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Article

Modulation of Metabolic Pathways and Protection against Cadmium-Induced Disruptions with Taxifolin-Enriched Extract

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Cite This: *ACS Omega* 2024, 9, 4057−4072

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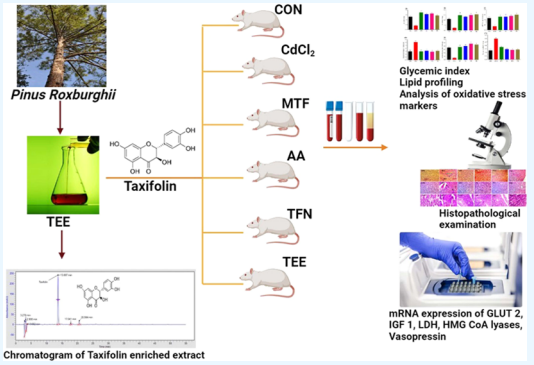
ABSTRACT: Cadmium, a ubiquitous environmental pollutant, has been implicated in the disruption of various metabolic pathways, contributing to the development of insulin resistance, glucose intolerance, and associated metabolic disorders. This study aimed to investigate the cadmium chloride ($CdCl_2$) exposure on metabolic pathways and to assess the potential therapeutic efficacy of the taxifolin-enriched extract in mitigating these disruptions by modulating biochemical pathways. Taxifolin-enriched extract (TEE) was prepared from *Pinus roxburghii* bark using a green extraction method. About 60 Wistar albino rats were divided into six groups: the control group (n = 10), the $CdCl_2$ group (30 mg/kg) (n = 10), and four groups (each comprises n = 10) treated with 30 mg/kg $CdCl_2$ in combination with metformin (100 mg/kg), ascorbic acid, taxifolin (30 mg/kg), and TEE (30 mg/kg), respectively. After the treatment period of 1 month, a comprehensive assessment of metabolic biomarkers and gene expressions that regulate the metabolism of carbohydrates and lipids was conducted to evaluate the impact of $CdCl_2$ exposure and the potential protective effects of TEE. The results revealed that $CdCl_2$ exposure significantly increased ($P < 0.001$) serum levels of α-glucosidase, α-amylase, insulin, G6PC, hexokinases, TGs, LDL, HMG-CoA reductase, and pro-inflammatory cytokines such as IL-6 and TNF-α. Conversely, $CdCl_2$ exposure led to a reduction in HDL, antioxidant enzyme levels, phosphofructokinases, and glucose-6-phosphatase dehydrogenase. However, the administration of TEE alongside $CdCl_2$ substantially mitigated ($P < 0.001$) these fluctuations in metabolic and inflammatory biomarker levels induced by $CdCl_2$ exposure. Both TEE and taxifolin treatment effectively lowered the elevated levels of α-amylase, α-glucosidase, G6PC, insulin, TGs, HMG-CoA reductase, leptin, ALT, AST, blood urea nitrogen, creatinine, and pro-inflammatory cytokines while simultaneously enhancing levels of HDL cholesterol and antioxidant enzymes. Moreover, $CdCl_2$ exposure suppressed mRNA expression of critical metabolic biomarkers such as glucose transporter 2 (GLUT2), insulin-like growth factor 1 (IGF-1), lactate dehydrogenase, and HMG-CoA lyases while upregulating the mRNA expression of angiotensin receptor 2 and vasopressin, key metabolic biomarkers involved in glucose metabolism and insulin regulation. TEE demonstrated the potential to restore normal metabolic functions and reduce the adverse impacts caused by $CdCl_2$ exposure by mitigating disturbances in several metabolic pathways and restoring gene expression of critical metabolic biomarkers related to glucose metabolism and insulin regulation. Nevertheless, further investigation is warranted to comprehensively understand the underlying mechanisms and optimize the appropriate dosage and duration of TEE treatment for achieving the most effective therapeutic outcomes.



INTRODUCTION

The increasing concern regarding the toxicology of heavy metals has raised significant apprehension across various ecosystems on Earth.[1] Among these heavy metals, cadmium (Cd) is particularly noteworthy as a nonessential toxic element, often referred to as the "metal of the 20th century".[1,2] Cd finds extensive use in a diverse range of industrial applications, including galvanization, electroplating, battery manufacturing, electrical conduction, alloy formulation, pigment and plastic production, and phosphate fertilizer stabilization, thus making its omnipresence a source of considerable environmental apprehension. Cd is released into the environment as a byproduct of smelting operations, leading to its widespread contamination.[3] The human population primarily encounters cadmium through dietary intake, smoking, and to a lesser extent drinking water. Despite the absence of any recognized physiological role,

Received: November 14, 2023
Revised: December 21, 2023
Accepted: December 27, 2023
Published: January 9, 2024

emerging evidence suggests that Cd may function as a potent metallohormone.[4,5]

Cd has emerged as a prominent ecological pollutant, disseminating in both atmospheric and aquatic systems. The 20th century witnessed a disturbing increase in heavy metal toxicity.[6] The extensive environmental distribution of cadmium has heightened concerns regarding its disproportionate toxicity and adverse ecological consequences.[6,7] Notably, Cd accumulates significantly in hepatic and renal tissues, initiating severe cellular damage across multiple organ systems.[8,9] Cd finds widespread application in various industrial sectors, including paint manufacturing, silver-plating, battery production, and agricultural practices.[6] Cigarette smoke is a well-recognized source of Cd exposure, significantly elevating the risk of contact.[10] Consequently, public exposure to Cd released into the environment due to its diverse applications poses substantial health hazards.[4] Cd's deleterious effects extend to soft tissues and cellular structures, including the liver and kidneys.[11,12] Despite being primarily known for its nephrotoxicity, mounting evidence links cadmium exposure to the onset of type 2 diabetes mellitus (T2DM) and/or prediabetes.[13] Experimental studies involving both short-term and long-term *in vivo* Cd exposure have indicated its potential to induce hyperglycemia and disrupt glucose homeostasis.[13] Notably, individuals with elevated urinary cadmium levels tend to exhibit significantly lower fasting serum insulin levels compared to those without occupational Cd exposure.[14] Mechanisms through which Cd potentially influences glucose homeostasis encompass alterations in glucose transporter expression in adipocytes[15] as well as increased renal and hepatic gluconeogenesis.[16] These collective findings underscore Cd's multifaceted impact across various tissues, contributing to disrupted glucose homeostasis. The consumption of fruits and vegetables has been linked to a plethora of health benefits, attributed to their rich assortment of bioactive compounds.[15] Among these compounds, flavonoids, especially taxifolin, have emerged as pivotal players in mitigating various human ailments, including cancer, inflammation, cardiovascular diseases, and neurodegenerative disorders. Flavonoids, such as taxifolin, exhibit remarkable pharmacological actions and possess anticancer properties, including antiangiogenic effects, modulation of cytochrome P450 enzymes, neutralization of reactive oxidative species (ROS), and induction of apoptosis.[17] Pine trees, notably *Pinus roxburghii* (*P. roxburghii*), indigenous to the Himalayan region, hold significant ecological and pharmacological importance.[18] As members of the Pinus genus and Pinaceae family, these evergreen conifers make substantial contributions to their ecosystems.[19] Taxifolin, a flavonoid found in these trees, has garnered attention due to its broad pharmacological actions, including its potential to counteract angiogenesis, influence cytochrome P450 enzymes, modulate P-glycoprotein, mitigate ROS effects, regulate the cell cycle, and induce apoptosis.[20]

We hypothesized that exposure to cadmium chloride ($CdCl_2$), a pervasive environmental pollutant, induces impaired carbohydrate metabolism, insulin resistance, and diabetes, while the taxifolin-enriched extract from *P. roxburghii* holds therapeutic potential to counteract these effects. With the alarming rise in diabetes cases and its associated health burdens, understanding the impact of environmental pollutants like cadmium on metabolic health is crucial. Our aim was to rigorously investigate taxifolin's protective properties against $CdCl_2$-induced metabolic disturbances, offering insights into mechanisms related to glucose metabolism, oxidative stress, and inflammation. We further expanded our study objectives to assess the effects of $CdCl_2$ on metabolic parameters, elucidate taxifolin's therapeutic mechanisms, evaluate tissue histopathology, and examine gene expression patterns.

