Synergistic Effects of *Cinnamomum zeylanicum* and Atorvastatin in streptozotocin-Induced *In Vivo*

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Abstract

Background: Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia, leading to severe complications such as cardiovascular diseases, neuropathy, and nephropathy. While pharmacological interventions like atorvastatin are effective in managing hyperlipidemia, concerns regarding their adverse effects necessitate alternative or adjunctive therapies. *Cinnamomum zeylanicum* (CZ) has shown promise in diabetes management due to its bioactive compounds, which enhance insulin sensitivity and regulate lipid metabolism. Objective: This study investigates the anti-hyperglycemic and anti-hyperlipidemic effects of *Cinnamomum zeylanicum* in combination with atorvastatin in streptozotocin (STZ)-induced diabetic rats, assessing glucose levels, lipid profiles, liver function, and renal parameters. Methods: Thirty-five male Sprague-Dawley rats were divided into seven groups, including control, diabetic, CZ (250 mg/kg and 500 mg/kg), atorvastatin (10 mg/kg), and combination therapy groups. Diabetes and hyperlipidemia were induced using STZ and Triton X-100, respectively. Biochemical parameters were analyzed using enzymatic assays, and statistical significance was determined via ANOVA. Results: CZ at 500 mg/kg significantly reduced blood glucose (8.3 ± 14.4 mg/dL) and improved lipid profiles by lowering total cholesterol, triglycerides, and LDL levels. Combination therapy showed moderate improvements but was less effective than CZ alone. Liver function markers indicated hepatoprotective effects, while renal parameters remained stable, confirming CZ's safety. Conclusion: *Cinnamomum zeylanicum* demonstrates potent anti-diabetic and lipid-lowering effects, with a favorable safety profile. These findings support its potential as a complementary therapy for diabetes management, warranting further clinical investigations.

Keywords: *Cinnamomum zeylanicum*, Diabetes, Atorvastatin, Lipid Metabolism, Streptozotocin.

Significance | This study assesses the combined effects of Cinnamomum zeylanicum and atorvastatin on glucose regulation, lipid metabolism, and organ function in diabetic models.

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Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia, resulting from impaired insulin secretion, insulin action, or both. The global prevalence of diabetes has risen significantly, with an estimated 537 million adults affected in 2021, a number projected to reach 783 million by 2045 (Sun et al., 2022). Uncontrolled diabetes can lead to severe complications, including cardiovascular diseases, neuropathy, nephropathy, and retinopathy (American Diabetes Association, 2023). The primary goal of diabetes management is to maintain optimal blood glucose levels through pharmacological interventions and lifestyle modifications (Grundy et al., 2018).

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Streptozotocin (STZ)-induced diabetic rat models are widely employed in experimental studies to mimic the pathophysiology of human diabetes and assess potential therapeutic agents (Lee et al., 2003).

Cinnamomum zeylanicum, commonly known as Ceylon cinnamon, has been traditionally used for its medicinal properties, particularly in diabetes management. It contains bioactive compounds such as cinnamaldehyde, procyanidins, and polyphenols, which contribute to its anti-diabetic effects (Beheshti et al., 2024). Several studies have demonstrated that cinnamon supplementation improves insulin sensitivity, reduces fasting blood glucose levels, and exhibits hypolipidemic effects by lowering total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides (Ranasinghe et al., 2012; Cortez-Navarrete et al., 2023). Cinnamon's mechanisms of action are thought to involve enhancing glucose metabolism, modulating insulin receptor signaling, and exerting antioxidant properties .

Atorvastatin, a widely used statin, is known for its potent lipid-lowering effects by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This inhibition leads to reduced cholesterol synthesis and upregulation of LDL receptors, resulting in increased clearance of circulating LDL cholesterol (Nawrocki et al., 1995). Clinical guidelines recommend statins as first-line therapy for managing hyperlipidemia and preventing cardiovascular complications in diabetic patients (Grundy et al., 2018). Although atorvastatin effectively lowers cholesterol levels, concerns regarding its potential adverse effects, such as hepatotoxicity and myopathy, necessitate exploring alternative or adjunctive therapies.

