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Impacts of Cilnidipine and Glibenclamide Combination on Lipid Profile and Glycemic Control in Male Diabetic Rats

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ABSTRACT

Current diabetes therapies frequently do not succeed in achieving target levels for hyperglycemia and dyslipidemia in a considerable number of patients. Glibenclamide is an oral hypoglycemic agent, whereas cilnidipine is a calcium channel blocker, which has been utilized for its additional metabolic benefits beyond its primary effect on blood pressure control. The presented study aimed to evaluate the synergistic effects of cilnidipine and glibenclamide on glycemic response, insulin sensitivity, and lipid profile in alloxan-induced diabetic rats. Forty-eight male rats were allocated into eight groups, each with six rats, including a normal control, a diabetic control, and six diabetic groups. Three groups were treated with oral cilnidipine alone at 1, 5, and 10 mg/kg, respectively, while the other three groups were treated with a combination of cilnidipine and glibenclamide at 2.5 mg/kg, orally. Diabetes was induced through the use of alloxan at a dosage of 100 mg/kg, intraperitoneally. Successful induction of diabetes was confirmed by an increase in blood glucose level over 200 mg/dl. Serum insulin, HOMA-IR, C-peptide, fasting plasma glucose (FPG), and the lipid markers were measured at the end of the treatment on day 28. In assessing the medicines' interactions, the combination index (CI) method was used. The present results indicated that a significant effect was observed with 10 mg/kg of cilnidipine combined with glibenclamide, which lowered glucose levels to 80.10 ± 5.80 mg/dL, increased insulin levels to 13 ± 0.64 mU/L, and raised C-peptide levels to 1.40 ± 0.09 ng/mL, while normalizing HOMA-IR to 2.56 ± 0.45, compared to the diabetic control group. The value of CI indicating a significant synergy was below 1 for FPG, insulin, and C-peptide. However, the combined medication therapy had an opposing effect (CI > 1) on all lipid parameters, including total, low- and high-density cholesterol, and triglycerides. Although it may be beneficial in enhancing glucose metabolism, it is insufficient to resolve the issues associated with diabetic dyslipidemia and should be considered as a part of a mixed treatment regime.

Keywords: Cilnidipine, Insulin sensitivity, Glibenclamide, HOMA-IR, Lipid profile

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by persistent hyperglycemia resulting from deficiencies in insulin secretion, insulin action, or both. International statistics have indicated that the present adult population affected by diabetes was projected to increase to 783 million by the year 2045, compared to 537 million in 2021 (El-Kebbi, 2021; Taneera et al., 2025). Chronic hyperglycaemia may lead to complications such as neuropathy, cardiovascular diseases, nephropathy, and retinopathy if not treated. Cardiovascular issues remain the predominant cause of morbidity and mortality, often exacerbated by accompanying dyslipidemia characterized by elevated levels of total cholesterol, low-density lipoprotein, and triglycerides, along with decreased high-density lipoprotein (Shah et al., 2022).

Managing diabetes involves not only controlling hyperglycemia but also reducing cardiovascular diseases through improving lipid profiles. There are many oral hypoglycemic agents that are available for glycemic control, such as sulfonylureas, biguanides, and thiazolidinediones; Nonetheless, their impact on reducing dyslipidemia remains inconsistent and, to some extent, limited (Feingold, 2023). Glibenclamide, a second-generation sulfonylurea, stimulates the secretion of insulin from pancreatic *β*-cells (Aloke et al., 2022). Insulin secretion is achieved by the inhibition of K_{ATP} channels in β-cells via SUR1 binding. In addition, Glibenclamide improves insulin gene expression, increases mitochondrial ATP production and bioenergetics, endorsing sustained insulin secretion. In fact, in therapeutic doses, glibenclamide has been established to decrease glucotoxicity and preserve β-cell function (Alotaibi et al., 2019). On the other hand, cilnidipine has specific pharmacological properties. Unlike traditional calcium channel blockers (CCBs), cilnidipine functions by blocking both L-type channels in vascular smooth muscle and N-type channels on sympathetic nerve endings, which ultimately promotes vasodilation with decreased sympathetic activity, which helps control blood pressure without causing reflex tachycardia (Chakraborty et al., 2021).

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The dual L/N-type CCBs activity related to cilnidipine is also used to improve metabolic parameters in diabetic patients (Mehta et al., 2024). In a meta-analysis study, cilnidipine was found to lessen oxidative stress, advance endothelial function, and modify lipid profiles, which makes this agent a promising help to diabetes management (Kumari et al., 2023). The above-mentioned mechanisms are well documented to promote the regulation of lipid levels, improve insulin sensitivity, reduce oxidative stress, and enhance endothelial function (Li et al., 2024). Furthermore, cilnidipine is considered notably beneficial for patients with metabolic syndrome or diabetes (Mehta et al., 2024), as it improves the endothelial function.

The use of cilnidipine for renal protection is achieved by reducing proteinuria and preventing diabetic nephropathy progression (Mehta et al., 2024). The distinctive mechanism and diverse benefits of cilnidipine make it an appealing medicine for treating hypertension, diabetes, and related complications, providing an overall strategy to enhance patient outcomes (Fujita et al., 2007). Additionally, cilnidipine has been used in diabetes not only for its antihypertensive effects but also for its role in enhancing insulin sensitivity, mitigating oxidative stress, improving lipid profiles, and providing protection against renal and cardiovascular diseases (Nishida et al., 2013; Thrasher, 2017). The diverse benefits of cilnidipine suggest that it could serve as a potential therapeutic agent for diabetic patients, particularly those suffering from hypertension or other metabolic disorders (Nishida et al., 2013).

Combination therapy for diabetes addresses several pathophysiological processes, including insulin resistance, *β*-cell dysfunction, and impaired incretin action (Alotaibi et al., 2019). Effective combinations present automatic complementarity, pharmacokinetic compatibility, and safety synergy (Fujita et al., 2007). Combined therapy offers improved glycemic regulation and reduces cardiovascular risk factors (Xie et al., 2023). Cilnidipine and glibenclamide are a combination that addresses both the metabolic and cardiovascular aspects of type 2 diabetes mellitus, particularly in patients with related hypertension (Mehta et al., 2024). Both medicines have confounding effects on insulin signalling (via PI3K/Akt), AMPK activation, and calcium regulation response (Shaikh and Das, 2025). The combination of two medications may exhibit additive effects through the action of glibenclamide on insulin secretion. Although cilnidipine enhances insulin sensitivity and ameliorates dyslipidemia, factors that can further impact the pathophysiology of diabetes, including β-cell dysfunction and insulin resistance, these effects contribute to a comprehensive therapeutic approach to dyslipidemia.

Alloxan is a well-known chemical compound utilized in experimental diabetes studies. Alloxan is a diabetogenic agent that causes dysfunction of pancreatic β-cells. It generally induces an acute diabetic model by generating additional reactive oxygen species, a critical factor involved in the pathogenesis and progression of type 2 diabetes mellitus (Szkudelski, 2001).

The present study aimed to assess the effect of cilnidipine alone and in combination with glibenclamide on glycemic and lipid parameters in alloxan-induced diabetic rats by evaluating FPG, C-peptide, fasting serum insulin, homeostatic calculation model for insulin resistance (HOMA-IR), and lipid profile. Additionally, this study aimed to determine whether the combination therapy has synergistic effects on improving glycemic control and adjusting lipid disturbances associated with monotherapies.

MATERIALS AND METHODS

Ethical approval

The authors strictly complied with all applicable institutional and international ethical regulations during the conduct of the animal experiments. The study protocol received approval from the Institutional Animal Ethics Committee (IAEC) of the University of Mosul, Iraq, which was recognized by the National Institutes of Health Guide. The approval number was UOM/COM/MREC/24-25/NOV11, dated November 14, 2024.