MATERIALS AND METHODS

Chemicals and Reagent. $CdCl_2$ (purchased from Sigma-Aldrich), Taxifolin (sourced from Macklin Biochemical Co., China), Metformin, ascorbic acid, and normal saline were employed as chemical reagents in this study. Additionally, a Cybergreen master mix (WizPure, obtained from Wizbiosolutions, Korea), a cDNA kit (WizScript, also provided by Wizbiosolutions, Korea), primers (supplied by Thermo Fisher Scientific), and ELISA kits for the measurement of antioxidant enzymes (acquired from Elabscience ELISA kit) were utilized. Analytical-grade chemicals were employed exclusively throughout all of the experimental procedures.

Preparation of Taxifolin-Enriched Extract. A taxifolin-enriched extract (TEE) was meticulously prepared following a previously established methodology.[21] In a concise overview, the initial step involved the extraction of a crude sample using an environmentally friendly green extraction method, which involved soaking the source material in ethyl acetate. Subsequently, silica gel column chromatography was employed to facilitate fractionation of the obtained extract. The fractions thus obtained were subjected to further purification through a combination of column chromatography and preparative thin-layer chromatography (prep-TLC) techniques. This multistep procedure ensured the isolation and enrichment of taxifolin from the original source material with a high degree of precision and purity.

HPLC Analysis of Taxifolin-Enriched Extract. The taxifolin-enriched extract was analyzed by using HPLC with a binary gradient solvent system. The liquid chromatography setup included a C18 column (250×4.6 mm^2 internal diameter) with a 5 μm film thickness and an oven set at 30 °C. A Chromera HPLC system from PerkinElmer, equipped with a Flexar Binary LC pump and a UV/vis LC Detector (Shelton, CT), controlled by software version 4.2.6410, was employed for data analysis. The mobile phase consisted of solvent A (acetonitrile/methanol, 70:30) and solvent B (double-distilled water with 0.5% glacial acetic acid). UV spectra were recorded at 275 nm. Analyte identification was achieved by matching retention times and spiking samples with standards, and quantification was performed using the external standard method as described previously.[22]

Animals and Treatment Protocols. The experiments involving laboratory animals were conducted in strict accordance with the guidelines and approval of the Ethical Review Committee of Government College University, Faisalabad, Pakistan (GCUF/ERC/35). A total of 60 albino rats, comprising both male and female individuals with an average body weight ranging from 150 to 200 g, were utilized in the study. These rats were housed in dedicated cages within the animal facility located in the Faculty of Pharmaceutical Sciences at Government College University, Faisalabad, Pakistan. During the acclimatization phase, the rats were allowed to adapt to their environment, which was maintained at a temperature of 25 ± 5 °C, with humidity levels ranging from 45% to 70%. The photoperiod followed a standard 12 h light and 12 h dark cycle. The rats were provided with a standard rodent diet during this period. The experimental groups were defined as follows:

https://doi.org/10.1021/acsomega.3c08989
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Group 1: Named CON, they received oral administration of normal saline.

Group 2: Named as $CdCl_2$, it was treated with $CdCl_2$ at a dose of 30 mg/kg of body weight to induce metabolic impairment.

Group 3: Named as MTF, treated with metformin at a dose of 100 mg/kg of body weight 1 h before the administration of $CdCl_2$ (30 mg/kg).

Group 4: Named as AA, treated with ascorbic acid (AA) at a dose of 100 mg/kg 1 h before the administration of $CdCl_2$ (30 mg/kg).

Group 5: Named as TFN, treated with taxifolin (TFN) at a dose of 30 mg/kg, also 1 h before the administration of $CdCl_2$ (30 mg/kg).

Group 6: Named as TEE, treated with taxifolin-enriched extract at a dose of 30 mg/kg of body weight 1 h before the administration of $CdCl_2$ (30 mg/kg).

Collection of Blood Samples. The collected blood samples were left undisturbed to undergo coagulation at room temperature for a duration of 20 min. Subsequently, the blood specimens were subjected to centrifugation at 3000*g* for 15 min. The resulting serum obtained post centrifugation was carefully preserved and stored at a temperature of −20 °C until it was ready for subsequent analytical procedures.

Effect of Treatment on Glycemia. Hyperglycemia is a physiological state characterized by an elevated concentration of glucose in the circulatory system. It serves as a pivotal indicator of the presence of DM. To monitor the blood glucose levels in all experimental animal groups, a glucometer (specifically, the Accu-Chek Performa model) was employed to measure the glucose concentration twice a week.

Assessment of Glucose Tolerance. A subgroup of 3−4 animals was systematically selected from each experimental group and housed individually in cages, with the intention of conducting an oral glucose tolerance test (OGTT). The OGTT was carried out 2 days prior to the conclusion of the cadmium exposure period. The chosen animals were subjected to a fasting period, refraining from both food and water consumption for a period spanning 4−6 h. Following this fasting interval, a baseline blood sample was obtained from each animal (0 min). Subsequently, an oral glucose challenge was administered via gavage, wherein an anhydrous glucose solution was delivered at a dosage of 1.75 g/kg of body weight.[23] Blood samples were collected at specific time intervals, namely, 30, 60, 90, and 120 min postglucose administration. Each blood sample consisted of 100 μL and was obtained from the tail veins of the animals. These collected samples were then subjected to centrifugation for a duration of 10 min at 400*g*. The resultant serum was carefully isolated and subsequently stored at a temperature of −70 °C, pending further analysis to determine the glucose and insulin levels.

Assessment of Insulin Tolerance. A subset of animals, specifically 3−4 individuals from each experimental group, were chosen to undergo an insulin tolerance test (ITT). These mice were subjected to a period of fasting, abstaining from food intake for a duration of 4−6 h. The subsequent ITT was conducted at 10 am without the use of anesthesia to maintain physiological condition. Basal glycemia was assessed by collecting blood samples from the tail vein at the commencement of the experiment (0 min). Following the baseline measurements, insulin was administered intraperitoneally at a dosage of 1 U/kg of body weight. Blood samples were subsequently collected at specified time intervals, including 0, 30, 60, 90, and 120 min postinsulin administration, with each sample being obtained from the tail vein. To evaluate insulin sensitivity, the glucose disappearance constant (kITT) was determined. This constant was calculated by performing a linear regression analysis on the Napierian logarithm of the glycemic values acquired during the 0−120 min period of the test.

Assessment of Pyruvate Tolerance. An additional cohort of 3−4 animals was incorporated into the study and subjected to the Pyruvate tolerance test (PTT). In this experimental protocol, the mice were subjected to a fasting period lasting 12 h and the subsequent test was conducted at 8 AM. To ensure the animals' comfort and compliance, anesthesia was administered using sodium pentobarbital at a dose of 60 mg/kg of body weight, delivered intraperitoneally. Following the induction of anesthesia, a pyruvate solution with a concentration of 0.25 g/mL was administered intraperitoneally to the animals at a dosage of 2 g/kg of body weight. Glycemia levels were monitored by collecting blood samples from the tail vein at multiple time points, including baseline (0 min) and subsequently at 30, 60, 90, and 120 min following the pyruvate administration. To assess hepatic glucose production, the area under the curve of glycemia following the pyruvate challenge was calculated using GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA).[24] This analysis provided insights into the animals' response to pyruvate and their hepatic glucose production capacity.

Estimation of Carbohydrate-Metabolizing Enzymes. The serum levels of carbohydrate-metabolizing enzymes, specifically α-glucosidase (Catalog Number: E-EL-R1083, Elabscience), α-amylase (Catalog Number: E-EL-R2544, Elabscience), skeletal muscle hexokinase (Catalog Number: E-EL-RR0502, Elabscience), glucose-6-phosphate dehydrogenase (G6PDH) (Catalog Number: E-BC-K056-M, Elabscience), liver tissue phosphofructokinase (Catalog Number: E-BC-K612-M, Elabscience), and liver tissue glucose-6-phosphatase (G6PC) (Catalog Number: E-EL-M1362, Elabscience), were quantified utilizing the ELISA method. The measurements were conducted using a Microplate ELISA reader (Bio Tek Instruments, Inc.).

Estimation of Antioxidant Capacity. Liver tissues were promptly excised and subsequently homogenized in phosphate-buffered saline (PBS) at a pH of 7.4. Following homogenization, centrifugation was carried out and the resulting supernatant was collected for the assessment of various biochemical parameters. The activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in the tissue were determined in accordance with the manufacturer's instructions, utilizing commercially available assay kits.