Despite the individual benefits of *Cinnamomum zeylanicum* and atorvastatin, limited studies have investigated their combined effects on diabetes and hyperlipidemia. Herbal remedies, including cinnamon, have gained popularity due to their perceived safety, cost-effectiveness, and potential synergistic effects with conventional pharmaceuticals (Pezzani et al., 2019). The combination of cinnamon and atorvastatin may offer a novel approach to managing diabetes and its associated lipid abnormalities by integrating the glucose-lowering and antioxidant properties of cinnamon with the lipid-lowering effects of atorvastatin (Alsoodeeri et al., 2020).

This study aims to evaluate the anti-hyperglycemic and anti-hyperlipidemic effects of *Cinnamomum zeylanicum* in combination with atorvastatin in STZ-induced diabetic rats. By assessing blood glucose levels, lipid profiles, liver function markers, and renal parameters, this research seeks to determine whether the combination therapy provides superior benefits compared to monotherapies. The findings may contribute to the development of integrative therapeutic strategies for diabetes management, promoting the use of natural compounds alongside conventional pharmacological agents.

2. Materials and Methods

2.1 Animal Model and Grouping

Thirty-five male Sprague-Dawley rats, each weighing between 250 and 300 g, were obtained and acclimatized for one week under controlled environmental conditions, including a 12-hour light/dark cycle, a temperature of 22 ± 2°C, and a humidity level of 55–65%. During the acclimatization period, the rats were provided with standard laboratory chow and water ad libitum. Following acclimatization, the animals were randomly assigned into seven groups, with five rats per group. The negative control group received no treatment and was maintained on a normal diet. The positive control group was induced with diabetes and hyperlipidemia but did not receive any treatment. The treatment groups included the CZ 250 mg/kg group, which received *Cinnamomum zeylanicum* aqueous extract at 250 mg/kg, and the CZ 500 mg/kg group, which received *Cinnamomum zeylanicum* aqueous extract at 500 mg/kg. Additionally, an atorvastatin group was treated with atorvastatin at 10 mg/kg. Two combination treatment groups were also included: one received CZ at 250 mg/kg along with atorvastatin at 10 mg/kg, while the other received CZ at 500 mg/kg in combination with atorvastatin at 10 mg/kg.

2.2 Induction of Diabetes and Hyperlipidemia

Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) at 50 mg/kg, prepared freshly in 0.1 M citrate buffer (pH 4.5). Hyperlipidemia was induced through an intraperitoneal injection of Triton X-100 at 100 mg/kg (Khoshnoud et al. 2019). Rats were monitored for 72 hours post-STZ injection, and hyperglycemia was confirmed via fasting blood glucose levels (≥11 mmol/L).

2.3 Preparation of Treatments

Aqueous extracts of *Cinnamomum zeylanicum* (CZ) bark were prepared by decoction followed by freeze-drying to obtain a fine powder (Ahmad et al. 2015). The extract was dissolved in distilled water for oral administration. Atorvastatin was dissolved in 0.9% physiological saline. Treatments were administered orally once daily using a gavage needle for seven consecutive days.

2.4 Blood Sample Collection and Biochemical Analyses

At the end of the treatment period, the rats were fasted overnight and subsequently anesthetized using ketamine (50 mg/kg) and xylazine (10 mg/kg) to minimize distress. Blood samples were collected via cardiac puncture and transferred into EDTA-coated tubes for plasma separation and plain tubes for serum separation. Various biochemical parameters were analyzed to assess metabolic and organ function. Blood glucose levels were measured using an Accu-Chek® glucometer. The lipid profile, including total