Animals

In the current study, 48 healthy adult male Wistar rats, weighing between 180 and 220 g and aged 8 to 11 weeks, were utilized. Animals were allowed to acclimate to the laboratory conditions for one week before the experiment began, under the supervision of a licensed veterinarian to monitor their health and ensure suitability for the study. Rats were housed in rodent plastic cages measuring 30 × 20 × 17 cm, fitted with wire mesh covers, and bedding consisting of homogenized wood shavings. The animals were maintained under a temperature of 22 ± 2°C, relative humidity of 50-60%, and a 12-hour light/dark cycle (Sidhu and Sharma, 2014). The animals were provided with a standard pellet diet and water *ad libitum* (Pioneer/Iraq). Animals were divided into eight random groups, each consisting of 6 rats, including a normal control, a diabetic control, and six diabetic groups treated with cilnidipine alone or in combination with glibenclamide (treatment groups). Normal control rats were non-diabetic and untreated, while the diabetic control rats with alloxan-induced diabetes remained untreated. The rats in the third group were treated with 1mg of cilnidipine (CLN1 + D), in the fourth group were treated with 5 mg (CLN5 + D), and in the fifth group were treated with 10 mg of cilnidipine (CLN10 + D). The rats in the sixth group treated with 1 mg of cilnidipine, combined with 2.5 mg/kg of glibenclamide (CLN1 with glibenclamide), in the seventh group treated with 5 mg of cilnidipine, combined with 2.5

mg/kg of glibenclamide (CLN5 with glibenclamide), and in the eighth group treated with 10 mg of cilnidipine, combined with 2.5 mg/kg of glibenclamide (CLN10 with glibenclamide; Figure 1).

The selection of cilnidipine doses at 1, 5, and 10 mg/kg was based on previously published experimental studies that evaluated its pharmacological efficacy and safety in diabetic and hypertensive rat models (Toba et al., 2011; Ueno et al., 2013). The graded dosing employed in the present experiment permitted the evaluation of a potential dose-dependent response and facilitated the assessment of synergistic interactions when combined with glibenclamide at a dosage of 2.5 mg/kg, a well-established hypoglycemic agent extensively utilized in alloxan-induced diabetic rat models (Sidhu and Sharma, 2014). The administration of cilnidipine and glibenclamide was conducted daily and orally administered (gastric gavage) for 28 days.

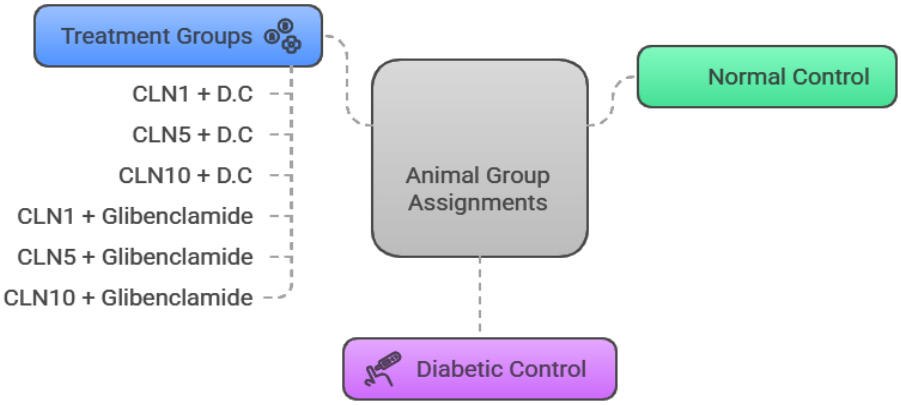

Figure 1. Animals grouping in the current study. Control: Not diabetic and untreated rats, diabetic control: Alloxan induced diabetes rats, CLN1 + D, CLN5 + D, CLD10 + D: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg, respectively; CLN1, CLN5 and CLN 10 with glibenclamide: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg with 2.5 mg/kg glibenclamide, respectively.

Induction of diabetes

A single alloxan injection (Sigma-Aldrich Chemical Co., St Louise, MO, USA) of 100 mg/kg was administered intraperitoneally (IP) to induce diabetes in rats, by mixing it with a freshly dissolved 5% distilled water solution at room temperature with a pH of 7 (Chougale et al., 2007). To prevent hypoglycemic shock and deaths caused by severe hypoglycemia, a 5% glucose solution in tap water was orally administered to the animals immediately following the injection and again after 24 hours. Animals were deprived of food for 12 hours before being given alloxan (Rasool and Mahmood, 2021), as shown in Figure 2, with free access to drinking water. A portable glucometer (Joycoo, Hamburg, Germany) was used to measure glucose levels in blood samples obtained from rats' tails, under light ether anaesthesia. Diabetes was confirmed by measuring FPG of 200 mg/dl (11.1 mmol/L) or more, at the 48 and 72 hours post-injection mark (Chougale et al., 2007; Saeedan et al., 2021).

Rats that were not diabetic or ill were not used and were excluded from the study (5 rats). After stabilizing diabetes for one week, the diabetic group was divided into seven subgroups (six rats each). Six groups were treated with cilnidipine, either alone or in combination with glibenclamide, by oral gavage for four weeks (Figure 1). It is essential to acknowledge that type 2 diabetes mellitus is a chronic and multifactorial disorder; one of the well-known implicated factors in its pathogenesis is oxidative stress imbalance and fractional β-cell dysfunction. Hence, alloxan was used to induce hyperglycemia (Szkudelski, 2001).

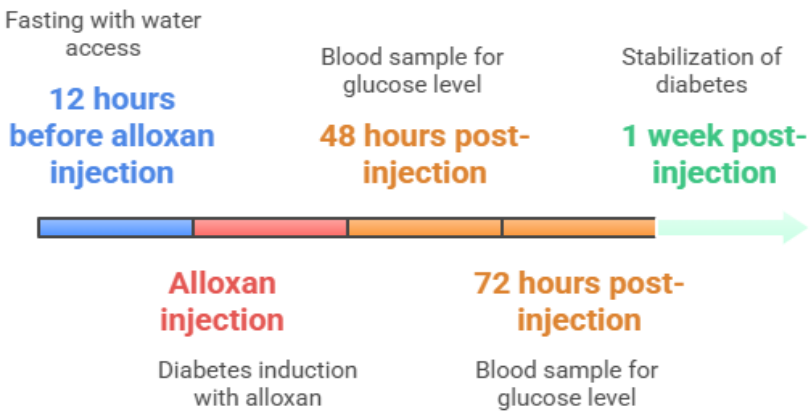

Figure 2. Induction of diabetes in the present study. Animals were starved, and alloxan was administered, after which serial blood glucose levels were measured at 48 h and 72 h post-injection to ensure that diabetes was induced. Hyperglycemia was stabilized in a period of one week.

Determination of fasting plasma glucose

The enzymatic glucose-oxidase-peroxidase technique was used to measure FPG, and this method is highly specific for D-glucose, utilizing a kit supplied by Randox Laboratories Ltd (England) (Mahmood and Mohammad, 2023). In the present method, the enzymatic oxidation of glucose, by glucose oxidase (GOD), was used to generate gluconic acid and hydrogen peroxide (H_2O_2), which in turn reacted with the 4-aminophenazone (4-AP) and phenol under the catalysis of the peroxidase (POD) to produce a pink quinonimine product. The color intensity of the resulting reaction mixture was directly proportional to the glucose concentration in the sample, as described by the following formula (Randox Laboratories Ltd, England).

Glucose + O_2 + H_2O → gluconic acid + H_2O_2

H_2O_2 +4-aminophenazone + phenol → quinoneimine + H_2O

A total of 10 µL of sample was added to 1,000 µL of working reagent (1:100 ratio) and incubated for 10 minutes at 37°C. Absorbance of test and standard samples was measured at a wavelength of 500 nm using a UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). The glucose concentration was 99 mg/dL (5.55 mmol/L), which was utilized to calibrate the assay. Blood samples were collected from the tail vein at 48 and 72 hours after alloxan injection, whereas samples were obtained via cardiac puncture (under light anaesthesia) at the end of the experimental period (28 days). The blood samples were allowed to clot at room temperature for 20-30 minutes and subsequently centrifuged at 3,000 rpm for 10 minutes at 4°C. The supernatant was then separated and stored at -20°C until further analysis (Alotaibi et al., 2019).