Estimation of Biomarkers of Lipid Profile. To assess the influence of heavy metal exposure on dyslipidemia, several key lipid control biomarkers, including high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TGs), were quantified from serum samples. The quantification of HMG-CoA reductase, a hepatic enzyme involved in cholesterol biosynthesis, was conducted utilizing a microplate ELISA reader (BioTek Instruments, Inc.) in accordance with the manufacturer's instructions and a commercially available ELISA kit (Catalog Number: E-EL-H2472, Elabscience).

Estimation of Inflammatory Biomarkers. The serum concentrations of inflammatory biomarkers, namely, interleukin-6 (IL-6) (Catalog Number: E-EL-R0015, Elabscience) and tumor necrosis factor-α (TNF-α) (Catalog Number: E-EL-R0019, Elabscience), as well as adipokines, specifically, adiponectin (Catalog Number: E-BC-K013-S, Elabscience) and leptin (Catalog Number: 201905, Elabscience), were

https://doi.org/10.1021/acsomega.3c08989
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Table 1. List of Primer Sequences with Accession Numbers Used in Gene Expression Analysis

primer name	primer type	sequencing requirement	accession number
insulin-like growth factor 1 (IGF-1)	forward	GCTCCAAAGCAGACAAAATACCC	XM_0391022225.1
	reverse	GGTCTGGGCACAAAGATGGA	
glucose transporter 2 (GLUT2)	forward	GCAGCCTTGGTTAAGAAGGTCA	XM_039101783.1
	reverse	CTTCTGACATGTTGCGTGCG	
lactate dehydrogenase	forward	TCCTCAGCGTCCCATGTATC	XM_039082293.1
	reverse	TCCATAGAAACCCTGCTGCA	
angiotensin II receptors	forward	ATCCAAGATGACTGCCCCAA	NM_031009.2
	reverse	CCACAGTGGCAAAGTCAACA	
HMG-COA lyases	forward	CTAAAGTTGCTGAGGTCGCC	XM_039110832.1
	reverse	GCTTGGCCATAGGTGTCATG	
vasopressin	forward	GTTGCTGGCTTCCTTGAACA	NM_053019.2
	reverse	TGGGCTCCGGTTGTTAGAAT	

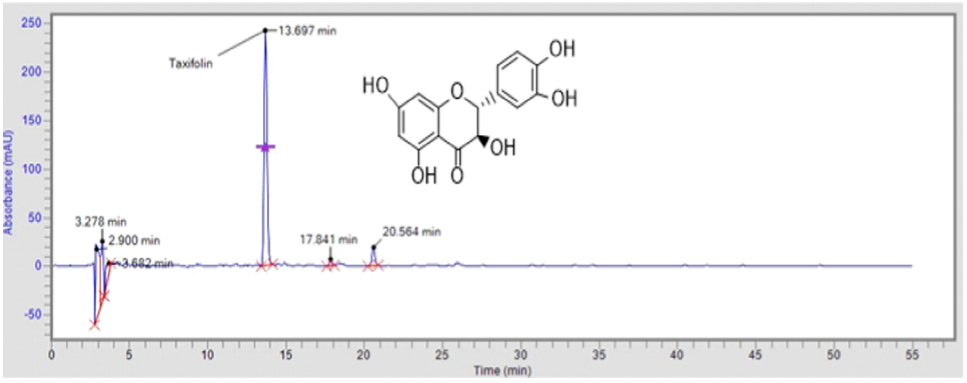

Figure 1. Chromatogram of the Taxifolin-enriched extract.

quantified using commercially available ELISA kits. These measurements were conducted with the aid of microplate ELISA.

Estimation of Gene Expression Involved in Carbohydrate and Lipid Metabolism. Following the homogenization of liver tissue, total RNA extraction was carried out employing Trizol reagent (Thermo Fisher Scientific, MA) following the manufacturer's guidelines. The quantification of the total RNA yield was performed using a NanoDrop 2000c spectrophotometer (Thermo Scientific) at an absorbance of 260 nm. Furthermore, the quality of RNA was assessed by visual examination on a 1% agarose gel. Subsequently, 2 μg of total RNA was subjected to reverse transcription utilizing the RevertAid cDNA synthesis kit from Thermo Fisher Scientific. The amplification process was executed using the Applied Biosystems Real-Time PCR System, with a final reaction volume of 20 μL. The expression levels of the specified genes (Table 1) were analyzed during the polymerase chain reaction (PCR). For the PCR procedure, the denaturation step was carried out at 95 °C for a duration of 15 s for all three genes. This was followed by 40 cycles consisting of denaturation at 95 °C for 5 s and annealing/extension at 60 °C for 30 s.

Histopathological Examination. Small segments of freshly excised liver, pancreas, and kidney tissues were promptly submerged in a 10% formalin solution and subsequently embedded in paraffin wax to facilitate morphological studies. The paraffin-embedded sections were subjected to staining using hematoxylin and eosin. Each prepared slide was thoroughly examined under a light microscope to conduct specific morphological assessments.

RESULTS

HPLC Analysis of Taxifolin-Enriched Extract. Methanol underwent column chromatography fractionation to isolate taxifolin and subsequent confirmation of taxifolin presence and purity using thin-layer chromatography (TLC). Quantification of taxifolin concentration was accomplished through high-performance liquid chromatography (HPLC) with UV/vis detection, revealing a concentration of 0.16 mg/mL (Figure 1). This process ensures the extraction, isolation, and precise measurement of taxifolin, making it a valuable resource for potential pharmaceutical applications or research endeavors.

Effect of Treatment on Body Weight and Glycemic Markers. The body weight of the animals was assessed on a weekly basis both prior to the initiation of the treatment, during the treatment period, and upon its completion. It was observed that the administration of $CdCl_2$ significantly elevated ($P < 0.001$) the body weight of animals in the treated group when compared to the CON animals (Figure 2A). However, the treatment with TEE led to a noticeable decline ($P < 0.05$) in body weight within the designated groups as compared to the $CdCl_2$-treated group (Figure 2A). To investigate the impact of the treatment on glycemia, blood glucose levels were measured before, during, and after the treatment regimen. Fasting and random blood glucose measurements for all animals in their respective groups revealed that $CdCl_2$ administration significantly increased fasting blood glucose levels ($P < 0.001$) and random blood glucose levels ($P < 0.001$) in comparison to the CON group (Figure 1B,C). However, it became evident that during the final week of the experiment, AA, TFN, and TEE induced a significant progressive hypoglycemic effect when compared to that of the $CdCl_2$-treated group, as reflected in both