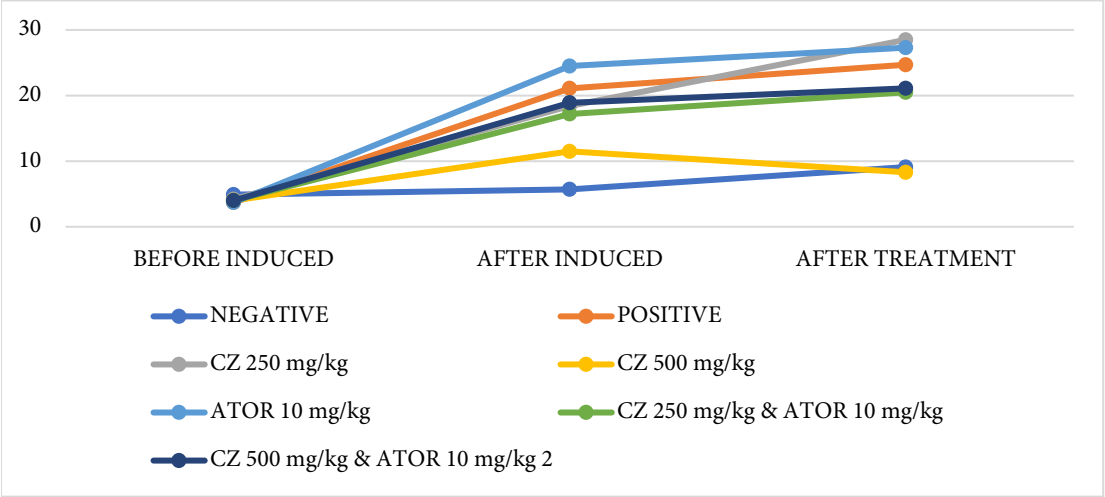

Figure 1. Effect of Cinnamon Zeylanicum on blood glucose fluctuations before and after inducing Streptozotocin and Triton X-100, and after treatments.

Table 1. Effect of Cinnamon Zeylanicum on blood glucose after treatment

GROUP	GLUCOSE LEVEL
Negative control group	9.1 ± 2.6
Positive control group	24.7 ± 15.7
Cz 250 mg/kg	28.5 ± 16.0
Cz 500 mg/kg	8.3 ± 14.4
Atorvastatin 10 mg/kg	20.5 ± 16.2
Cz 250 mg/kg & Atorvastatin10 mg/kg	21.1 ± 20.0
Cz 500 mg/kg & Atorvastatin10 mg/kg	27.3 ± 15.4
p = 0.224, (p > 0.05)	

