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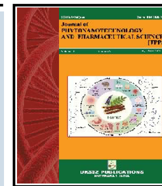

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Comparative evaluation of repaglinide, caffeic acid, and *Coffea arabica* L. bean aqueous extract on glycemic control and oxidative stress in streptozotocin induced type 2 diabetic rats

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Abstract

The present study aimed to evaluate the synergistic antidiabetic and antioxidant effects of repaglinide in combination with caffeic acid in streptozotocin (STZ)-induced diabetic rats. Type 2 diabetes was induced in male Sprague-Dawley rats by a single intraperitoneal injection of STZ (65 mg/kg), followed by nicotinamide (110 mg/kg). The animals were treated orally with repaglinide (0.15 or 0.30 mg/kg), caffeic acid (40 mg/kg), their combinations, and *C. arabica* aqueous extract (CAAE, 100 mg/kg) for 28 days. Biochemical parameters including blood glucose, lipid profile (cholesterol, triglycerides, HDL, LDL, VLDL), and oxidative stress markers (superoxide dismutase, SOD, and malondialdehyde, MDA) were estimated. STZ-induced diabetic rats exhibited significant hyperglycemia, dyslipidemia, body-weight loss, and oxidative stress compared to normal controls. Treatment with repaglinide and caffeic acid significantly reduced fasting blood glucose, improved oral glucose tolerance, and restored lipid profiles and antioxidant enzyme activities compared to diabetic controls. The combination therapy showed superior efficacy to either drug alone, indicating a synergistic interaction that enhances insulin secretion and mitigates oxidative injury. CAAE produced comparable effects, supporting the role of natural phenolic compounds such as caffeic acid in glucose regulation. These findings suggest that combining repaglinide with caffeic acid may offer a promising therapeutic approach for managing type 2 diabetes mellitus by targeting both glycemic control and oxidative stress.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is associated with impaired metabolism of carbohydrates, lipids, and proteins, and leads to severe complications such as nephropathy, neuropathy, retinopathy, and cardiovascular disease (Hashim *et al.,* 2024; Hashim *et al.,* 2023). The increasing prevalence of diabetes globally poses a major health problem and requires novel therapeutic approaches that can effectively manage blood glucose levels while minimizing adverse effects. Among the various experimental models, streptozotocin (STZ)-induced diabetes in rodents remains a widely accepted model for investigating the pathophysiology of diabetes and evaluating the efficacy of new antidiabetic agents due to their ability to selectively destroy pancreatic β-cells through oxidative stress mechanisms (Shoaib *et al.,* 2020). Oxidative stress plays a pivotal role in the development and progression of diabetes and its complications. Elevated levels of reactive oxygen species (ROS) and lipid peroxidation products such as malondialdehyde (MDA) contribute to cell and tissue damage, particularly in pancreatic β-cells, which are highly susceptible to oxidative insult. Therefore, the use of antioxidants in conjunction with conventional antidiabetic drugs has gained increasing attention as a strategy to improve oxidative stress and improve glycemic control (Singh *et al.,* 2024).

Caffeic acid, a naturally occurring phenolic compound widely distributed in plants such as coffee (*Coffea arabica* L.), exhibits potent antioxidant, anti-inflammatory and antidiabetic properties. Previous studies have demonstrated that caffeic acid can improve glucose metabolism, enhance insulin sensitivity, and modulate lipid profiles in diabetic models. Its ability to eliminate free radicals and reduce lipid peroxidation makes it a promising adjunct in diabetes management (Yusuf *et al.,* 2019). Repaglinide, a short-acting insulin secretagogue belonging to the meglitinide class, acts by stimulating pancreatic β-cells to release insulin through closure of ATP-sensitive potassium channels. Although, repaglinide effectively reduces postprandial glucose levels, monotherapy may not adequately address the oxidative component of diabetes. Therefore, combining repaglinide with a potent antioxidant such as caffeic acid may provide a synergistic benefit by simultaneously targeting hyperglycemia and oxidative stress (Mohajan and Mohajan, 2024).