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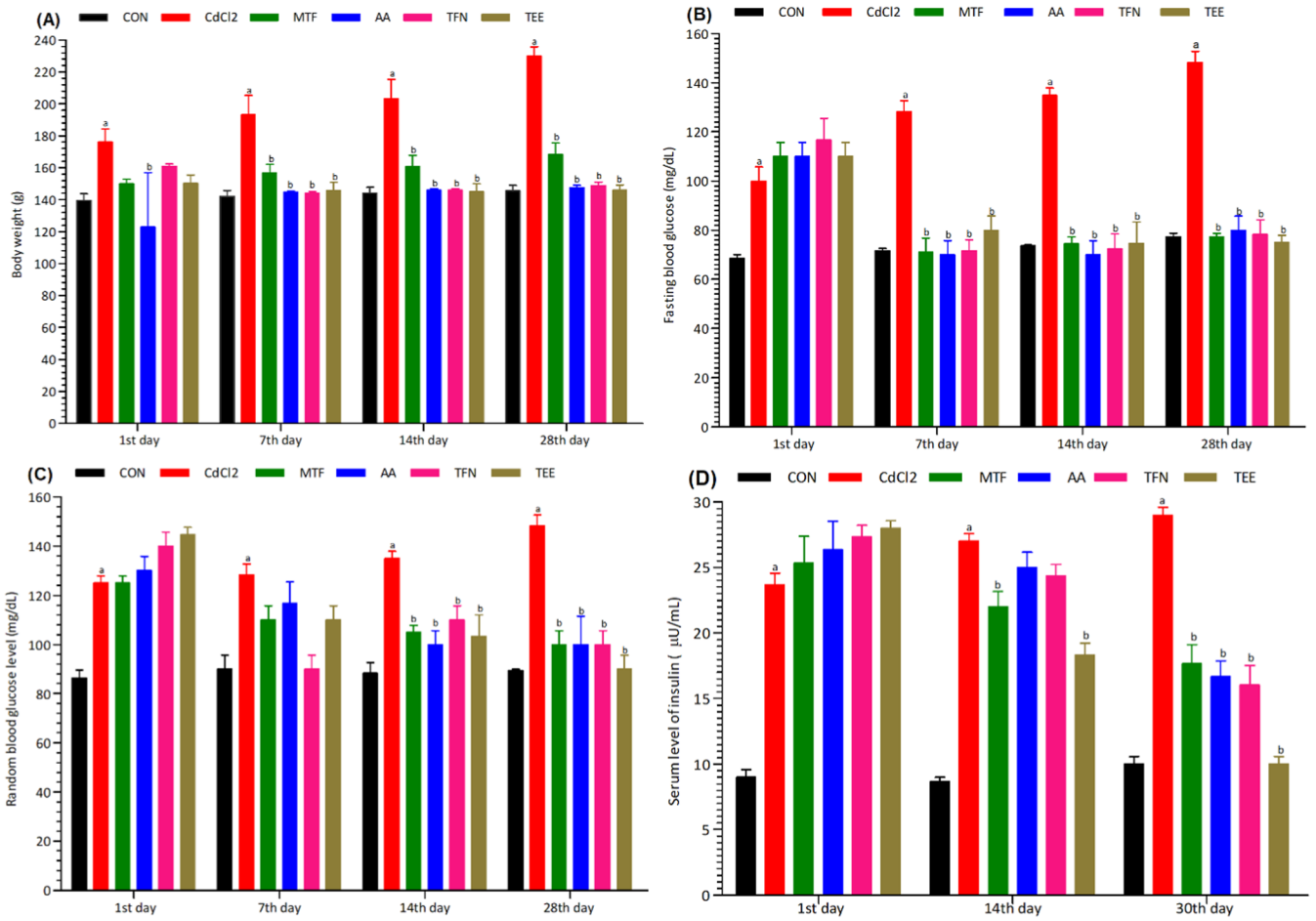

Figure 2. Evaluation of the impact of treatment and $CdCl_2$ on (A) body weight, (B) fasting blood glucose, (C) random blood glucose, and (D) serum insulin levels. The data is presented as mean ± SD. Significance levels were determined using a two-way ANOVA with Bonferroni post-test for multiple comparisons. In the notation used, "a" denotes a significant difference when compared to the control group, while "b" indicates a significant difference when compared to the $CdCl_2$-treated group.

fasting and random blood glucose levels (Figure 2B,C). Furthermore, the serum levels of insulin were measured before the commencement of the experiment, during the treatment period, and upon its completion. It was observed that $CdCl_2$ treatment significantly increased the serum insulin levels ($P < 0.001$) in the $CdCl_2$-treated group when compared to the CON group (Figure 1D). However, during the course of treatment, the serum insulin levels in the TFN and TEE groups exhibited a significant decrease ($P < 0.001$) in comparison to the $CdCl_2$-treated group (Figure 2D).

Effect of Treatment on Glucose Tolerance. In the last week, just before the completion of the treatment period, OGTT was conducted to assess the influence of TFN and TEE on blood glucose levels following an overnight fasting period. Prior to the administration of a defined quantity of glucose based on the animals' body weight, fasting blood sugar and serum insulin levels were determined. Subsequently, blood glucose levels were measured at predetermined time intervals of 30, 60, 90, and 120 min. The results revealed that the $CdCl_2$-treated group exhibited the highest blood glucose levels ($P < 0.001$) after 30 min, and these elevated levels persisted up to the 120 min mark, in contrast to the CON-, AA-, TFN-, and TEE-treated groups (Figure 3A). Conversely, the treatment with TEE, TFN, and AA demonstrated a progressive hypoglycemic effect ($P < 0.001$) when compared to the $CdCl_2$-treated group (Figure 3A).

Effect of Treatment on Insulin Sensitivity and Resistance. In the final week of the experiment, just before its completion, an insulin tolerance test was conducted. Following a fasting period of 4−6 h, animals received a predetermined quantity of insulin, and their blood glucose levels were monitored at 0, 30, 60, 90, and 120 min intervals. The results indicated that the $CdCl_2$-treated group exhibited significantly higher blood glucose levels ($P < 0.001$) compared to the CON group (Figure 2B). However, treatment with TEE markedly reduced blood glucose levels ($P < 0.001$) in the TEE group when compared to the $CdCl_2$-treated group. Additionally, it also led to a reduction in the area under the curve (AUC) (Figure 3B,D). To assess insulin resistance based on fasting blood glucose levels and serum insulin levels, the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated. It was evident that the $CdCl_2$-treated group exhibited significantly greater insulin resistance ($P < 0.001$) compared to the CON group (Figure 3C). However, treatment with TFN, TEE, and AA improved $CdCl_2$-induced insulin resistance in the TFN, TEE, and AA groups, respectively, similar to that in the MTF-treated animals in the MTF group (Figure 3C).

Effect of Treatment on Pyruvate Tolerance. To assess the impact of TEE on gluconeogenesis, animals underwent a fasting period of 6−8 h to deplete hepatic glycogen reserves. Following this fasting period, pyruvate was administered as a

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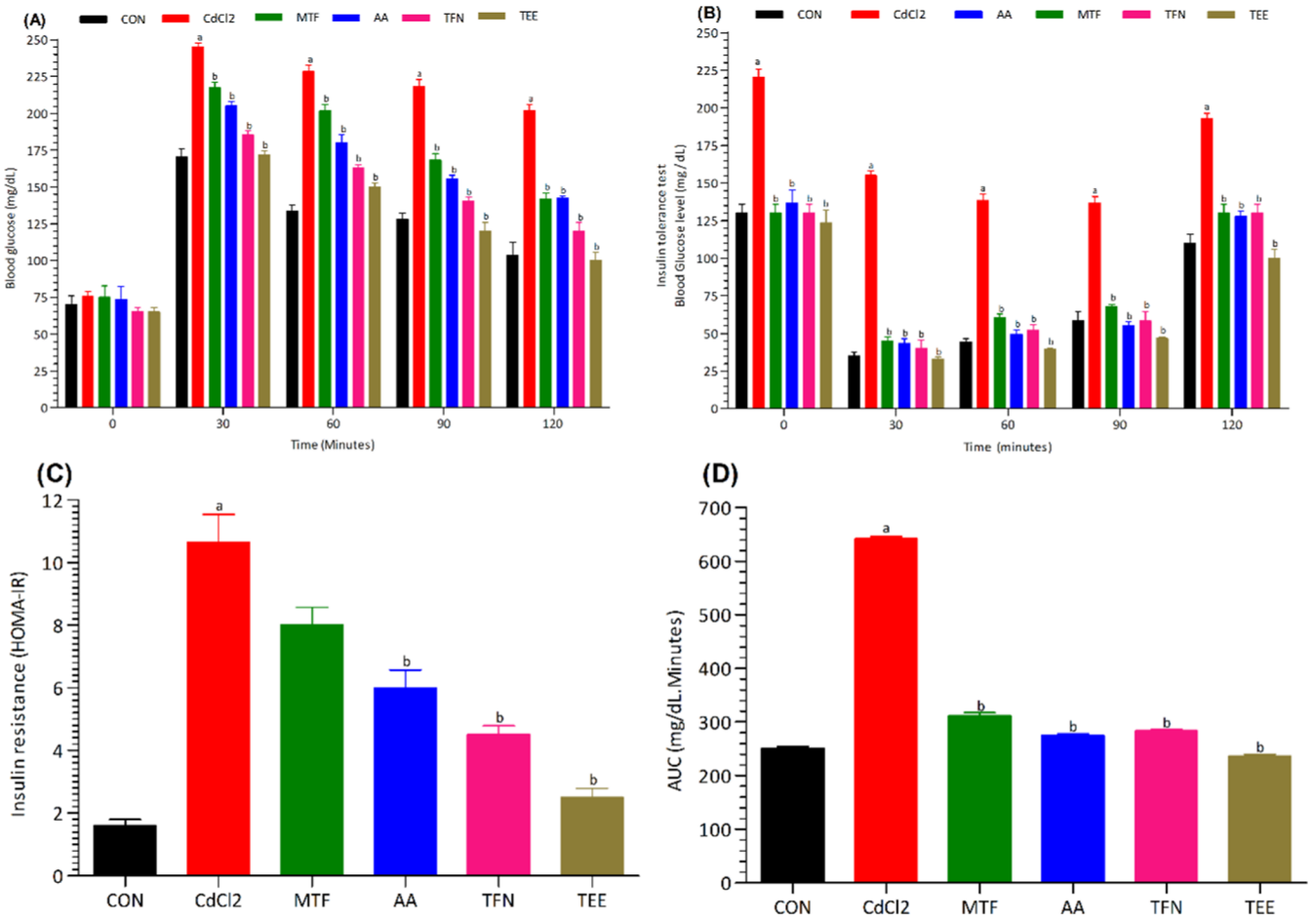