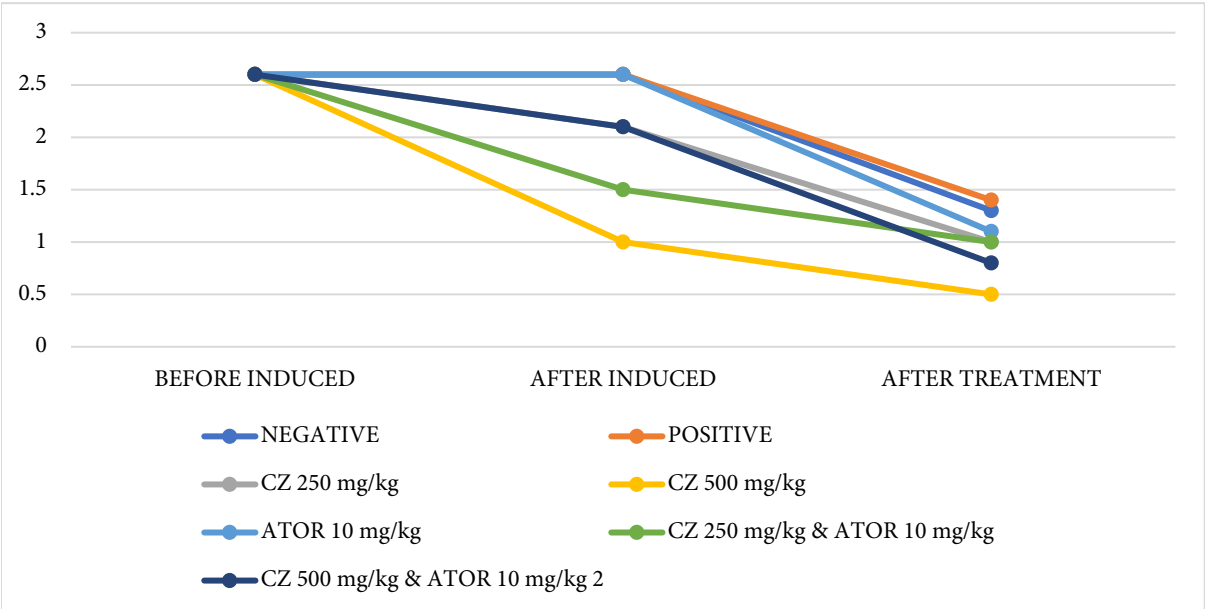

Figure 2. Effect of Cinnamon Zeylanicum on Cholesterol fluctuations before and after inducing Streptozotocin and Triton X-100, and after treatments.

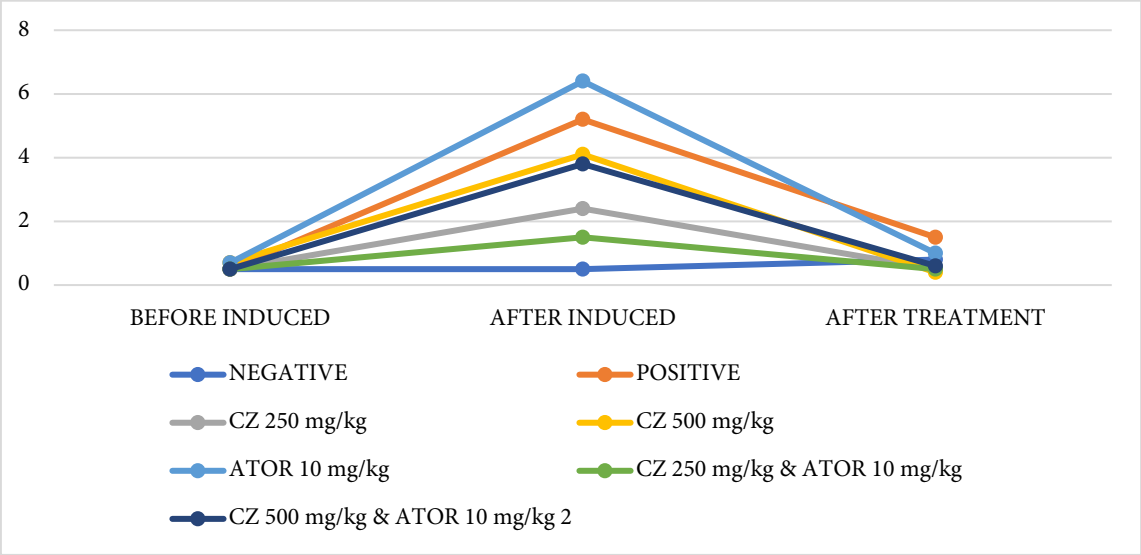

Figure 3. Effect of Cinnamon Zeylanicum on Triglyceride fluctuations before and after inducing Streptozotocin and Triton X-100, and after treatments.