The present study was designed to evaluate the antidiabetic and antioxidant potential of repaglinide in combination with caffeic acid in streptozotocin-induced diabetic rats. In addition, an aqueous extract

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of *C. arabica* (CAAE) was included for comparative assessment. The study investigates changes in biochemical parameters such as serum glucose, lipid profile (cholesterol, triglycerides, HDL, LDL and VLDL), and oxidative stress, including superoxide dismutase (SOD) and malondialdehyde (MDA). The findings are intended to elucidate whether the combination of repaglinide and caffeic acid offers enhanced glycemic control and oxidative protection compared to monotherapy, suggesting a potential combinational therapeutic approach to diabetes management.

2. Materials and Methods

2.1 Drugs and Chemicals

The entire chemicals used are of analytical grade.

2.2 Methods of preparations

2.2.1 Preparation of coffee bean extract

The coffee beans were selected from *Coffea arabica* L. coffee was washed with water and sodium hypochlorite 1% (v/v), and subsequently the stability process was carried out at a temperature of 120°C for 5 min. The aqueous extract was made using a decoction, which weighed 15 g of coffee beans and added 100 ml of distilled water to boil for 15 min. Then it was proceeded to filter through sterile gauze. The extract was poured into glass jar amber. The dose was obtained in dry matter (Z³otek *et al.,* 2016).

2.2.2 Preparation of 0.1 M citrate buffer

192 mg of citric acid was suspended in 10 ml of distilled water to make a 0.1M citric acid solution and 294 mg of tri sodium citrate was also added in 10 ml of distilled water to prepare the 0.1M solution. Then 4.5 ml of citric acid was mixed with 5.5 ml of tri sodium citrate solution. Finally, the pH (4.5) was adjusted with citric acid. Then, 4.5 ml of citric acid was assorted with 5.5 ml of the previously prepared tri sodium citrate solution previously prepared. Finally, the pH (4.5) was adjusted with citric acid.

2.2.3 Preparation of the STZ solution

The STZ (65 mg/kg b.w., i.p) was prepared by dissolving it in ice cold citrate buffer (pH 4.5, 0.1 M).

2.3 Animals

Adult male Sprague Dawleyrats body weight (120-200 g) were procured from Central Drug Research Institute (CDRI), Lucknow and housed in the Animal house facility, Faculty of Pharmacy, Integral University, Lucknow. Polypropylene cages were used to keep the animals (5 in each cage). The laboratory was maintained at 12 h light and 12 h dark cycle and all the animals had a free right to take standard pellet diet and drinking water *ad libitum*. The animal house temperature was maintained at 23 ± 2°C and relative humidity was also maintained at (50 ± 15%). Seven days was given to the animals for acclimatization. Ethical clearance was obtained from Institutional animal Ethical Committee (IAEC) (Approval No: IU/IAEC/18/17) Integral University, Lucknow.

2.4 Experimental design

2.4.1 Induction of diabetes

Rats were deprived of food but not water from the prior evening. Streptozotocin (STZ) was dissolved in ice cold citrate buffer (pH 4.5, 0.1 M) and intraperitoneally injected within 5 min at 65 mg/kg body weight followed by the i.p. administration of NIC (110 mg/kg, NIC) after 15 min. Blood glucose was analyzed after 72 h of injection. Diabetic animals were given water along with 5% glucose 24 h after STZ injection to prevent mortality because of hypoglycemia. After 72 h blood was taken from the tail vein and was analyzed using glucometer (ACCU CHECK). Animals showed plasma glucose elevated than 250 mg/dl were deemed as diabetic and were used for diabetic studies.

The following diabetic animal (after induction) was used to evaluate the effect of repaglinide (0.15, 0.30 mg/kg) with body weight of caffeic acid (40mg / kg) and *C. arabica*aqueous extract (100mg / kg) against STZ induced diabetic rats. For this purpose, Sprague-Dawley rats were divided into 7 groups (5 animals in each) and the initial body weight of all groups of animals was recorded. The treatment period for each group of animals was 28 days (Table 1). At the end of the study, the final body weight of all animals was recorded. After that, the animal was anaesthetized with thiopentone sodium, and the blood sample was collected by retro orbital puncture for the estimation of blood sugar and other biochemicals.