Figure 3. Impact of treatment and $CdCl_2$ on (A) blood glucose level, (B) insulin tolerance test blood glucose level, (C) Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), and (D) area under the curve (AUC). Data are presented as mean ± SD. Significance levels were determined using a two-way ANOVA for (A, B) and one-way ANOVA for (C, D), followed by Bonferroni post-test for multiple comparisons. In the notation used, "a" indicates a significant difference when compared to the control group, while "b" denotes a significant difference when compared to the $CdCl_2$-treated group.

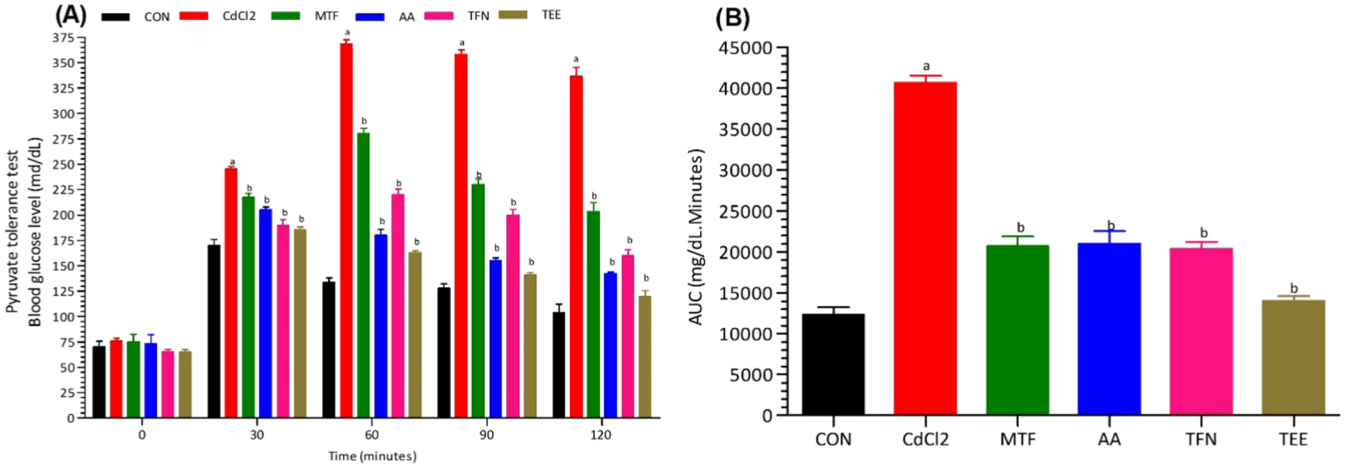

Figure 4. Impact of treatment and CdCl2 on (A) pyruvate tolerance test and (B) the area under the curve for the pyruvate tolerance test. Data is presented as mean ± SD. Significance levels were determined using (A) a two-way ANOVA and (B) a one-way ANOVA, followed by Bonferroni post-test for multiple comparisons. In the notation used, "a" indicates a significant difference when compared to the control group, while "b" denotes a significant difference when compared to the $CdCl_2$-treated group.

substrate to the animals. Pyruvate significantly elevated blood glucose levels in the $CdCl_2$-treated group after 30 min when compared to the CON group ($P < 0.001$) (Figure 4A). In the $CdCl_2$ group, blood sugar levels remained persistently elevated from 30 to 120 min and did not return to basal levels. However, treatment with TEE markedly decreased blood glucose levels to

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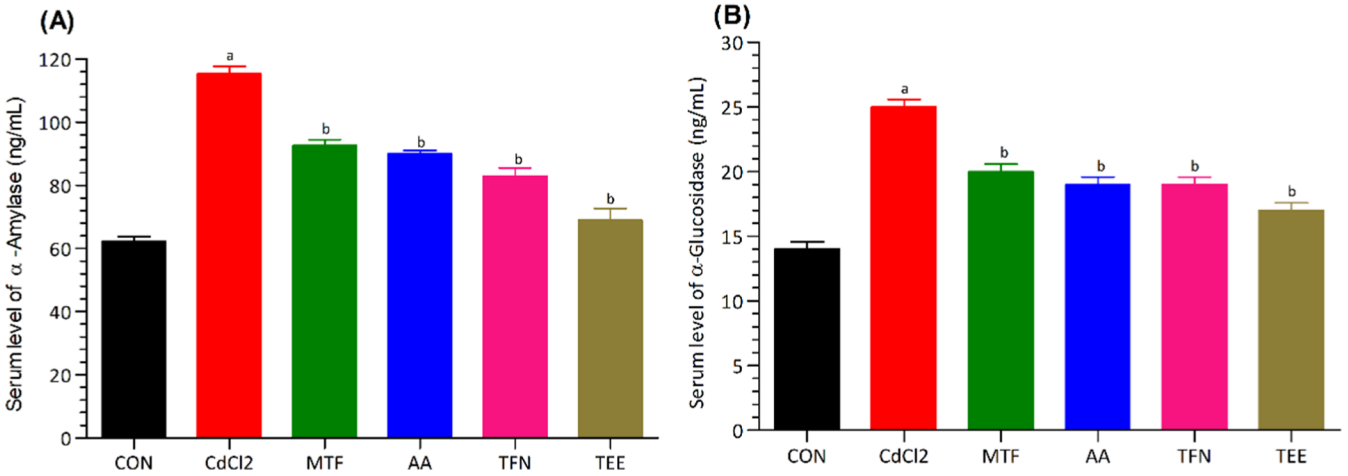

Figure 5. Impact of treatment and $CdCl_2$ on (A) α-amylase and (B) α-glucosidase. Data are presented as mean ± SD. Significance levels were determined using a one-way ANOVA, followed by Bonferroni post-test for multiple comparisons. In the notation used, "a" indicates a significant difference when compared to the control group, while "b" denotes a significant difference when compared to the $CdCl_2$-treated group.