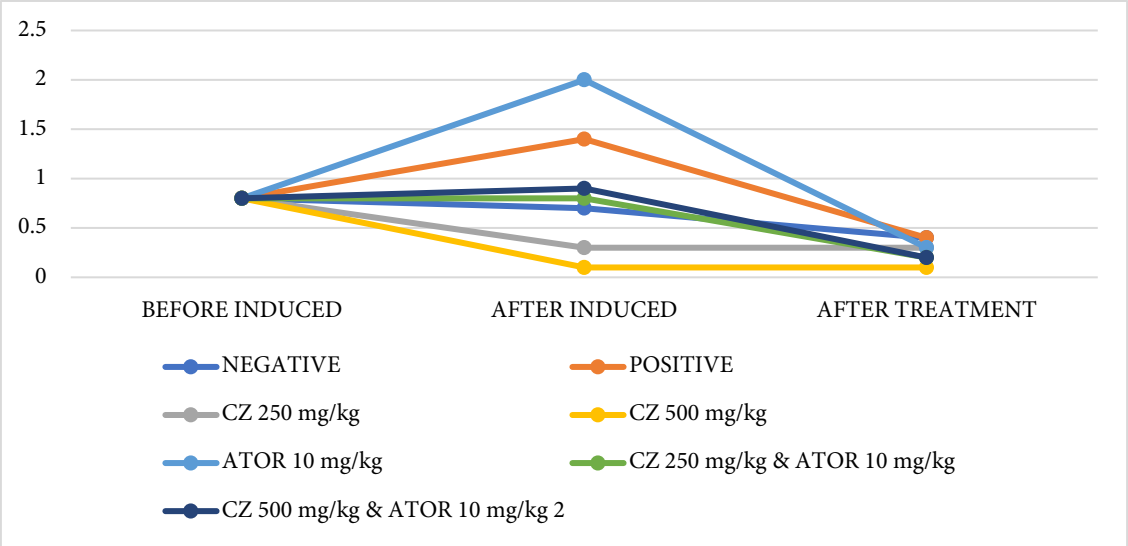

Figure 4. Effect of Cinnamon Zeylanicum on LDL fluctuations before and after inducing Streptozotocin and Triton X-100, and after treatments.

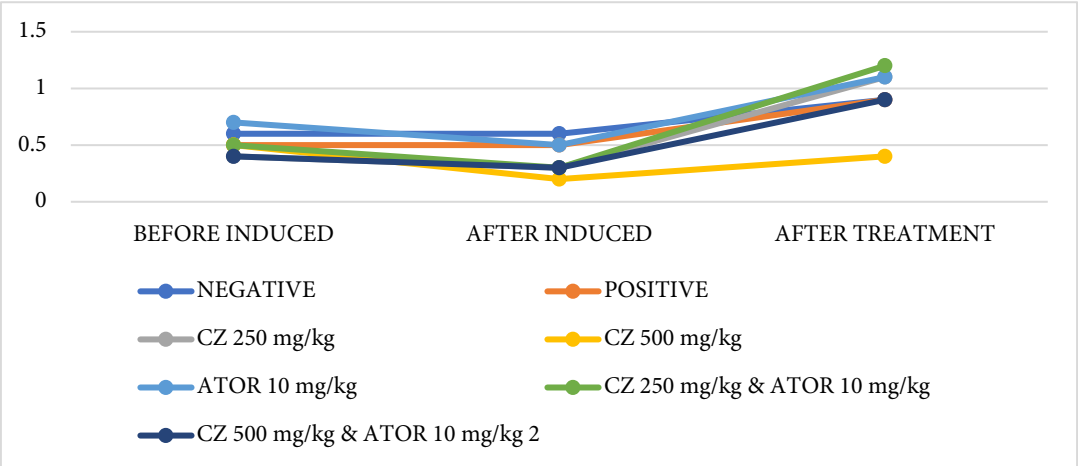

Figure 5. Effect of Cinnamon Zeylanicum on HDL fluctuations before and after inducing Streptozotocin and Triton X-100, and after treatments.

Table 2. Effect of Cinnamon Zeylanicum on lipid profile after treatment

GROUP	TC	LDL	HDL	TG
Negative control group	1.3 ± 0.3	0.4 ± 0.1	0.9 ± 0.1	0.8 ± 0.2
Positive control group	1.4 ± 0.2	0.4 ± 0.1	0.9 ± 0.3	1.5 ± 0.6
Cz 250 mg/kg	1.0 ± 0.6	0.3 ± 0.2	1.1 ± 0.6	0.5 ± 0.4
Cz 500 mg/kg	0.5 ± 0.7	0.1 ± 0.1	0.4 ± 0.6	0.4 ± 0.5
Atorvastatin 10 mg/kg	1.1 ± 0.7	0.3 ± 0.2	1.1 ± 0.7	1.0 ± 0.7
Cz 250 mg/kg & Atorvastatin10 mg/kg	0.8 ± 0.8	0.2 ± 0.2	0.9 ± 0.9	0.6 ± 0.8
Cz 500 mg/kg & Atorvastatin10 mg/kg	1.0 ± 0.6	0.2 ± 0.1	1.2 ± 0.8	0.5 ± 0.3
	p = 0.261, (p>0.05)	p = 0.064, (p>0.05)	p = 0.630, (p>0.05)	p = 0.056, (p>0.05)

