehicle control on day 3 **(Table 6)**. Subsequently, the calculation of HOMA-IR and HOMA-β on day 30 (post-treatment) indicated sustenance of IR (insulin resistance) and loss of pancreatic-β cell function in STZ-treated rats relative to the vehicle control group. On day 3 we observed that plasma insulin levels were significantly declined in the STZ-treated groups relative to the vehicle control group. Afterward, measurement of circulating insulin levels on day 30 showed a significant decrease in the STZ group relative to the vehicle control group. These results indicated the development and sustenance of type 2 diabetes in Wistar rats in response to a single injection of nicotinamide and STZ.

Treatment with glimepiride (1 mg/kg, *p.o.*) and glimepiride eutectic mixtures (1 mg/kg, *p.o.*) in separate groups for 28 days daily significantly attenuated STZ-induced IR, pancreatic-β cell dysfunction, and improved circulating insulin levels relative to STZ and vehicle treatments. Administration of glimepiride alone (standard orally active anti-diabetic drug) and glimepiride eutectic mixtures (1 mg/kg, *p.o.*) in different groups of STZ-treated diabetic animals caused a significant decrease in HOMA-IR, an increase in HOMA-β, and improvement in plasma insulin levels. Interestingly, the GL+GA mixture showed better improvement in IR, pancreatic-β cell function, and insulin levels when juxtaposed to other GL eutectic mixtures in the STZ prototype of type 2 diabetes. This improvement in symptoms of type 2 diabetes by GL-GA eutectic mixture was comparable to the standard drug, GL.

Effect of glimepiride and prepared eutectic mixtures on STZ-induced oxidative and nitrosative stress in diabetic rats

In the present study, administration of STZ on day 1 caused a significant increase in blood lipid peroxidation contents (TBARS) and total nitrite levels, and a decrease in GSH content and catalase activity in comparison to the vehicle control group. Oral treatment with glimepiride and glimepiride eutectic mixtures (1 mg/kg) for 28 days daily significantly attenuated STZ-induced oxidative and nitrosative stress, and improved the GSH content and catalase activity relative to rats that received STZ and vehicle-only **(Figure 9)**. Hence, the study provides insightful evidence into the oxidative stress parameters in diabetic rats and the ameliorative potential of eutectic mixtures of glimepiride. In the case of lipid peroxidation, the significant increase in thiobarbituric acid reactive substances (TBARS) in the plasma of diabetic rats further corroborates the heightened oxidative stress in diabetes. The marked decrease in GSH levels, along with the activities of key antioxidant enzymes such as catalase in the plasma of diabetic rats, emphasizes the significant oxidative stress induced by STZ-induced type 2 diabetes. Reduced glutathione and catalase are critical components of the cellular antioxidant defense system. Their decreased levels suggest an overwhelmed and impaired antioxidant defense in diabetic rats, which aligns with previous research indicating oxidative stress as a pivotal factor in the pathogenesis of diabetes and its complications. Lipid peroxidation is a deleterious process resulting in cellular damage and oxidative stress. The observed reduction in TBARS levels following chronic treatment with both the standard drug and the eutectic formulations indicates the efficacy of these treatments in mitigating oxidative damage. Notably, the reduction was more pronounced with the glimepiride eutectic mixtures, predominantly the GL+GA eutectic, reinforcing its superior antioxidative capability. The treatment with eutectic mixtures, particularly the GL+GA formulation, significantly ameliorated GSH levels and catalase activity, indicating a potent antioxidative effect.

Elevated nitrite levels, indicating increased nitric oxide production and oxidative stress, were observed in the plasma of diabetic rats. The standard drug and its eutectics significantly reduced nitrite levels in diabetic rats, suggesting it effectively decreases nitric oxide production or enhances its degradation, thereby reducing oxidative stress. This effect may be due to the inhibition of inducible nitric oxide synthase (iNOS) expression.