Table 1: Treatment schedule

Groups (n = 5)	Treatment	Dosage, route of administration,and duration
I	Vehicle (normal saline)	10 ml/kg, p.o once a day for 28 days
II	STZ	65 mg/kg, i.p., STZ single dose
III	Diabetic rats + Repaglinide	0.3 mg/kg, p.o once a day for 28 days
IV	Diabetic rats + Caffeic acid	40 mg/kg, p.o once a day for 28 days
V	Diabetic rats + CAAE	100 mg/kg, p.o once a day for 28 days
VI	Diabetic rats + Repaglinide + Caffeic acid	0.15 mg/kg + 40mg/kg, p.o once a day for 28 days
VII	Diabetic rats + Repaglinide + Caffeic acid	0.3 mg/kg + 40mg/kg, p.o once a day for 28 days

Note: n: number of animals, NC: Normal control, DC: Diabetic control, STD: Standard, STZ: Streptozotocin, i.p: intraperitoneal, p.o: per oral, CAAE: Aqueous extract of *C. arabica*.

2.4.2 Blood glucose estimation

On the first and final day of treatment, blood sugar measurements were taken using tail's vein blood (by ACCUCHEK-ACTIVE kit made by Roche, Germany).

2.4.3 Oral glucose tolerance test

OGTT was performed to measure glucose tolerance. After an overnight fasting, dextrose solution (40% wt/vol.) was administered intragastrically to the rat at a dose of 2.5 g/kg body weight and blood

sugar was checked at time points of 0, 30, 60 and 120 min. Glucose levels were considered at specific intervals. The glucose tolerance (OGT) was measured by calculating the area under curve (AUC) for glucose by the trapezoidal method.

AUC = (basal glycaemia + glycaemia 0.5 h) × 0.25 + (glycaemia 0.5 h + glycaemia 1 h) × 0.25 + (glycaemia 1 h + glycaemia 2 h) × 0.5

2.4.4 Estimation of triglycerides

Serum triglyceride levels were estimated using the ERBA triglycerides diagnostic kit based on the GPO-Trinder enzymatic colorimetric method. The assay involves enzymatic hydrolysis of triglycerides and subsequent colorimetric measurement of the quinonimine dye formed. The absorbance was read at 546 nm, and the triglyceride concentration was calculated according to the manufacturer's instructions.

2.4.5 Estimation of cholesterol

Serum total cholesterol was estimated using the ERBA cholesterol diagnostic kit based on the Trinder enzymatic colorimetric method. The assay involves enzymatic hydrolysis and oxidation of cholesterol followed by color development through the Trinder reaction. Absorbance was measured at 510 nm, and cholesterol concentration was calculated as per the manufacturer's instructions.

2.4.6 Estimation of HDL cholesterol

Serum HDL-cholesterol was estimated using the ERBA HDL-cholesterol diagnostic kit based on the phosphotungstic acid enzymatic method. In this assay, non-HDL lipoproteins are selectively precipitated, and HDL-cholesterol in the supernatant is determined colorimetrically. Absorbance was measured at 578 and 630 nm, and HDL-cholesterol concentration was calculated according to the manufacturer's protocol.

2.4.7 Estimation of LDL cholesterol

Using Friedwald's equation**:** LDL cholesterol = total cholesterol – Triglycerides/5 – HDL-cholesterol

2.4.8 Estimation of VLDL cholesterol

VLDL-cholesterol = Triglycerides/5

2.4.9 Evaluation of oxidative biomarkers in the pancreas

2.4.9.1 Preparation of the pancreatic supernatant

After the last dose of treatment, the animals were sacrificed using pentobarbitone sodium (150 mg/kg). The pancreas was carefully isolated from each animal, washed in cold 0.9% saline, weighed and homogenized with 10 times (10%, w/v) ice cold potassium phosphate buffer (0.1 M, pH 7.4) in a teflon glass homogenizer. The homogenate was centrifuged at 1000 × g at 4 °C for 3 min to obtain the supernatant for biochemical estimation.

2.4.9.2 Superoxide dismutase (SOD)

The activity of SOD in pancreatic homogenates was determined spectrophotometrically by the pyrogallol autoxidation method as described by Marklund and Marklund (1974). The assay is based on the enzyme's ability to inhibit the oxidation of pyrogallol at alkaline pH. The change in absorbance was recorded at 420 nm, and one unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of pyrogallol autoxidation (Kannan *et al.,* 2022).