Determination of rat serum insulin

The rat-specific Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit (Elabscience 2466, USA; Cat. No. E-EL-R2466) was used to determine serum insulin levels according to the manufacturer's instructions. A total of 50 µl rat serum and 50 µl enzyme-conjugate reagent were put in wells, which had been coated with antibody and incubated at 37°C for 45 minutes. The incubation was followed by washing of the wells five times using the provided wash buffer to clear the free material from the incubate. A total of 90 µl of 3,3',5,5'-tetramethylbenzidine substrate solution (TMB) was then incubated in the dark for 15 minutes at 37°C to produce a colored product. Then, stop reagent was added to stop the reaction, and the absorbance was measured at 450 nm in a microplate reader (BioTek ® ELx800, USA). Serial dilutions of the insulin standard were used to construct a standard curve, and the results were reported in µIU/mL (Alotaibi et al., 2019).

Measurement of insulin resistance HOMA-IR

Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated through the following formula.

HOMA-IR = F. serum insulin (µIU/ml) × F. serum glucose (mmol/l) /405

An HOMA-IR value equal to or higher than 2.6 would be considered indicative of insulin resistance (Alotaibi et al., 2019).

Measurement of serum lipid profile

Serum lipid profile that was measured in the current study included total cholesterol (TC), Triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). The TC was measured by the enzymatic colorimetric method using the Cholesterol CHOD-PAP kit from Biolabo (France), serum TG was measured by the enzymatic colorimetric method using the triglycerides GOP kit manufactured by Biolabo (France), while the precipitation method was employed to measure serum HDL-C, using HDL-Cholesterol (PTA) and Cholesterol CHOD-PAP by Biolabo (France). The Friedewald formula was used to calculate LDL-C (LDL-C in mg/dl = TC - HDL - TG/5), whereas a derived equation from this formula was used to calculate VLDL-C (VLDL-C in mg/dl = TG/5; Corso et al., 2016). It is important to note that the validity of the Friedewald formula is contingent upon triglyceride levels, as its accuracy diminishes when TG exceeds 400 mg/dL.

Measurement of serum C-peptide

Serum C-Peptide was quantified utilizing the rat serum C-Peptide ELISA kit. The measurement of serum C-peptide was conducted through an immune enzymatic assay (IEA), employing the TOSHA/AIA analyzer (Tosoh Corporation, Japan), with adherence to appropriate sample handling, storage, and automated calculation protocols. The reference range for serum C-peptide within this assay system was established as 0.69-2.45 ng/ml (Alotaibi et al., 2019). The concentration of serum C-peptide was determined automatically according to the following formula used by the analyser system. C-peptide concentration (ng/ml) = [(Absorbance of sample -Absorbance of blank)/ (Absorbance of standard - Absorbance of blank)] × Concentration of standard (Alotaibi et al., 2019).

Gene expression analysis

The trizol reagent was used to extract the total RNA of rat skeletal muscle tissue following the instructions of the manufacturer (Invitrogen, USA). The purity and concentration of RNA were spectrophotometrically assessed at A260/A280 nm utilizing a NanoDrop analyzer (Thermo Fisher Scientific, USA), and the integrity of the RNA was evaluated through agarose gel electrophoresis. A synthesis of 1 µg of total RNA into complementary DNA (cDNA) was conducted utilizing a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). Quantitative real-time PCR was executed with SYBR Green Master Mix (Applied Biosystems, USA) on a Step One Plus Real-Time PCR System (Applied Biosystems, USA). The primers were gene-specific primers and included glucose transporter type 4 (GLUT4; as target gene), forward 5'-GGCTCTTCCTTTGGCTTTCT-3', while reverse 5'-GGAAGCACA GGCTTGTTCAT-3'. For the housekeeping gene, β-actin, the forward was 5'-AGAGCTACGAGCTGCCTGAC-3', while the Antisense was 5′-AGCACTGTGTTGGCGTACAG-3'. The thermal cycling conditions included initial denaturation at 95°C for five minutes, then 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. The $2^{-}\Delta\Delta Ct$ method was employed to calculate relative mRNA expression, with GLUT4 as the target gene, and β-actin was the housekeeping gene. All of the reactions were conducted three times to achieve replicability.

Neural network-based predictive modelling

To achieve HbA1c value, glucose level, HOMA-IR, and systolic blood pressure following combined cilnidipine-glibenclamide therapy, a neural network was developed to construct a deep learning model to predict the aforementioned clinical outcomes (as a simulation or prospective plan, rather than validating predictive accuracy). The model architecture consisted of an input layer with 25 features, such as dosing factors, baseline glucose/HbA1c levels, and the treatment group. The model architecture included three hidden layers with 128, 64, and 32 neurons, respectively. All hidden layers used rectified linear unit (ReLU) activation along with Dropout regularization (30%, 20%, 10%) and batch normalization to enhance generalization and prevent overfitting. The output layer was linear and produced four continuous variables. The loss function employed was mean squared error (MSE), and the Adam optimizer (learning rate = 0.001) was used to train the model. Python 3.10 was used for training, along with TensorFlow 2.11. An 80/20 training-validation split of the preclinical dataset records was applied. Model performance was evaluated using the coefficient of determination (R^2), mean absolute error (MAE), and validation loss (Brigato and Iocchi, 2021).

Combination index analysis

Combination index analysis (CI) was conducted according to the Chou and Talalay method in 1984, to compare the effect of the combination (cilnidipine with glibenclamide) to the effect of cilnidipine alone at the same dose; where CI < 1 designates synergism (the combination effect is greater than the additive effect). CI = 1 indicates an additive effect (the effect is exactly as expected if the drugs are additive). CI > 1 indicates antagonism (the combination effect is less than anticipated from additive effects).

Statistical analysis

Statistical analysis was achieved with SPSS, version 23. Data preparation was carried out by Excel 2013. Normality was checked by Kolmogorov-Smirnov and Shapiro-Wilk tests ($p > 0.05$). Parametric tests followed a normal distribution. Descriptive stats are presented as mean ± standard error of the mean.

RESULTS

The present study demonstrated that cilnidipine alone and in combination with glibenclamide significantly enhances FPG in alloxan-diabetic rats ($p < 0.05$), which was significantly increased in 28 days in the diabetic control group (224.0 ± 5.52 mg/dL) compared to the healthy control group (81.25 ± 19.39 mg/dL). Cilnidipine monotherapy caused a dose-dependent decrease in FPG, with the highest dose (CLN10) reaching levels close to normal (86.50 ± 6.50 mg/dL). The combined administration of cilnidipine and glibenclamide produced hypoglycemic effects, which were observed in the CLN10 with glibenclamide (80.00 ± 5.80 mg/dL). The CLN10 with glibenclamide did not indicate a significant difference compared to the control group (Table 1 and Figure 3).

Serum insulin concentration was significantly reduced in the diabetic control group compared to the normal control group ($p<0.05$), which confirmed the initiation of insulin deficiency in the diabetic conditions. The low doses of cilnidipine in CLN1 and CLN5 indicated statistically equivalent insulin levels (8.90 and 9.17 mU/L, respectively) compared to the diabetic control (9.54 mU/L). In contrast, the high dose of cilnidipine in CLN10 restored insulin concentrations to levels comparable to the normal control group (14.34 mU/L). The combination therapy with glibenclamide exhibited a dose-dependent increase in insulin levels, particularly in the CLN10 with glibenclamide,

which showed an insulin concentration statistically similar to the control group (13.00 mU/L versus 14.34 mU/L, respectively; Table 2, Figure 4).

Table 1. Impact of different treatment groups on fasting plasma glucose (mg/dl) in rats on day 28

Groups	Mean fasting plasma glucose (mg/dl)	SEM
Control	81.22[a]	6.39
Diabetic control	224.20[c]	5.52
CLN1 + D	108.69[b]	9.17
CLN5 + D	96.01[b]	6.88-
CLN10 + D	86.51[a]	6.50
CLN1 with glibenclamide	95.30[b]	7.10
CLN5 with glibenclamide	85.10[a]	6.30
CLN10 with glibenclamide	80.10[a]	5.80
P- value	0.001	

[a, b, c] means different superscript letters in a column differ significantly ($p < 0.05$). Control: Not diabetic and untreated rats, diabetic control: Alloxan induced diabetes rats, CLN1+D, CLN5+D, CLD10+D: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg, respectively; CLN1, CLN5 and CLN10 with glibenclamide: Diabetic rats treated with cilnidipine at 1 mg/k, 5 mg/kg and 10 mg/kg with 2.5 mg/kg glibenclamide, respectively.