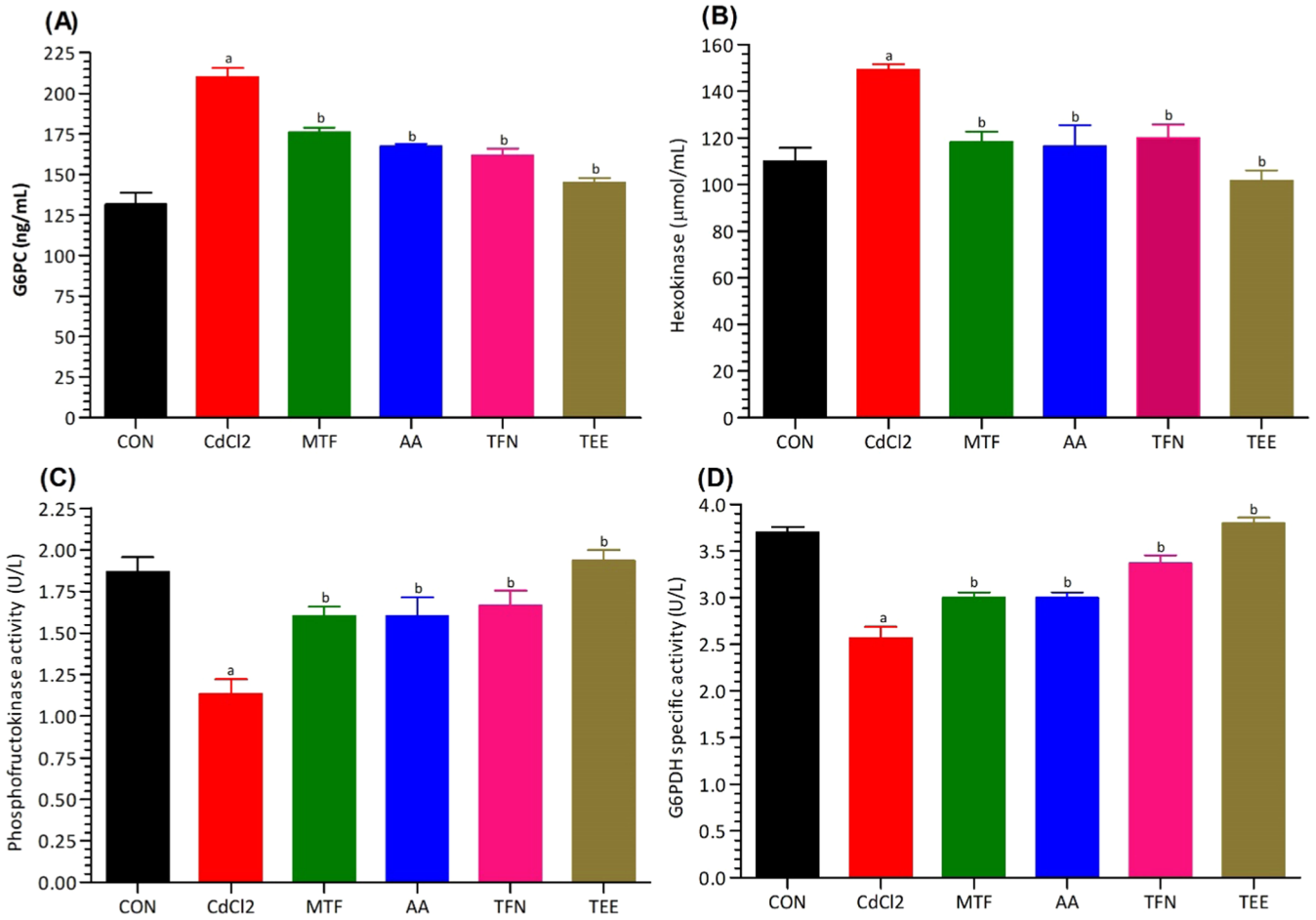

Figure 6. Impact of treatment and $CdCl_2$ on the serum levels of (A) glucose-6-phosphatase and (B) hexokinase, as well as on the activity of (C) phosphofructokinase and (D) glucose-6-phosphate dehydrogenase. Data is presented as mean ± SD. Significance levels were determined using a one-way ANOVA, followed by Bonferroni post-test for multiple comparisons. In the notation used, "a" indicates a significant difference when compared to the control group, while "b" denotes a significant difference when compared to the $CdCl_2$-treated group.

basal values after 120 min ($P < 0.001$) (Figure 4A). TFN, AA, and MTF also progressively decreased blood glucose levels after 120 min compared to the $CdCl_2$-treated group (Figure 4A). To further evaluate the effect of TEE on blood glucose levels, an analysis of the area under the curve (AUC) was performed. In the $CdCl_2$-treated group, the AUC was significantly higher compared to the CON group ($P < 0.001$) (Figure 4B). However, treatment with TEE significantly reduced both blood glucose levels and the AUC compared to the $CdCl_2$-treated group ($P < 0.001$) (Figure 4B). Similarly, TFN, AA, and MTF also

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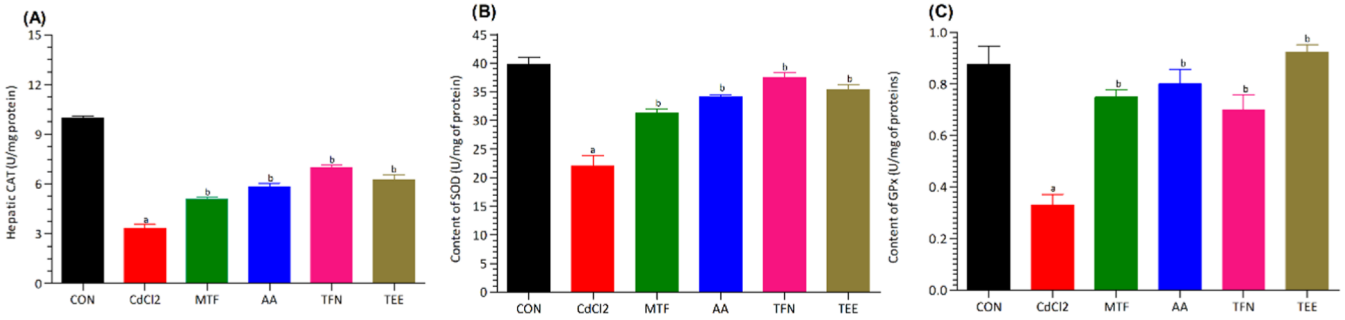

Figure 7. Impact of treatment and $CdCl_2$ on first-line antioxidant enzymes, namely, (A) catalase, (B) superoxide dismutase, and (C) glutathione peroxidase. Data is presented as mean ± SD. Significance levels were determined using a one-way ANOVA, followed by Bonferroni post-test for multiple comparisons. In the notation used, "a" indicates a significant difference when compared to the control group, while "b" denotes a significant difference when compared to the $CdCl_2$-treated group.

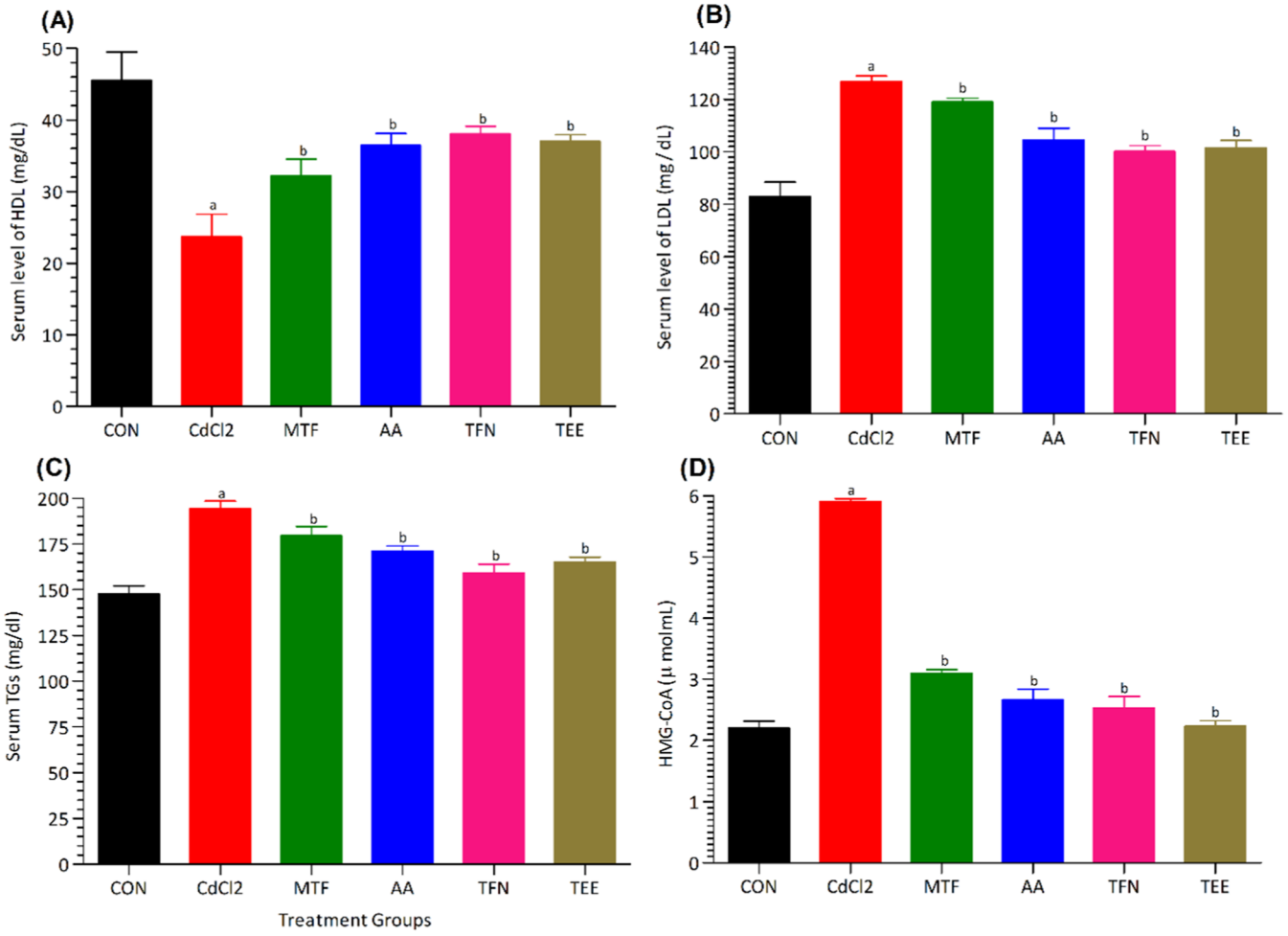

Figure 8. Impact of treatment and $CdCl_2$ on the serum levels of (A) HDL, (B) LDL, (C) TGs, and (D) HMG-CoA reductase. Data is presented as mean ± SD. Significance levels were determined using a one-way ANOVA, followed by Bonferroni post-test for multiple comparisons. In the notation used, "a" indicates a significant difference when compared to the control group, while "b" denotes a significant difference when compared to the $CdCl_2$-treated group.