2.4.9.3 Estimation of thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation in pancreatic tissue was estimated by measuring thiobarbituric acid reactive substances (TBARS) according to the method of Ohkawa *et al.* (1979). The assay is based on the formation of a pink chromogen resulting from the reaction between malondialdehyde (MDA), a lipid peroxidation product, and thiobarbituric acid (TBA) under acidic and high-temperature conditions. The absorbance of the supernatant was measured at 540 nm, and the concentration of MDA was calculated using a molar extinction coefficient of 1.56×10 u M^{-1} cm^{-1} (Kherouf *et al.,* 2021).

2.5 Statistical analysis

Data were represented as mean ± SEM of four animals in each faction. Statistical analysis was performed by using one-way analysis of variance (ANOVA) followed by Dunnett: compare all vs control (Graph Pad Instat, USA).

Table 2: Change in body weight

Groups/treatment	Change in body weight (g)			
	At week 0	At week 4 (g)	% body weight variation	
			% weight gain	% weight loss
NC	137.5 ± 5.20	153.7 ± 6.25	11.78 ± 2.6	
DC	136.2 ± 2.6	129 ± 2.61		$5.2 \pm 1.4^{**}$
RG (0.30 mg)	134 ± 2.3	141.1 ± 3.4	$7.4 \pm 2.1^{\#\#}$	-
CA	126.7 ± 3.1	156 ± 1.5	$19.21 \pm 2.5^{\#\#}$	-
CAAE	128.8 ± 2.1	159 ± 6.2	$12.6 \pm 3.2^{\#\#}$	
RG (0.15 mg) + CA	134.5 ± 1.7	159 ± 2.6	$13.72 \pm 6.7^{\#\#, a}$	-
RG (0.30 mg) + CA	136.8 ± 6.5	151 ± 3.13	$8.7 \pm 2.7^{\#\#, a}$	-

All values were expressed as mean ± SEM. Significant difference between various groups (ANOVA) and individual comparison was done by Dunnett's t-test. $^{**}p<0.01$ = Significant, when compared with normal control (NC), $^{\#\#}p<0.01$ = Significant when compared with diabetic control (DC). $^{a}p<0.01$ = Significant when compared with repaglinide (0.30 mg) standard.

3. Results

3.1 Effect of repaglinide with caffeic acid on body weight

There was significance (p<0.01) decrease (p<0.01) in blood glucose level in the diabetic control group when compared to the normal group. Blood glucose level was significantly (p<0.01) in treated groups (*i.e.,* RG 0.30 mg, RG 0.15 mg + CA, RG 0.30 mg + CA, CAAE, CA, respectively), compared with diabetic control (DC). Although, therewas a significant increase (p<0.01) in combination of RG + CA (both doses) compared with repaglinide (0.30 mg) alone (Table 2).

3.2 Effect of repaglinide with caffeic acid on serum blood glucose level

There was significance (p<0.01) increase (p<0.01) in blood glucose level in the diabetic control group when compared to the normal group. Blood glucose level was significantly decreased (p<0.01) in treated groups (*i.e.,* RG 0.30 mg, RG 0.15 mg + CA, RG 0.30 mg + CA, CAAE, CA, respectively), compared with diabetic control (DC). Although, it was significantly decreased (p<0.01) in combination with RG + CA (both doses) compared with repaglinide (0.30 mg) alone (Table 3).

3.3 Effect of repaglinide with caffeic acid on oral glucose tolerance (OGT)

There was significance (p<0.01) increase (p<0.01) in the diabetic control group when compared to the normal group. AUC decreased significantly (p<0.01) in treated groups (*i.e.,* RG 0.30 mg, RG 0.15 mg + CA, RG 0.30 mg + CA, CAAE, CA, respectively), compared with diabetic control (DC). Although, it was significantly decreased (p<0.01) in combination with RG + CA (both doses) compared with repaglinide (0.30 mg) alone (Table 3).

Table 3: Effect of repaglinide with caffeic acid on serum blood glucose level

Groups/treatment	Blood glucose concentration (mg/dl)		AUC (mg/dl)
	Initial blood glucose level	Final blood glucose level	
NC	65.7 ± 0.85	67.7 ± 2.0	72.25 ± 1.55
DC	395.7 ± 64.7	350.05 ± 5.2**	308.8 ± 1.14**
RG (0.30 mg)	254.7 ± 4.2	226.4 ± 3.4##	162.5 ± 0.65##
CA	333.2 ± 19.6	326.2 ± 2.5##	153.75 ± 0.54##,a
CAAE	312 ± 6.9	280.4 ± 3##	149.78 ± 0.88##,a
RG (0.15 mg) + CA	327 ± 3.4	293.7 ± 3.3##,a	175.05 ± 0.71##
RG (0.30 mg) + CA	375.7 ± 17.3	223.0 ± 2.7##,a	161 ± 3.02##

All values were expressed as Mean ± S.E.M. **p<0.01=Significant, when compared with normalcontrol (NC), ##p<0.01=Significant when compared with diabetic control (DC), ap<0.01=Significant when compared with repaglinide (0.30 mg) standard.