Effect of glimepiride and prepared eutectic mixtures on STZ-induced inflammation in diabetic rats

The anti-inflammatory effects of glimepiride and its eutectic mixtures on diabetic rats by measuring TNF-α, IL-6, and IL-1β levels **(Figure 10)**. STZ-induced diabetic rats exhibited significantly elevated levels of these pro-inflammatory markers compared to vehicle controls, indicating a robust inflammatory response. Elevated TNF-α exacerbates insulin resistance, IL-6 promotes chronic inflammation and metabolic dysregulation, and IL-1β contributes to beta-cell destruction and impaired insulin secretion. Treatment with the standard drug, glimepiride, and its eutectic mixtures (dose 1 mg/kg, *p.o.*) significantly reduced these inflammatory markers in STZ-treated animals in comparison to animals that received STZ and vehicle only, thus demonstrating their potent anti-inflammatory properties. These findings highlight the potential of both the standard drug and its eutectic mixtures in managing diabetes-related inflammation. By reducing inflammation, these treatments may improve insulin sensitivity, preserve beta-cell function, and enhance glycemic control. The superior efficacy of the eutectic mixtures underscores the importance of optimizing drug formulations for better clinical outcomes. Interestingly, the GL+GA eutectic mixture showed a significantly more potent anti-inflammatory activity relative to the other mixtures in the STZ-induced type 2 diabetes rat prototype. This anti-inflammatory potential of the

GL+GA mixture was comparable to the standard drug.

Table 1 Comparison of melting endotherm and heat of fusion of glimepiride and its binary mixtures

	GL	GL-GA	GA	GL-CA	CA	GL-TH	TH	GL-AA	AA
Melting point (℃)	214.59	186.09	262.10	189.40	236.08	172.01	179.71	166.17	193.19
ΔH_{fus} (J/g)	222.50	-90.50	637.7	-72.70	133.6	-19.22	135.5	-16.05	229.5

Table 2 Peaks positions showing 2θ values in PXRD patterns of glimepiride, coformers and their binary mixtures

Formulations	2θ position
GL	6.4°, 13.59°, 16.86°, 18.31°, 19.34°, 21.39°
GL-GA	6.4°, 13.4°, 13.8°, 14.66°, 16.47°, 18.18°, 19.18°
GA	14.68°, 15.12°, 18.45°, 19.79°
GL-AS	6.4°, 10.4°, 13.9°, 14.62°, 16.67°, 19.14°, 21.06°, 21.44°, 21.96°, 22.25°, 22.90°, 23.13°
AS	10.45°, 13.90°, 16.10°, 17.40°, 21.04°, 21.27°, 22.70°, 23.41°
GL-TH	6.4°, 12.4°, 13.1°, 13.4°, 18.12°, 19.13°, 19.82°, 20.63°, 21.05°, 21.96°, 23.11°, 25.24°, 25.4°, 26.33°, 28. 26°, 28.8°
TH	20.73°, 21.75°, 21.63°, 24.12°, 26.42°, 29.53°
GL-CA	11.88°, 13.48°, 14.06°, 17.18°, 18.17°, 19.12°, 21.14°, 21.50°, 22.04°, 22.33°, 25.32°, 26.48°
CA	12.98°, 13.00°, 20.9°, 26.48°

Table 3 Solubility study of different eutectic mixture formulations containing glimepiride in 0.1N HCl and phosphate buffer pH 6.8

S. No.	Formulation	Solubility(mg/ml) ± SD (0.1 N HCl)	Solubility(mg/ml) ± SD (Phosphate Buffer. pH 6.8)	Fold solubility increase in 0.1 N HCl	Fold solubility increase in Phosphate Buffer 6.8
1	GL	0.94 ± 0.004	0.49 ± 0.004	-	-
2	GL-GA	13.157±0.108	31.027±0.108	13.99	63.32
3	GL-AA	5.481±0.011	16.028±0.054	5.83	12.30
4	GL-TH	2.202±0.108	7.931±0.162	2.34	16.18
5	GL-CAF	1.756±0.027	3.747±0.108	1.868	7.65