significantly reduced the AUC compared to the $CdCl_2$-treated group ($P < 0.001$) (Figure 4B).

Effect of Treatment on Carbohydrate Metabolism. It was observed that $CdCl_2$ administration significantly impaired carbohydrate metabolism, leading to elevated levels of α-amylase and α-glucosidase in the $CdCl_2$-treated group (Figure 5A,B). However, treatment with TEE significantly reduced the levels of α-amylase and α-glucosidase in the TEE group more effectively when compared to the TFN treatment in the TFN group (Figure 5A,B).

G6P activity was significantly increased ($P < 0.001$) in the $CdCl_2$-treated group due to $CdCl_2$ treatment. However, treatment with TEE induced a notable decline, restoring glucose-6-phosphatase levels to a normal range in the TEE group (Figure 6A). Taxifolin, ascorbic acid, and metformin also successfully reversed abnormally elevated levels of G6P (Figure

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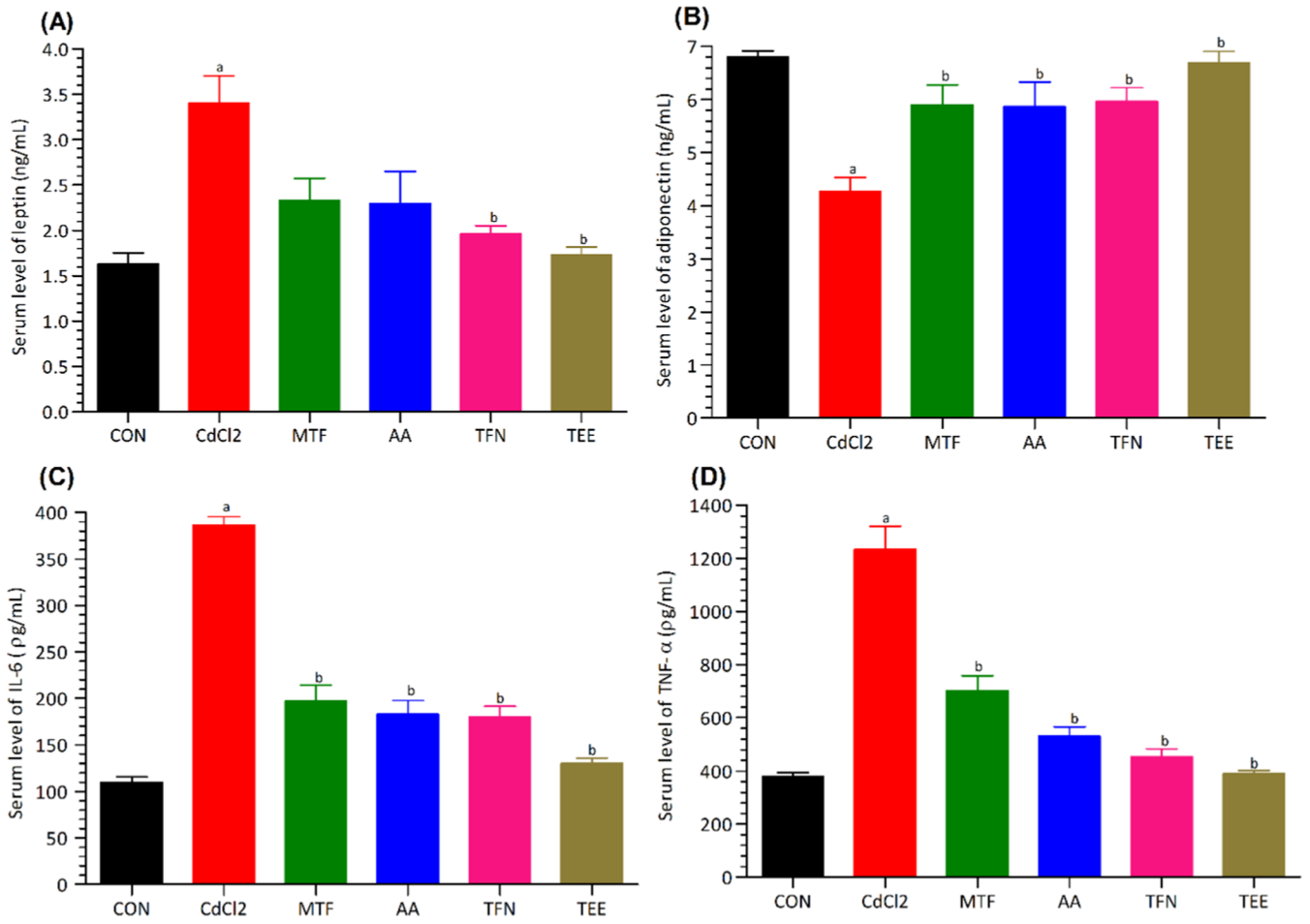

Figure 9. Impact of treatment and $CdCl_2$ on serum levels of (A) leptin, (B) adiponectin, (C) IL-6, and (D) TNF-α. The data is presented as mean ± SD. Significance levels were determined using a one-way ANOVA, followed by Bonferroni post-test for multiple comparisons. In the notation used, "a" indicates a significant difference when compared to the control group, while "b" denotes a significant difference when compared to the $CdCl_2$-treated group.

6A). $CdCl_2$ treatment significantly increased hexokinase levels ($P < 0.001$) in the $CdCl_2$-treated group (Figure 6B). Nonetheless, administration of TEE, TFN, MTF, and AA significantly reduced the elevated hexokinase levels in the TEE, TFN, MTF, and AA groups (Figure 6B). Phosphofructokinase activity was markedly decreased ($P < 0.001$) in the $CdCl_2$-treated group compared to the control group (CON) (Figure 6C). However, treatment TEE significantly ($P < 0.001$) restored phosphofructokinase activity in the TEE group (Figure 6C). TFN, MTF, and AA also improved the decreased phosphofructokinase activity in the TFN, MTF, and AA groups (Figure 6C). Similarly, TEE treatment significantly increased ($P < 0.001$) the activity of G6PDH in the TEE group compared to the $CdCl_2$-treated group (Figure 6D). TFN, AA, and MTF also increased the G6PDH activity in the TFN, AA, and MTF groups, respectively (Figure 6D).

Effect of Treatment on Antioxidant Enzymes Capacity. TEE exhibited significant antioxidant potential by modulating the activity of the first-line antioxidant enzymes. It was observed that $CdCl_2$ treatment led to a decrease in the levels of CAT, SOD, and GPx in the $CdCl_2$-treated group (Figure 7A−C). However, treatment with taxifolin-enriched extract markedly restored these antioxidant enzyme levels ($P < 0.05$) in the TEE group (Figure 7A−C). In the TFN group, TFN also improved the levels of antioxidant enzymes, although to a comparatively lesser extent when compared with the TEE in the TEE group (Figure 7A−C).