Table 4: Effect of caffeic acid on serum triglyceride level

Groups	Triglyceride (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I	74.81 ± 0.51	185.25 ± 1.49	77.83 ± 0.99	97.6 ± 0.79	36.61 ± 0.25
II	209.26 ± 0.27**	331.04 ± 0.67**	22.16 ± 0.65**	266.55 ± 0.75**	67.69 ± 0.8**
II	136.13 ± 0.53##	272.14 ± 0.48##	45.83 ± 0.75##	164.03 ± 0.6##	47.27 ± 0.64##
IV	130.31 ± 0.25##,a	226.08 ± 1.21##,a	42.80 ± 0.77##,a	156.93 ± 0.42##,a	44.41 ± 0.23##,a
V	111.55 ± 0.24##,a	209.64 ± 0.30##,a	55.85 ± 0.47##,a	131.58 ± 0.26##,a	41.71 ± 0.25##,a
VI	144.28 ± 0.16##	249.51 ± 0.29##	42.50 ± 0.55##	178.77 ± 1.1##	52.5 ± 0.34##
VII	151.57 ± 0.23##	233.77 ± 0.53##	39.94 ± 0.48##	196.4 ± 0.52##	54.57 ± 0.52##

All values were expressed as mean ± SEM. Significant difference between various groups (ANOVA) and individual comparison was done by Dunnett's t-test. **p<0.01=Significant, when compared with normal control (NC), ##p<0.01=Significant when compared with diabetic control (DC), ap<0.01=Significant when compared with repaglinide (0.30 mg) standard.

3.4 Effect of caffeic acid on serum triglyceride level

There was significance (p<0.01) increase (p<0.01) in the level of triglyceride in the diabetic control group compared with normal group. The triglyceride level was significantly decreased (p<0.01) in the treated groups (*i.e.,* RG 0.30 mg, RG 0.15 mg + CA, RG 0.30 mg + CA, CAAE, CA, respectively), compared with diabetic control (DC). Although, it was significantly decreased (p<0.01) in combination with RG + CA (both doses) compared with repaglinide (0.30 mg) alone (Table 4).

3.5 Effect of caffeic acid on serum cholesterol level

There was significance (p<0.01) increase (p<0.01) in the cholesterol level in the diabetic control group when compared to the normal group. Cholesterol level was significantly decreased (p<0.01) in treated groups (*i.e.,* RG 0.30 mg, RG 0.15 mg + CA, RG 0.30 mg + CA, CAAE, CA, respectively), compared with diabetic control (DC). Although, it was significantly decreased (p<0.01) in combination with RG + CA (both doses) compared with repaglinide (0.30 mg) alone (Table 4).

3.6 Effect of caffeic acid on serum HDL level

There was significance ($p<0.01$) decrease ($p<0.01$) in HDL level in the diabetic control group when compared to the normal group. HDL level increased significantly ($p<0.01$) in treated groups (*i.e.*, RG 0.30 mg, RG 0.15 mg + CA, RG 0.30 mg + CA, CAAE, CA, respectively) compared with diabetic control (DC). Although, there was asignificant increase ($p<0.01$) in combination of RG + CA (both doses) compared with repaglinide (0.30 mg) alone (Table 4).

3.7 Effect of caffeic acid on serum LDL level

There was significance ($p<0.01$) increase ($p<0.01$) in the level of LDL in the diabetic control group compared with normal group. LDL level was significantly decreased ($p<0.01$) in treated groups (*i.e.,* RG 0.30 mg, RG 0.15 mg + CA, RG 0.30 mg + CA, CAAE, CA respectively), compared with diabetic control (DC). Although, it was significantly decreased ($p<0.01$) in combination with RG+CA (both doses) compared with repaglinide (0.30 mg) alone (Table 4).