Table 4 In vitro Drug Release of eutectic formulations containing glimepiride

Time (in minutes)	GL	GL-GA	GL-AA	GL-TH	GL-CA
0	0.00±0.05	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000
5	7.63±0.24	88.55±1.216	19.40±0.877	28.10±0.730	32.46±0.644
10	5.13±0.06	83.58±0.973	17.99±0.421	26.28±0.243	33.16±0.843
15	4.87±0.08	80.77±1.115	17.57±0.421	25.72±0.243	30.77±0.644
20	3.56±0.02	76.56±0.730	16.87±0.644	25.01±0.243	28.81±0.243
25	4.28±0.09	72.49±0.243	16.73±0.421	23.47±0.421	27.82±0.243
30	4.22±0.09	71.78±0.487	15.74±0.243	21.64±0.243	26.28±0.243
45	5.14±0.04	60.83±0.877	12.23±0.243	19.68±0.421	25.01±0.487
60	5.34±0.03	61.95±0.644	11.11±0.487	17.99±0.421	24.17±0.243
120	5.98±0.03	66.73±1.216	9.85±0.644	16.59±0.243	22.21±0.421

Table 5 Estimated pharmacokinetic parameters after oral administration of GL and its eutectic mixture formulations to Wistar rats

	$T_{1/2}$ (min)	T_{max} (min)	C_{max} (µg ml^{-1})	AUC (min µg ml^{-1})	Improvement
GL	561.52	240	65.86	21595.7	-
GL-GA	579.70	120	99.36	23879.56	1.39
GL-AA	679.57	120	96.03	23537.28	1.33
GL-TH	692.33	120	95.08	23248.29	1.31
GL-CA	620.41	120	90.26	21562.67	1.24

Table 6 Effects of glimepiride (GL) eutectic formulations on plasma insulin, homeostatic model assessment (HOMA)-IR, and homeostatic model assessment (HOMA)-β in type 2 diabetic rats.

Parameter	Measurement time	Animal groups						
		Vehicle Control	STZ	STZ+GL	STZ+GL+GA	STZ+GL+TH	STZ+GL+AA	STZ+GL+CA
Plasma insulin (μIU/ml)	Day 3	17.61 ± 1.068	9.983 ± 1.107#	8.238± 0.5568#	9.148 ± 0.7197#	8.248 ± 0.4787#	8.318 ± 0.5311#	8.678 ± 0.4569#
	Day 30	19.24 ± 1.811	6.763 ± 0.682#	16.28 ± 1.702*	16.61 ± 2.031*	14.31 ± 1.746*	14.123 ± 1.600*	14.47 ± 0.326*
HOMA-IR	Day 3	3.060 ± 0.405	9.651 ± 0.509#	10.22 ± 0.495#	10.19 ± 0.656#	10.75 ± 0.703#	10.08 ± 0.715#	10.10 ± 0.858#
	Day 30	3.262 ± 0.387	10.27 ± 0.811#	4.515 ± 0.311*	3.902 ± 0.383*	4.541 ± 0.29*	4.391 ± 0.413*	5.106 ± 0.574*
HOMA-β	Day 3	220.7 ± 6.462[a]	14.00 ± 1.439#	9.558 ± 0.901#	11.75 ± 1.165#	10.29 ± 0.968#	10.54 ± 1.037#	10.71 ± 1.239#
	Day 30	213.5 ± 7.495	13.30 ± 1.865#	34.06 ± 2.242*	37.62 ± 1.76*	28.97 ± 1.184*	28.04 ± 1.72*	29.98 ± 1.688*

Data is expressed as mean ± SEM. # $p < 0.05$ *vs* Vehicle Control; * $p < 0.05$ *vs* STZ. Statistical analysis was performed by one-way ANOVA followed by Tukey's multiple comparison test. STZ, Streptozotocin; GL, Glimepiride; GA, Gallic acid; TH, Thiourea; AA, Ascorbic acid; CA, Caffeine.

CONCLUSION

Solvent-drop grinding of glimepiride with selected co-formers led to the formation of eutectic formulations. Lowering of melting points of products was observed in DSC analysis. Solubility in 0.1N HCl and phosphate buffer 6.8 improved as compared to pure glimepiride. The most significant improvement in physicochemical parameters was attained in the GL-GA binary mixture which is confirmed by pharmacokinetic and pharmacodynamic profiles of all the prepared formulations.

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