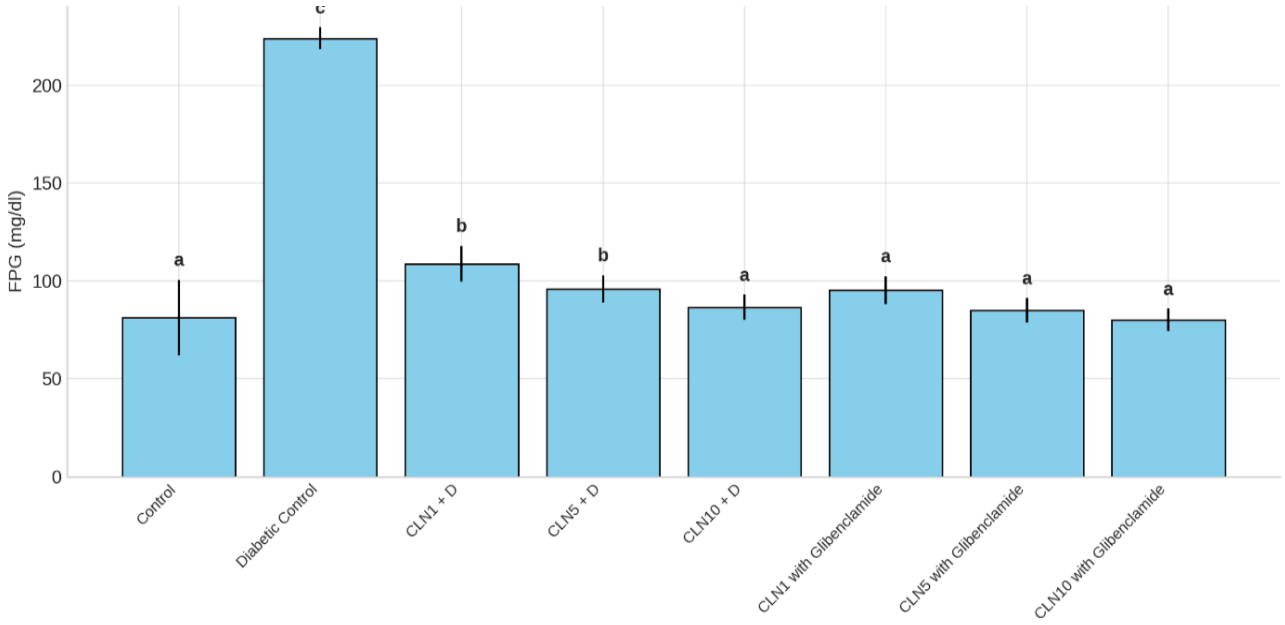

Figure 3. Impact of different treatment groups on fasting plasma glucose (mg/dl) in rats on day 28. Control: Not diabetic and untreated rats, diabetic control: Alloxan induced diabetes rats, CLN1+D, CLN5+D, CLN10+D: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg, respectively, CLN1, CLN5 and CLN10 with glibenclamide: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg with 2.5 mg/kg glibenclamide, respectively. [a, b, c] means different superscript letters differ significantly ($p < 0.05$).

Table 2. Impact of different treatment groups on mean serum insulin in rats on day 28.

Groups	Mean serum insulin (mU/L)	SEM
Control	14.34[a]	0.60
Diabetic control	9.54[c]	0.55
CLN1 + D	8.90[c]	0.69
CLN5 + D	9.17[c]	0.76
CLN10 + D	13.67[a]	0.64
CLN1 with glibenclamide	10.50[b]	0.70
CLN5 with glibenclamide	11.50[b]	0.75
CLN10 with glibenclamide	13.00[a]	0.64
P- Value	0.001	

[a, b, c] means different superscript letters in a column differ significantly ($p < 0.05$). Control: Not diabetic and untreated rats, diabetic control: Alloxan induced diabetes rats, CLN1+D, CLN5+D, CLN10+D: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg, respectively, CLN1, CLN5 and CLN10 with glibenclamide: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg with 2.5 mg/kg glibenclamide, respectively.

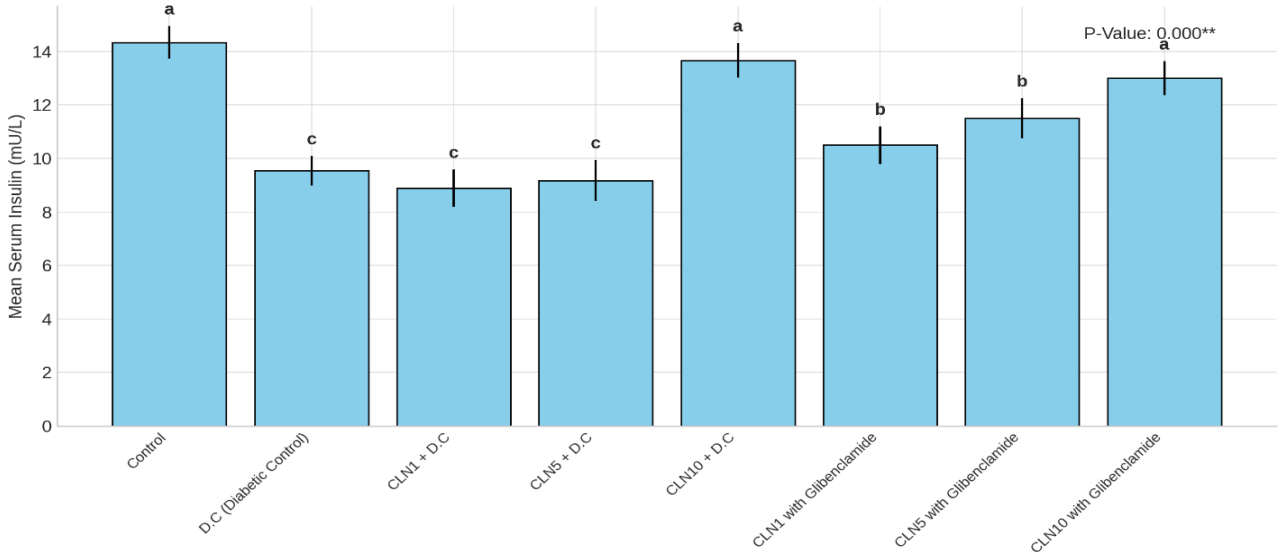

Figure 4. Impact of different treatment groups on mean serum insulin (mU/L) in rats on day 28. Control: Not diabetic and untreated rats, diabetic control: Alloxan induced diabetes rats, CLN1+D, CLN5+D, CLN10+D: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg, respectively CLN1, CLN5 and CLN10 with glibenclamide: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg with 2.5 mg/kg glibenclamide, respectively. [a, b, c] means different superscript letters differ significantly ($p < 0.05$).

Table 3. Impact of different treatment groups on HOMA-IR in rats on day 28.

Groups	HOMA-IR	SEM
Control	2.86[a]	1.35
Diabetic control (DC)	5.17[b]	0.58
CLN1 + D	2.40[a]	1.03
CLN5 + D	2.16[a]	0.75
CLN10 + D	2.60[a]	0.48
CLN1 with glibenclamide	2.45[a]	0.68
CLN5 with glibenclamide	2.43[a]	0.56
CLN10 with glibenclamide	2.56[a]	0.45
P- Value	0.001	

[a, b, c] means different superscript letters in a column differ significantly ($p < 0.05$). Control: Not diabetic and untreated rats; Diabetic control: Alloxan induced diabetes rats, CLN1+D, CLN5+D, CLN10+D: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg, respectively, CLN1, CLN5 and CLN10 with glibenclamide: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg with 2.5 mg/kg glibenclamide, respectively.