3.8 Effect of repaglinide with caffeic acid on serum VLDL level

There was significance ($p<0.01$) increase ($p<0.01$) in the level of VLDL in the diabetic control group compared with normal group. The VLDL level was significantly decreased ($p<0.01$) in the treated groups (that is, RG 0.30 mg, RG 0.15 mg + CA, RG 0.30 mg + CA, CAAE, CA, respectively), compared with diabetic control (DC). Although, it was significantly decreased ($p<0.01$) in combination with RG + CA (both doses) compared with repaglinide (0.30 mg) alone (Table 4).

3.9 Effect of caffeic acid on serum superoxide dismutase (SOD) level

There was significance ($p<0.01$) decrease ($p<0.01$) in the SOD level in the diabetic control group when compared to the normal group. The SOD level was significantly increased ($p<0.01$) in the treated groups (*i.e.,* RG 0.30 mg, RG 0.15 mg + CA, RG 0.30 mg + CA, CAAE, CA, respectively), compared with diabetic control (DC). Although, there was a significant increase ($p<0.01$) in combination of RG + CA (both doses) compared with repaglinide (0.30 mg) alone (Table 5).

Table 5: Serum level of SOD and MDA levels

Groups /Treatment	SOD (mg/dl)	MDA
NC	144.55 ± 1.91	25.84 ± 0.76
DC	74.07 ± 1.69**	48.99 ± 0.89**
RG (0.30 mg)	123.26 ± 0.93$^{\#\#}$	34.69 ± 0.83$^{\#\#}$
RG (0.15 mg) + CA	136.25 ± 1.91$^{\#\#,a}$	33.25 ± 0.69$^{\#\#,a}$
RG (0.30 mg) +CA	141.73 ± 2.47$^{\#\#,a}$	31.1 ± 0.52$^{\#\#,a}$
CAAE	128.37 ± 1.82$^{\#\#}$	27.89 ± 0.72$^{\#\#}$
CA	129.08 ± 0.61$^{\#\#}$	28.92 ± 0.73$^{\#\#}$

All values were expressed as mean ± SEM. The significant difference between various groups (ANOVA) and the individual comparison was performed using the Dunnett t-test.$^{**}p<0.01$ = Significant, compared with normal control (NC),$^{\#\#}p<0.01$ = Significant compared with Diabetic control (DC), $^{a}p<0.01$ = Significant compared with repaglinide (0.30 mg).

3.10 Effect of caffeic acid on serum MDA (malondialdehyde) level

There was significance ($p<0.01$) increase ($p<0.01$) in MDA level in the diabetic control group when compared to the normal group. The MDA level decreased significantly ($p<0.01$) in treated groups (*i.e.,* RG 0.30 mg, RG 0.15 mg + CA, RG 0.30 mg + CA, CAAE, CA, respectively), compared with diabetic control (DC). Although, it was significantly decreased ($p<0.01$) in combination with RG + CA (both doses) compared with repaglinide (0.30 mg) alone (Table 5).

4. Discussion

The present study investigated the combined effect of repaglinide and caffeic acid on glycaemic control, lipid metabolism, and oxidative stress in streptozotocin (STZ)-induced diabetic rats. The findings demonstrate that the combination therapy significantly improved biochemical parameters compared to the diabetic control group and produced superior results compared to either agent alone. These results suggest a synergistic antidiabetic and antioxidant interaction between repaglinide and caffeic acid.

STZ-induced diabetes is a well-established experimental model that mimics the pathophysiological characteristics of type 2 diabetes mellitus, primarily through β-cell destruction, resulting in impaired insulin secretion and elevated blood glucose levels. In the current study, STZ-treated rats showed marked hyperglycemia, dyslipidaemia, body weight loss, and elevated oxidative stress such as malondialdehyde (MDA), confirming successful induction of diabetes. These findings align with previous reports that highlight that STZ induces oxidative injury and lipid peroxidation within pancreatic tissues, leading to β-cell necrosis and metabolic imbalance (Lukman *et al.,* 2024).