Table 3. Effect of Cinnamon Zeylanicum on liver function tests

GROUP	T.BIL	ALT	AST
Negative control group	1.0 ± 0.5	61.2 ± 20.6	68.0 ± 21.5
Positive control group	1.5 ± 0.4	113.0 ± 56.7	229.6 ± 143.4
Cz 250 mg/kg	1.1 ± 0.6	175.6 ± 114.4	250.0 ± 194.0
Cz 500 mg/kg	1.1 ± 0.3	87.5 ± 13.4	147.5 ± 27.6
Atorvastatin 10 mg/kg	1.2 ± 0.4	180.8 ± 33.4	166.8 ± 61.0
Cz 250 mg/kg & Atorvastatin10 mg/kg	1.3 ± 0.3	187.0 ± 60.7	221.3 ± 131.4
Cz 500 mg/kg & Atorvastatin10 mg/kg	1.4 ± 0.2	120.0 ± 81.4	206.3 ± 100.5
	p = 0.420, (p>0.05)	p = 0.079, (p>0.05)	p = 0.311, (p>0.05)

Table 4. Effect of Cinnamon Zeylanicum on renal functions

GROUP	UREA	CREATININE
Negative control group	5.1 ± 0.7	74.0 ± 10.2
Positive control group	9.1 ± 2.4	74.4 ± 10.4
Cz 250 mg/kg	11.9 ± 1.2	67.8 ± 10.1
Cz 500 mg/kg	7.1 ± 0.8	78.0 ± 24.0
Atorvastatin 10 mg/kg	8.6 ± 0.9	60.8 ± 1.5
Cz 250 mg/kg & Atorvastatin10 mg/kg	8.0 ± 1.9	67.3 ± 7.5
Cz 500 mg/kg & Atorvastatin10 mg/kg	11.8 ± 4.3	61.3 ± 7.8
	p = 0.248, (p>0.05)	p = 0.191, (p>0.05)

cholesterol, triglycerides, LDL, and HDL levels, was determined using enzymatic kits (Roche Diagnostics, Germany). Liver function was evaluated by measuring alanine transaminase (ALT), aspartate transaminase (AST), and bilirubin levels, while renal function was assessed through serum urea and creatinine analysis to determine any potential nephrotoxic effects.

2.5 Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS (version 26.0, IBM Corporation, Armonk, NY, USA). Differences between groups were assessed using one-way ANOVA followed by Tukey's post hoc test for pairwise comparisons. A p-value of <0.05 was considered statistically significant.

2.6 Ethical Considerations

The study protocol was approved by the University Ethics Committee (UEC) (approval number EA-L3-01-IMS-2024-10-0028), adhering to the guidelines set forth by the National Institutes of Health (NIH) for the care and use of laboratory animals. All efforts were made to minimize animal suffering and reduce the number of animals used.

3. Results and Discussion

The administration of *Cinnamomum zeylanicum* (CZ) at a dose of 500 mg/kg significantly reduced blood glucose levels in streptozotocin-induced diabetic rats, as reflected by a decrease in blood glucose to 8.3 ± 14.4 mg/dL, compared to the positive control group with blood glucose levels of 24.7 ± 15.7 mg/dL (Table 1). This reduction in blood glucose supports the well-documented hypoglycemic effects of cinnamon, particularly *Cinnamomum zeylanicum* (Ranasinghe et al., 2012). The mechanism underlying these effects is likely associated with bioactive compounds such as cinnamaldehyde, which has been shown to enhance insulin sensitivity and glucose metabolism in diabetic models (Cortez-Navarrete et al., 2023). These findings are consistent with previous studies that observed improved glucose homeostasis following the administration of cinnamon or its extracts in diabetic animal models (Lee et al., 2003).