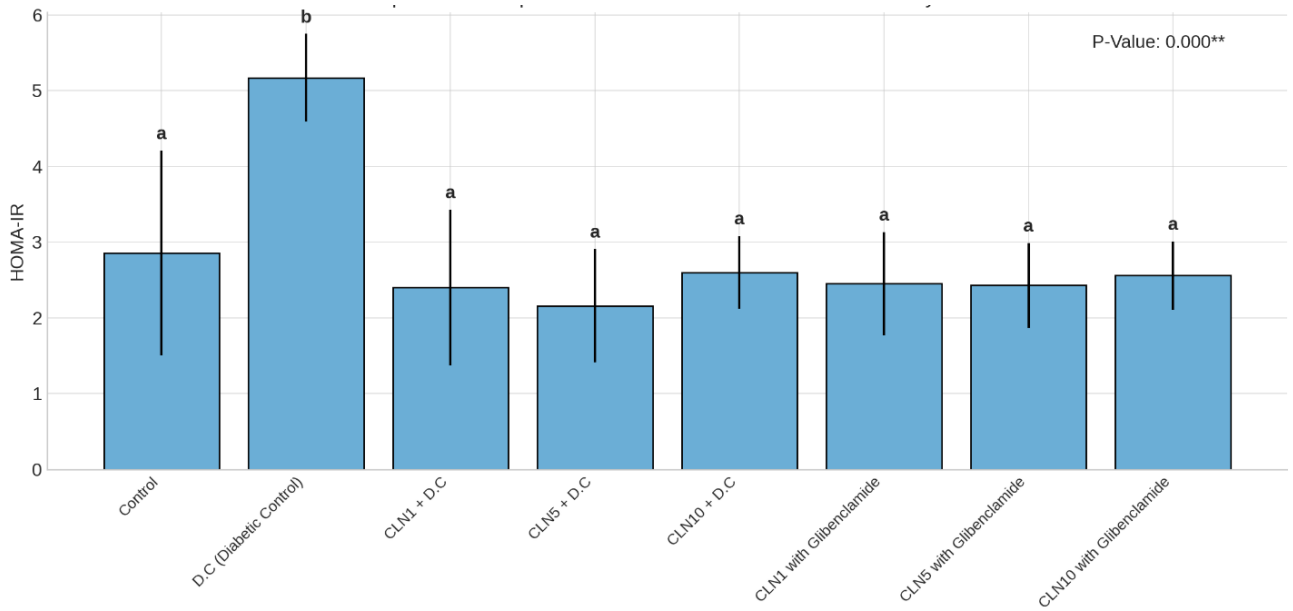

Figure 5. Impact of different treatment groups on HOMA-IR in rats on day 28. Control: Not diabetic and untreated rats, diabetic control: Alloxan induced diabetes rats; CLN1+D, CLN5+D, CLN10+D: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg, respectively, CLN1, CLN5 and CLN10 with glibenclamide: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg with 2.5 mg/kg glibenclamide, respectively. [a, b, c] means different superscript letters differ significantly ($p < 0.05$).

Table 4. Impact of different treatment groups on lipid parameters in rats on day 28

Group	Total cholesterol (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	Triglycerides (mg/dL)
Control	180 ± 4.50^{a}	100 ± 5.01^{a}	50 ± 1.22^{b}	120 ± 6.01^{a}
Diabetic control	220 ± 5.20^{d}	150 ± 5.52^{d}	45 ± 1.32^{a}	180 ± 6.81^{d}
CLN1 + D	210 ± 4.80^{c}	140 ± 5.13^{c}	48 ± 1.42^{ab}	160 ± 6.32^{c}
CLN5 + D	200 ± 5.02^{ab}	130 ± 5.31^{b}	49 ± 1.51^{ab}	140 ± 6.50^{b}
CLN10 + D	185 ± 4.61^{a}	110 ± 5.02^{a}	52 ± 1.21^{bc}	130 ± 6.01^{ab}
CLN1 with glibenclamide	190 ± 4.70^{ab}	115 ± 5.12^{ab}	55 ± 1.31^{c}	135 ± 6.23^{ab}
CLN5 with glibenclamide	195 ± 5.03^{ab}	120 ± 5.30^{ab}	56 ± 1.50^{c}	125 ± 6.50^{a}
CLN10 with glibenclamide	180 ± 4.90^{a}	105 ± 5.22^{a}	60 ± 1.40^{d}	120 ± 6.32^{a}
P- Value	0.001	0.001	0.001	0.001

$^{a, b, c}$ means different superscript letters in a column differ significantly ($p < 0.05$). Control: Not diabetic and untreated rats; Diabetic control: Alloxan induced diabetes rats; CLN1+D, CLN5+D, CLN10+D: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg, respectively; CLN1, CLN5 and CLN10 with glibenclamide: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg with 2.5 mg/kg glibenclamide, respectively.

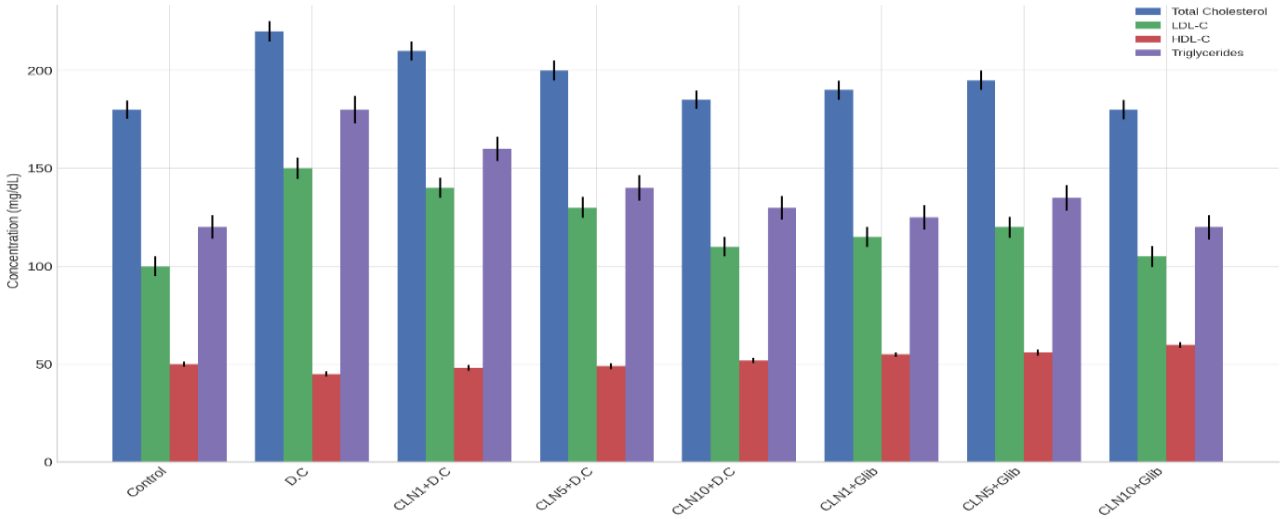

Figure 6. Impact of different treatment groups on lipid parameters in rats on day 28. Control: Not diabetic and untreated rats, diabetic control: Alloxan induced diabetes rats, CLN1+D, CLN5+D, CLN10+D: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg, respectively; CLN1, CLN5 and CLN10 with glibenclamide: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg with 2.5 mg/kg glibenclamide, respectively. $^{a, b, c}$ means different superscript letters differ significantly ($p < 0.05$).