Administration of repaglinide alone produced a significant reduction in blood glucose levels and partial restoration of body weight, reflecting its insulinotropic mechanism that promotes glucose utilisation and suppresses hepatic glucose output. However, the combination of repaglinide with caffeic acid (0.15 mg/kg or 0.30 mg/kg + 40 mg/kg) further reduced fasting blood glucose and improved oral glucose tolerance compared to the monotherapy group. This enhancement can be attributed to the potent antioxidant property, which may protect pancreatic β-cells from oxidative damage, thus increasing the insulin release effect (Tang *et al.,* 2024).

The observed improvement in lipid profiles, characterised by reduced levels of total cholesterol, triglycerides, LDL, and VLDL and elevated HDL concentrations further supports the beneficial metabolic effect of the combination treatment. Dyslipidaemia is a common metabolic derangement in diabetes, increasing the risk of cardiovascular complications. Restoration of lipid homeostasis in treated animals suggests that combination therapy improves lipid metabolism, possibly through enhanced insulin sensitivity and modulation of key lipid-regulating enzymes (Kane *et al.,* 2021). Interestingly, the aqueous extract of *C. arabica* (CAAE) demonstrated comparable effects to the repaglinide caffeic acid group, indicating that bioactive constituents such as caffeic acid within the extract contribute to its hypolipidemic potential.

Oxidative stress plays a central role in the pathogenesis of diabetes and its associated complications (Singh *et al.,* 2022). In the present study, diabetic rats exhibited significant reductions in superoxide dismutase (SOD) activity and elevated levels of malondialdehyde

(MDA), indicating increased oxidative burden. Treatment with repaglinide and caffeic acid markedly restored SOD levels and reduced MDA concentrations, suggesting effective scavenging of reactive oxygen species and inhibition of lipid peroxidation. The antioxidant enhancement observed with combination therapy may stem from the polyphenolic structure of caffeic acid, which donates hydrogen atoms to neutralise free radicals, along with possible indirect up-regulation of endogenous antioxidant enzymes.

In general, the results indicate that caffeic acid complements repaglinide therapy not only by amplifying glucose lowering efficacy but also by mitigating oxidative stress, a key contributor to β-cell dysfunction. Significant restoration of body weight and normalisation of biochemical indices demonstrate systemic metabolic improvement. These findings are consistent with previous studies reporting that natural antioxidants improve the therapeutic efficacy of oral hypoglycemic agents by attenuating oxidative damage and improving insulin dynamics.

5. Conclusion

The findings of the present study clearly demonstrate that the combination of repaglinide and caffeic acid exerts a synergistic antidiabetic effect in streptozotocin-induced diabetic rats. Co-administration of these agents significantly improved glycaemic control, increased glucose tolerance, and normalised serum lipid profiles compared to diabetic controls and monotherapy groups. Additionally, the combination effectively restored antioxidant enzyme activity (SOD) and reduced lipid peroxidation (MDA), indicating strong protection against oxidative stress in pancreatic and systemic tissues.

These results suggest that caffeic acid potentiates the therapeutic efficacy of repaglinide by improving insulin secretion and attenuating oxidative injury, providing dual benefits in diabetes management. The comparable results observed with *C. arabica* aqueous extract further support the role of natural polyphenols as valuable adjuncts in the treatment of diabetes. Future studies should focus on molecular mechanisms, long-term safety, and possible clinical translation of this combination to establish it as a novel and effective strategy for the management of type 2 diabetes mellitus and its associated metabolic complications.

Availability of data and material

All data are provided within the manuscript.

Authorship contribution statement

Mohammad Nasiruddin: Contributed to conceptualization, methodology design, data curation, and writing the original draft of the manuscript. **Badruddeen:** Contributed to supervision, conceptualization, validation, and critical review and editing of the manuscript. **Mohammad Khalid:** Contributed to data analysis, interpretation, and validation of results. **Anas Islam:** Contributed to investigation, methodology execution, and software handling. **Juber Akhtar:** Contributed to resources, formal analysis, and manuscript proofreading. **Mohammad Irfan Khan:** Contributed to experimental work, visualization, and data presentation. **Mohammad Ahmad:** Contributed to literature review, data organization, and editing of the final draft. **Mohd Muazzam Khan:** Contributed to project administration, validation, and final manuscript approval.

Consent for publication

All authors gave their full consent for publication and submission to this journal.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Ethics approval

Ethical clearance was obtained from Institutional animal Ethical Committee (IAEC) (Approval No: IU/IAEC/18/17) Integral University, Lucknow.

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None

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