In addition to glucose regulation, CZ at 500 mg/kg also exhibited significant improvements in lipid profile parameters. CZ administration resulted in notable reductions in total cholesterol (0.5 ± 0.7 mmol/L), triglycerides (0.4 ± 0.5 mmol/L), and low-density lipoprotein (LDL) cholesterol (0.1 ± 0.1 mmol/L), without affecting high-density lipoprotein (HDL) levels (Table 2) (Figures 2, 3, 4, 5). These findings align with prior research that demonstrated the hypolipidemic effects of cinnamon in hyperlipidemic animal models (Beheshti et al., 2024). The potential lipid-lowering mechanism of cinnamon is linked to its ability to modulate lipid metabolism, likely through regulation of genes involved in lipid biosynthesis and the antioxidant activity that reduces oxidative stress. The ability of CZ to maintain HDL levels, often referred to as "good" cholesterol, further emphasizes its favorable lipid profile, which is crucial for cardiovascular health (Grundy et al., 2018).

The combination of CZ and atorvastatin, though showing moderate reductions in glucose and lipid levels, was less effective than CZ alone. This suggests that atorvastatin, a statin drug that inhibits HMG-CoA reductase and thereby reduces cholesterol synthesis (Nawrocki et al., 1995), does not exhibit synergistic effects with CZ to the extent that might be expected. While atorvastatin effectively lowers cholesterol through its action on LDL receptors, the additional benefits provided by CZ, such as its antioxidant and anti-inflammatory properties, may contribute to its superior efficacy when used independently (Pezzani et al., 2019). Thus, while atorvastatin remains a standard treatment for hyperlipidemia, the results suggest that CZ could serve as a complementary agent for improving glucose and lipid control in diabetic patients.

Regarding liver function, the positive control group showed elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, indicative of liver damage. However, these elevations were significantly mitigated in the CZ-treated group (Table 3). These results suggest that CZ has hepatoprotective properties, a finding supported by previous studies which noted that cinnamon extract prevented liver damage in hyperlipidemic and diabetic models (Abdelgadir et al., 2020). This hepatoprotective effect is likely due to the antioxidant properties of cinnamon, which can neutralize free radicals and reduce oxidative stress within liver tissues (Ahmad et al., 2015).

Renal function parameters, including urea and creatinine levels, remained stable across all treatment groups (Table 4), suggesting that CZ does not induce nephrotoxicity, further supporting its safety profile as a therapeutic agent. This is a crucial aspect, as many diabetic treatments can have adverse effects on kidney function. The absence of nephrotoxicity observed in this study highlights CZ's potential for long-term use in diabetic patients without the risk of kidney damage, which is often a concern with other pharmacological treatments (Qamar et al., 2023).

4. Conclusion

In conclusion, the findings of this study suggest that *Cinnamomum zeylanicum* (CZ) has significant anti-hyperglycemic and anti-hyperlipidemic effects, making it a promising adjunct therapy for managing diabetes and related metabolic disorders. CZ's ability to reduce blood glucose and lipid levels, alongside its hepatoprotective and non-nephrotoxic properties, supports its potential clinical application. Future studies with longer treatment durations and larger sample sizes are necessary to confirm the long-term efficacy and safety of CZ, as well as to explore its synergistic potential with other therapeutic agents such as atorvastatin.

Author contributions

S.R., H.A.A., S.R.M., A.S., and R.T. contributed to the conceptualization and design of the study. N.A.B.Y. and I.A. were responsible for methodology development and data collection. S.M.A., J.K., and N.S.K. performed data analysis and interpretation. N.A.B.Y. and S.M.A. drafted the manuscript, while all authors reviewed and approved the final version.

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Competing financial interests

The authors have no conflict of interest.

References

Alsoodeeri, F. N., Alqabbani, H. M., & Aldossari, N. M. (2020). Effects of cinnamon (Cinnamomum cassia) consumption on serum lipid profiles in albino rats. Journal of Lipids. https://doi.org/10.1155/2020/8469830

Ahmad, I., Hussain, N., & Amjad, M. (2015). Therapeutic potential of cinnamon in diabetes management. Phytotherapy Research, 29(6), 871-881.