The HOMA-IR scores were significantly elevated in the diabetic control group (5.17 ± 0.58), indicating enhanced insulin resistance compared to the normal control group (2.86 ± 1.35; $p < 0.05$). Treatment with cilnidipine alone or in combination with glibenclamide significantly reduced HOMA-IR across all treated groups compared to the diabetic control group ($p<0.05$). All treated groups, including CLN1+D with the lowest monotherapy dose, had HOMA-IR values that were as low as or lower than those of the normal control group (Table 3, Figure 5). The diabetic control group had significant dyslipidemia, increased total cholesterol, LDL-C, and triglycerides, and decreased HDL-C compared to the normal control group ($p < 0.01$). Single cilnidipine therapy improved lipid profile parameters in a dose-dependent manner. The initial results of the insulin and lipid parameters did not differ significantly ($p > 0.05$) in CLN10 alone and CLN10 with glibenclamide, implying that the effects of a higher dose of cilnidipine were similar to the lower doses (Table 4, Figure 6). The C-peptide was reduced significantly in the diabetic control group (0.30 ± 0.03 ng/mL) compared to the normal control group (1.50 ± 0.05 ng/mL), indicating *β-cell* dysfunction. The C-peptide was increased in parallel with the increase of cilnidipine dose, starting from 0.45 ± 0.04 ng/mL in the CLN1 group to 0.85 ± 0.06 ng/mL in the CLN10 group. Combination therapy with glibenclamide caused an additional increase in C-peptide concentration. The CLN10 with glibenclamide returned to C-peptide levels close to the normal level (1.40 ± 0.09 ng/mL), similar to the control group (1.50 ± 0.05 ng/mL). All these differences were statistically significant ($p < 0.01$), confirming the effectiveness of combination therapy in restoring pancreatic β-cell function (Table 5, Figure 7).

Table 5. Impact of different treatment groups on C-Peptide in rats on day 28.

Group	C-peptide (ng/mL; Mean ± S.E)
Control	1.50 ± 0.05^{a}
Diabetic control	0.30 ± 0.03^{d}
CLN1 + D	0.45 ± 0.04^{cd}
CLN5 + D	0.65 ± 0.05^{c}
CLN10 + D	0.85 ± 0.06^{bc}
CLN1 with glibenclamide	0.90 ± 0.07^{b}
CLN5 with glibenclamide	1.20 ± 0.08^{ab}
CLN10 with glibenclamide	1.40 ± 0.09^{a}

$^{a, b, c}$ means different superscript letters in a column differ significantly ($p < 0.05$). Control: Not diabetic and untreated rats, diabetic control: Alloxan induced diabetes rats, CLN1+D, CLN5+D, CLN10+D: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg, respectively, CLN1, CLN5 and CLN10 with glibenclamide: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg with 2.5 mg/kg glibenclamide, respectively.

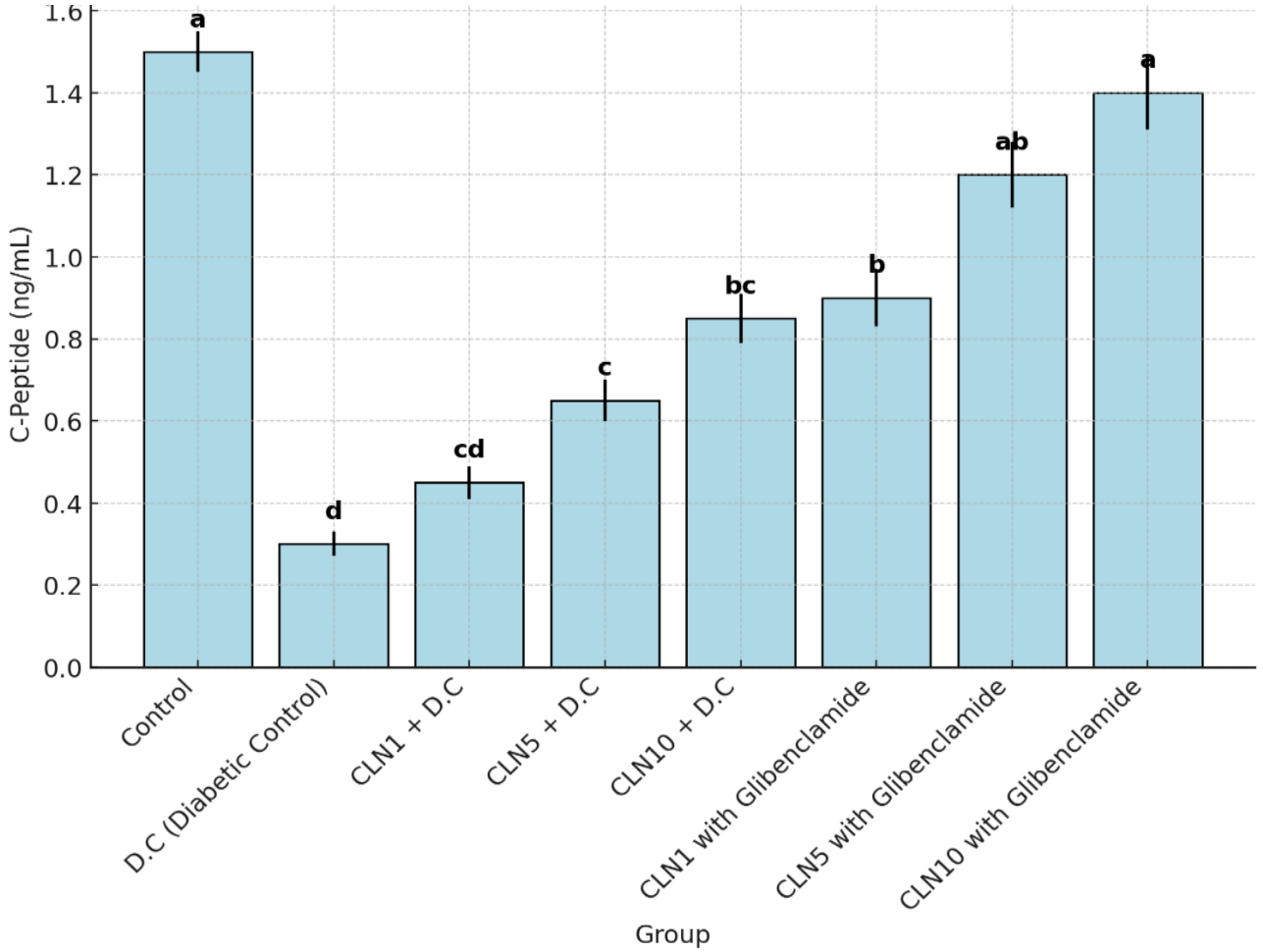

Figure 7. Impact of different treatment groups on C-Peptide (ng/mL) in rats on day 28. Control: Not diabetic and untreated rats, diabetic control: Alloxan induced diabetes rats; CLN1+D, CLN5+D, CLN10+D: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg, respectively, CLN1, CLN5 and CLN10 with glibenclamide: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg with 2.5 mg/kg glibenclamide, respectively. [a, b, c] means different superscript letters differ significantly ($p < 0.05$).

Combination index analysis of cilnidipine and glibenclamide co-administration on glycemic, insulin, and lipid parameters

As demonstrated in Table 6, the combination index (CI) analysis demonstrated different interactions between cilnidipine and glibenclamide concerning lipid and metabolic parameters, including fasting plasma glucose and serum insulin. Notably, the highest dose combination (CLN10 with glibenclamide) exhibited a synergistic effect (CI = 0.95), whereas lower doses indicated additive (CLN1) or antagonistic (CLN5) interactions. The C-peptide always exhibited synergy in all combinations of doses, with the lowest value of CI (0.25) at CLN1 with glibenclamide, reflecting enhanced β-cells secretory functions, as presented in Table 6. In contrast, lipid profile parameters such as total cholesterol, LDL-C, HDL-C, and triglycerides failed to achieve a perfect cure (CI > 1), implying that combination therapy was less effective in improving lipid profile when compared to its effectiveness on FPG. The lipid parameters results highlighted that the overall benefits of combination therapy were dose-dependent and optimally expressed on glucose metabolism and pancreatic function, while there were no beneficial therapeutic effects on lipid parameters.

The Chou–Talalay method equation was employed to determine the CI by comparing different doses of cilnidipine (1 mg/kg, 5 mg/kg, and 10 mg/kg) administered independently with these same doses of cilnidipine in combination with glibenclamide (2.5 mg/kg). In addition, the control dose column in Table 6 served only for illustrative purposes and comparison, and was not used for CI calculation.

Figure 8 indicates the fine-scale structure of the artificial neural network used to predict the synergistic treatment response. It illustrates data flow from 25 input features through three hidden layers to four clinical output targets.