Beheshti, A. S., Qazvini, M. M., Abeq, M., Abedi, E., Fadaei, M. S., Fadaei, M. R., Rahimi, V. B., & Askari, V. R. (2024). Molecular, cellular, and metabolic insights of cinnamon (*Cinnamomum zeylanicum*) advantages in diabetes and related complications: Condiment or medication? Naunyn-Schmiedeberg's Archives of Pharmacology. https://doi.org/10.1007/s00210-024-03644-0

Cortez-Navarrete, M., Pérez-Rubio, K. G., & Escobedo-Gutiérrez, M. de J. (2023). Role of cinnamon in type 2 diabetes treatment. Pharmaceuticals, 16(4), 515.https://doi.org/10.3390/ph16040515

Grundy, S. M., Stone, N. J., Bailey, A. L., Beam, C., Birtcher, K. K., Blumenthal, R. S., … & Wilson, P. W. (2018). 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: A report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. Retrieved from https://www.ahajournals.org/doi/10.1161/CIR.0000000000000625

Khoshnoud, M. J., Sabahi, Z., Moein, M., Rashedinia, M., & Pourshahsavari, S. (2019). Attenuation of hyperlipidemia in diabetic and Triton X-100 induced hyperlipidemic rats by Thymus daenensis Celak extract. DOAJ (Directory of Open Access Journals). https://doi.org/10.30476/tips.2019.81594.1001

Lee, J.-J., Yi, H.-Y., Yang, J.-W., Shin, J.-S., Kwon, J.-H., & Kim, C.-W. (2003). Characterization of streptozotocin-induced diabetic rats and pharmacodynamics of insulin formulations. Bioscience, Biotechnology, and Biochemistry, 67(11), 2396-2401. Retrieved from https://www.jstage.jst.go.jp/article/bbb/67/11/67112396/_pdfhttps://doi.org/10.1271/bbb.67.2396

Nawrocki, J. W., Weiss, S. R., Davidson, M. H., Sprecher, D. L., Schwartz, S. L., Lupien, P., Jones, P. H., Haber, H. E., & Black, D. M. (1995). Reduction of LDL cholesterol by 25% to 60% in patients with primary hypercholesterolemia by atorvastatin, a new HMG-CoA reductase inhibitor. Arteriosclerosis Thrombosis and Vascular Biology, 15(5), 678-682. https://doi.org/10.1161/01.ATV.15.5.678

Nurinda, E., et al. (2022). Antidiabetic activity of cinnamon bark and ginger extract. Open Access Macedonian Journal of Medical Sciences, 10(8), 1-6.https://doi.org/10.3889/oamjms.2022.9462

Padugupati, S., Ramamoorthy, S., Thangavelu, K., Sarma, D. S., & Jamadar, D. (2021). Effective dose of streptozotocin to induce diabetes mellitus and variation of biophysical and biochemical parameters in albino Wistar rats. Journal of Clinical and Diagnostic Research, 15(6), 1-5. Retrieved from https://doi.org/10.7860/JCDR/2021/46729.15567

Pezzani, R., Salehi, B., Vitalini, S., Iriti, M., Zuñiga, F. A., Sharifi-Rad, J., Martorell, M., & Martins, N. (2019). Synergistic effects of plant derivatives and conventional chemotherapeutic agents: An update on the cancer perspective. Medicina (Kaunas), 55(4), 110.https://doi.org/10.3390/medicina55040110

Qamar, F., Sultana, S., & Sharma, M. (2023). Animal models for induction of diabetes and its complications. Journal of Diabetes & Metabolic Disorders, 22, 1021-1028. Retrieved from https://doi.org/10.1007/s40200-023-01277-3

Ranasinghe, P., Perera, S., Gunatilake, M., Abeywardene, E., Gunapala, N., Premakumara, S., Perera, K., Lokuhetty, D., & Katulanda, P. (2012). Effects of *Cinnamomum zeylanicum* (Ceylon cinnamon) on blood glucose and lipids in a diabetic and healthy rat model. Pharmacognosy Research, 4(2), 73-79.https://doi.org/10.4103/0974-8490.94719