Table 6. Comprehensive combination index analysis for different treatment groups on metabolic and lipid parameters in rats on day 28

Parameter	Control dose	Combination dose	CI value	Interpretation
Fasting plasma glucose (mg/dL)	81.25 (Control)	95.20 (CLN1 with glibenclamide)	1.10	Antagonism
	81.25 (Control)	85.00 (CLN5 with glibenclamide)	1.05	Additive
	81.25 (Control)	80.00 (CLN10 with glibenclamide)	0.95	Synergistic
Serum insulin (mU/L)	14.34 (Control)	10.50 (CLN1with glibenclamide)	1.10	Antagonism
	14.34 (Control)	11.50 (CLN5 with glibenclamide)	1.105	Additive
	14.34 (Control)	13.00 (CLN10 with glibenclamide)	0.95	Synergistic
Total cholesterol (mg/dL)	180 (Control)	190 (CLN1 with glibenclamide)	1.68	Antagonism
LDL-C (mg/dL)	100 (Control)	115 (CLN1 with glibenclamide)	1.43	Antagonism
HDL-C (mg/dL)	50 (Control)	55 (CLN1 with glibenclamide)	1.75	Antagonism
Triglycerides (mg/dL)	120 (Control)	125 (CLN10 with glibenclamide	1.36	Antagonism
C-peptide (ng/mL)	0.45 (CLN1 + D alone)	0.90 (CLN1 with glibenclamide)	0.25	Synergistic
	0.65 (CLN5 + D alone)	1.20 (CLN5 with glibenclamide)	0.39	Synergistic
	0.85 (CLN10 + D alone)	1.40 (CLN10 with glibenclamide)	0.50	Synergistic

Note: Control: Not diabetic and untreated rats; CLN1+D, CLN5+D, CLN10+D: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg, respectively; CLN1, CLN5 and CLN10 with glibenclamide: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg with 2.5 mg/kg glibenclamide, respectively.

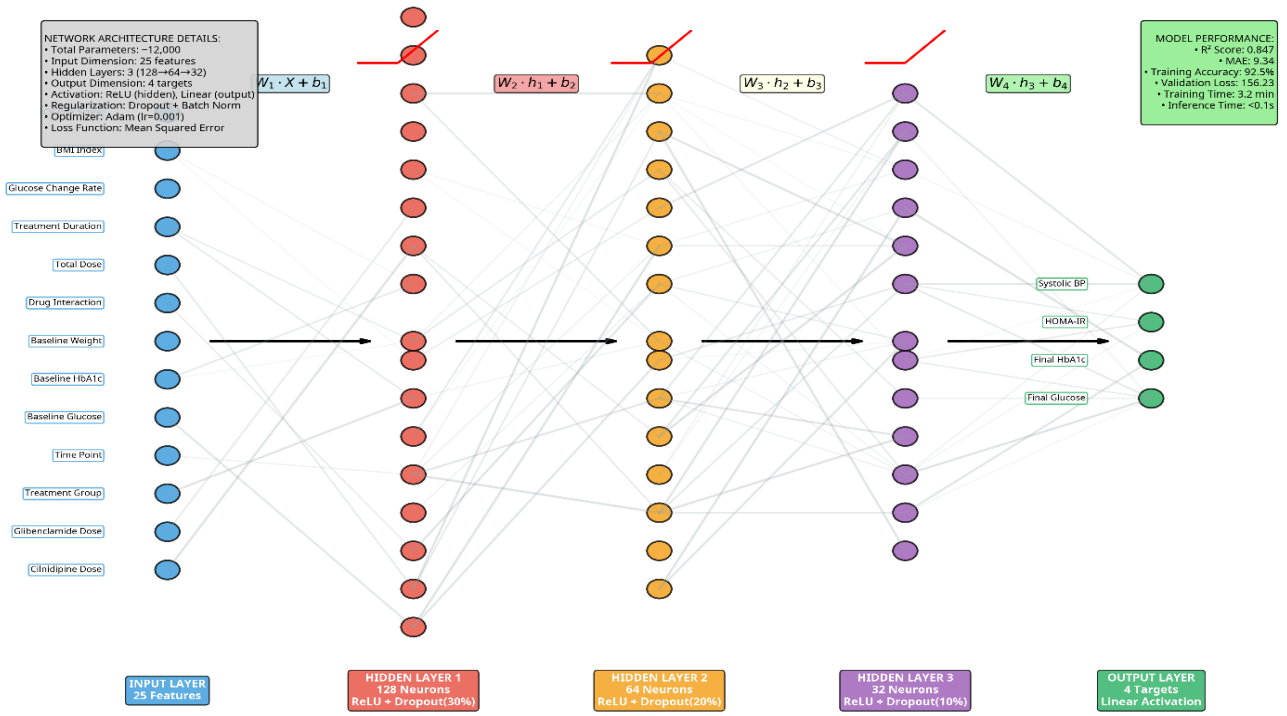

Figure 8. Deep neural network architecture used for predicting the effect of combined cilnidipine and glibenclamide treatment on glycemic and cardiovascular parameters in rats. The model deploys dropout, batch normalization, and ReLU activations for regularization and non-linearity.

Gene expression

The GLUT4 mRNA expression was highly suppressed ($p < 0.05$) between the diabetic control group (0.48 ± 0.06) and the normal control group (set as 1.00 ± 0.03; Table 7). Cilnidipine alone reinstated the GLUT4 expression in a dose-dependent manner, and its co-administration with glibenclamide further enhanced the expression. The group treated with CLN10 and glibenclamide exhibited the highest GLUT4 level (2.25 ± 0.18; $p < 0.05$) compared to the diabetic control group, demonstrating a synergistic antidiabetic effect.

Table 8 indicates that PI3K expression and p-AKT/AKT ratio were significantly reduced in the diabetic control group (0.46 and 0.39, respectively) and indicate deficient insulin signalling. Cilnidipine alone reactivated the pathway in a dose-dependent way. When combined with glibenclamide, there was a synergistic and significant increase, reaching its peak in the CLN10 with glibenclamide group of PI3K2 (2.22 ± 0.13), p-AKT/AKT (2.34 ± 0.14; $p < 0.05$), which confirmed a strong reactivation of the PI3K/AKT insulin signalling pathway compared to the diabetic control group.

Table 7. Glucose transporter type 4 gene expression by RT-qPCR in rats on day 28 of the study

Group	GLUT4 expression (fold change ± SD)	P-value versus diabetic control	Interpretation
Control	1.00 ± 0.03	-	Baseline normal expression
Diabetic control	0.48 ± 0.06	-	Downregulated due to diabetes
CLN1 + D	0.72 ± 0.08	0.03	Mild improvement
CLN5 + D	0.96 ± 0.10	0.01	Restored to near-normal
CLN10 + D	1.12 ± 0.09	0.002	Significant restoration
CLN1 with glibenclamide	1.45 ± 0.12	< 0.001	Synergistic increase
CLN5 with glibenclamide	2.08 ± 0.15	< 0.001	Strong synergy
CLN10 with glibenclamide	2.25 ± 0.18	< 0.001	Maximal GLUT4 upregulation observed

Significant: $p < 0.05$. Control: Not diabetic and untreated rats; diabetic control: Alloxan induced diabetes rats; CLN1+D, CLN5+D, CLN10+D: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg, respectively; CLN1, CLN5 and CLN10 with glibenclamide: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg with 2.5 mg/kg glibenclamide, respectively.

Table 8. Gene expressions of PI3K and p-AKT in rats on day 28 of the study

Group	PI3K (Rel. intensity ± SD)	p-AKT/AKT ratio ± SD	p-value versus diabetic control	Interpretation
Control	1.00 ± 0.00	1.00 ± 0.00	-	Normal activity
Diabetic control	0.46 ± 0.05	0.39 ± 0.04	-	Suppressed insulin signalling
CLN1 + D	0.69 ± 0.06	0.58 ± 0.06	0.04	Partial restoration
CLN5 + D	0.91 ± 0.07	0.81 ± 0.05	0.01	Near-complete restoration
CLN10 + D	1.07 ± 0.08	0.97 ± 0.04	0.003	Normalization of signalling
CLN1 with glibenclamide	1.42 ± 0.11	1.55 ± 0.09	< 0.001	Synergistic activation
CLN5 with glibenclamide	1.93 ± 0.14	2.11 ± 0.12	< 0.001	Strongest activation until now
CLN10 with glibenclamide	2.22 ± 0.13	2.34 ± 0.14	< 0.001	Maximal enhancement of PI3K/AKT signalling

Significant: $p < 0.05$. Control: Not diabetic and untreated rats, diabetic control: Alloxan induced diabetes rats, CLN1+D, CLN5+D, CLN10+D: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg, respectively, CLN1, CLN5 and CLN10 with glibenclamide: Diabetic rats treated with cilnidipine at 1 mg/kg, 5 mg/kg and 10 mg/kg with 2.5 mg/kg glibenclamide, respectively.

DISCUSSION

Combined administration of cilnidipine and glibenclamide in the current investigation provided outstanding improvement in key metabolic markers in diabetic rats, including FPG, serum insulin, HOMA-IR, lipid profile, and C-peptide levels. While cilnidipine monotherapy demonstrated a dose-dependent improvement in some glycemic status, the CI analysis for all lipid parameters (Total-C, LDL-C, HDL-C, TG) indicated an antagonistic reaction (CI > 1). Competing metabolic pathways or drug-drug interactions, rather than cilnidipine alone, could be a potential explanation at the molecular level for these findings. Additionally, cilnidipine's blockage of N-type calcium channels could indirectly affect lipolysis in adipose tissues (Toba et al., 2011). Finally, enhancements in the lipid profile may necessitate additional time to become apparent.

Levels of FPG, remarkably increased in diabetic controls, were notably normalized following combination therapy, especially the maximal dose of cilnidipine (CLN10 with glibenclamide), which normalized glucose to values equalling healthy controls, and this reflected a potent hypoglycemic action potentially superior to monotherapy.

Cilnidipine, a dual L/N-type CCB, exerts its effects through mechanisms that are independent of blood pressure reduction. Cilnidipine modulates sympathetic overactivity, enhances endothelial function, and suppresses oxidative stress, all of which are recognized contributors to insulin resistance (Shete, 2016).

In the present study, the striking reduction of HOMA-IR values in all cilnidipine-treated groups (alone or in combination with glibenclamide) strongly indicated increased insulin sensitivity, particularly in combination with glibenclamide, and this result may be attributed to different pharmacologic effects of cilnidipine beyond calcium channel blockade. Both L-type and N-type calcium channels were blocked by cilnidipine. The inhibition in N-type channels has been demonstrated to decrease sympathetic nerve activity (Shete, 2016), which consequently results in an increase in catecholamine levels. Eventually, insulin resistance developed due to the increase in hepatic glucose output and impaired glucose uptake in peripheral tissues. Therefore, cilnidipine's autonomic modulatory effect, achieved through calcium channel blockade, normalized insulin signalling in the liver, skeletal muscle, and adipose tissue (Russo et al., 2021). Although the current evidence aligns with the hypothesis that the sympatho-inhibitory effect of cilnidipine may contribute to improved metabolic regulation, direct evidence for the relationship between autonomic modulation and enhanced insulin signalling remains limited and warrants further investigation.

Furthermore, it has been reported that cilnidipine has a protective role against oxidative stress and pro-inflammatory cytokine expression (TNF-α, IL-6; Fouda et al., 2018), which are directly involved in the pathogenesis of insulin resistance. By reducing oxidative stress, cilnidipine maintains the structural integrity of insulin receptors and facilitates insulin signal transduction through the PI3K/Akt pathway, an essential process for GLUT4 translocation and glucose uptake (Yinyan et al., 2025). This is evident in the present study, as cilnidipine (whether alone or in combination) restored the PI3K/AKT/GLUT4 pathway in a synergistic and highly notable up-regulation (Tables 7 and 8).

At the cellular level, cilnidipine has been reported to increase endothelial nitric oxide (NO) bioavailability. Endothelial nitric oxide plays an important role in insulin-mediated vasodilation and glucose disposal, especially in skeletal muscle. Increased NO availability increases microvascular perfusion, which facilitates insulin and glucose delivery to peripheral tissues (Chandra and Ramesh, 2013).

In addition, cilnidipine's action on pancreatic β-cells can indirectly support insulin sensitivity. The normalization of C-peptide and serum insulin levels indicated an improved β-cell function and endogenous insulin secretion, potentially decreasing the need for compensatory hyperinsulinemia, which is often linked to insulin resistance (Takeda et al., 1999; Ueno et al., 2013; Kumari et al., 2023).

Blocking calcium channels decreases sympathetic overactivity, which is notorious for insulin signalling impairment. Cilnidipine facilitates nitric oxide-mediated vasodilation in vascular smooth muscles, thereby improving microvascular perfusion and enhancing glucose uptake in peripheral tissues. Additionally, cilnidipine possesses anti-inflammatory and antioxidant effects that improve the signalling in insulin receptors. The observed decrease in the HOMA-IR score with cilnidipine therapy could be explained by the above-mentioned coordinated actions. All in all, these benefits not only enhanced insulin receptor sensitivity but also promoted glucose uptake and utilization, thereby increasing the potential of cilnidipine as a metabolically active antihypertensive agent in patients with diabetes.

The model's capacity to incorporate multiple inputs, such as medicine interaction, dosing, and physiological baselines, indicates the complexity level of pharmacodynamic interactions that traditional models cannot have independently, and these findings validate the utility of deep learning in pharmacological synergy studies, particularly in identifying optimal combination doses and predicting patient-specific outcomes. The molecular results suggested that cilnidipine and glibenclamide acted synergistically to enhance insulin signalling and glucose uptake by upregulating the PI3K/AKT/GLUT4 pathway, providing a mechanistic basis for their antidiabetic activity. The ability of this combination to exceed typical levels of expression further highlights its potential not only in reversing insulin resistance but also in optimizing clinical glycemic control (Simon-Szabó et al., 2024).

Certain limitations should be acknowledged in the current study. The experiment was conducted exclusively on male Wistar rats, and sex-specific responses were not examined, which could influence metabolic responses. The second limitation was that the study lasted only 28 days, and the long-term effects on β-cell preservation, cardiovascular risk, and renal function were not assessed. While the CI had provided insights into pharmacodynamic interactions, molecular mechanisms such as markers of oxidative stress, inflammatory cytokines, or insulin receptor signalling pathways (excluding the insulin receptor signalling pathway, PI3K/AKT) and their downstream target (GLUT4) were not examined. Furthermore, the experiment was limited to monotherapies and two-medicine combinations; the inclusion of additional antidiabetic or lipid-lowering agents could potentially enhance efficacy.

CONCLUSION

The present study demonstrated that high doses of cilnidipine and its combination with glibenclamide could be a more integrated approach to diabetes and its comorbidities. A significant effect was observed in the combination of cilnidipine 10 mg/kg with glibenclamide, which resulted in reduced fasting plasma glucose (FPG), an increase in insulin and C-peptide levels, and the normalization of HOMA-IR. Furthermore, they demonstrated the highest levels of GLUT4 and a

significant upregulation of PI3K/p-AKT expression. However, this combination therapy exerted an opposing effect on all lipid parameters. The findings necessitated the exploration of the present results in clinical trials to establish the translational potential in diabetic patients. Future studies need to address these gaps by using long-term models, including females in the studies, investigating specific mechanistic pathways, such as whether it directly impacts the insulin cascade, testing glibenclamide alone for comparison purposes, and exploring its potential for clinical application in humans with diabetes.

DECLARATIONS

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Authors' contributions

Shatha Hani Mohammad designed and implemented the present study. Nada Satea Mahmood assisted in checking the manuscript, supervision, and ensuring necessary revisions and corrections. Abeer Mansour Abdel Rasool contributed to data analysis, interpretation, and writing the manuscript. All authors checked and approved the findings of the present study and the final edition of the submitted manuscript.

Competing interests

The authors have not declared any conflict of interest.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication or falsification, double publication, and redundancy, have been checked by all the authors. No artificial intelligence (AI) or generation was used for writing or preparing the manuscript.

Availability of data and materials

The data supporting the findings of the present study are available from the corresponding author upon reasonable request.

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