Effect of Treatment on Lipid Profile. The evaluation of lipid biomarkers, including HDL, LDL, triglycerides, and HMG-CoA reductase, was conducted. As a result of cadmium chloride administration, the serum levels of HDL significantly decreased when compared to the CON group (Figure 8A). However, treatment with TEE, TFN, MTF, and AA notably restored the abnormally decreased HDL levels in the TFN, MFT, and AA groups when compared to the $CdCl_2$ group (Figure 8A). Conversely, the serum levels of LDL, TGs, and HMG-CoA reductase significantly increased ($P < 0.05$) in the $CdCl_2$-treated group compared to the CON group (Figure 8B−D). Treatment with TEE, TFN, MTF, and AA effectively reversed these abnormal increases in these parameters in the TFN, MFT, and AA groups when compared to those in the $CdCl_2$ group (Figure 8B−D).

Effect of Treatment on Inflammatory Cytokines. In the $CdCl_2$-treated group, it was observed that the administration of $CdCl_2$ significantly increased the levels of leptin and adiponectin, as well as inflammatory cytokines (TNF-α and IL-6) when compared to the CON group (Figure 8A−D). However, treatment with TEE led to a significant reduction ($P < 0.05$) in the release of inflammatory markers IL-6 and TNF-α, thereby mitigating the inflammatory responses (Figure 9C,D).

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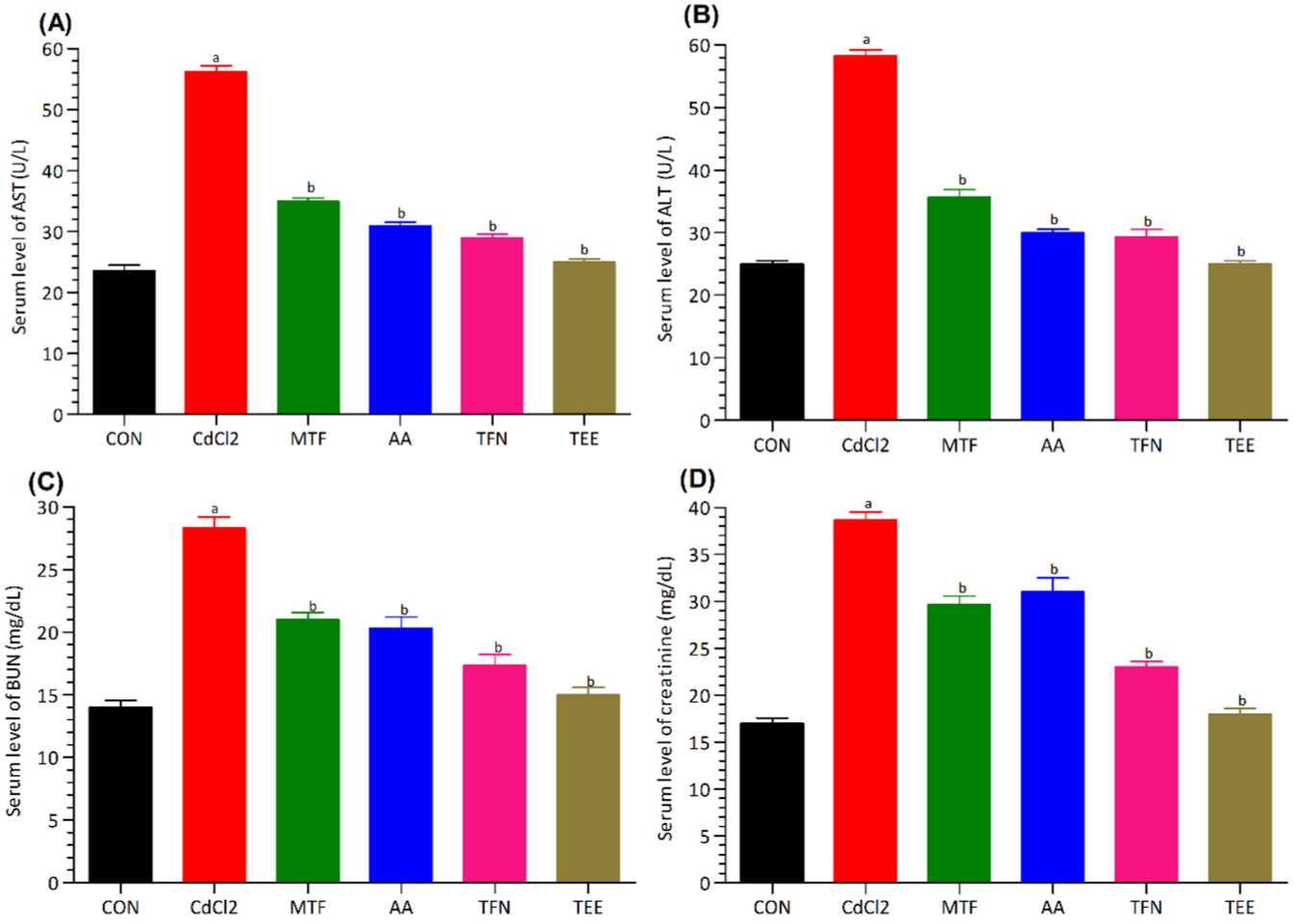

Figure 10. Impact of treatment and $CdCl_2$ on serum levels of (A) aspartate aminotransferase (AST), (B) alanine transaminase (ALT), (C) blood urea nitrogen (BUN), and (D) creatinine. Data is presented as mean ± SD. The level of significant difference was determined using one-way ANOVA with Bonferroni post-test for multiple comparisons. In the notation used, "a" indicates a significant difference when compared to the control group, while "b" denotes a significant difference when compared to the $CdCl_2$-treated group.

Additionally, TEE treatment decreased the level of leptin by reducing the excessive release of leptin and adiponectin from adipose tissues, as observed in the TEE group (Figure 9A,B). Similarly, TFN, AA, and MTF also decreased ($P < 0.05$) the liberation of inflammatory cytokines IL-6 and TNF-α in the TFF, MTF, and AA groups, although to a lesser extent compared to the taxifolin extract treatment in the TEE group (Figure 9C,D).

Effect of Treatment on Liver and Kidney Functions. $CdCl_2$ treatment markedly increased ($P < 0.05$) the levels of AST and ALT in the $CdCl_2$ group when compared to the CON group (Figure 10A,B). However, treatment with TEE exhibited a hepatoprotective effect by significantly reducing the levels of AST and ALT in the TEE group. In the TFN, MTF, and AA groups, the elevated levels of AST and ALT also improved but to a lesser extent compared to those in the TEE group (Figure 10A,B). $CdCl_2$ treatment significantly elevated ($P < 0.05$) the levels of blood urea nitrogen and serum creatinine in the $CdCl_2$ group in comparison to the CON group (Figure 10C,D). Nevertheless, treatment with TEE significantly ameliorated the increased levels of blood urea nitrogen and creatinine when compared to all other treatment groups (Figure 10C,D).

Effect of Gene Expression of Debilitated Carbohydrate and Lipid Metabolism-Associated Genes. $CdCl_2$ administration significantly downregulated ($P < 0.001$) the mRNA expression of IGF-1 and GLUT2 in the $CdCl_2$ group compared to the CON group (Figure 10A,B). However, treatment with TEE, TFN, AA, and MTF mitigated ($P < 0.001$) the mRNA expression of these genes, similar to the CON group (Figure 11A,B). $CdCl_2$ intoxication induced downregulation ($P < 0.001$) in mRNA expression of HMG-CoA lyase in the $CdCl_2$ group compared to the CON (Figure 11C). However, TEE markedly improved ($P < 0.001$) the downregulated expression of HMG-CoA lyase compared to the $CdCl_2$ group (Figure 11C). TFN, AA, and MTF also mitigated the expression, but to a lesser extent compared to TEE in the TEE group (Figure 11C). Angiotensin receptor II and lactate dehydrogenase expression were significantly upregulated ($P < 0.001$) in the $CdCl_2$ group compared to the control, TEE, TFN, AA, and MTF groups (Figure 10D,E). However, taxifolin-enriched extract treatment notably downregulated ($P < 0.001$) the mRNA expression of angiotensin receptor II and lactate dehydrogenase, similar to the control group in the TEE group (Figure 11D,E). Similarly, in mRNA expression of vasopressin, downregulation ($P < 0.001$) was observed in the $CdCl_2$ group compared to the control (Figure 10F). Taxifolin-enriched extract markedly improved ($P < 0.001$) the downregulated expression of vasopressin compared to the $CdCl_2$ group (Figure 11F). However, TFN, AA, and MTF also lessened the

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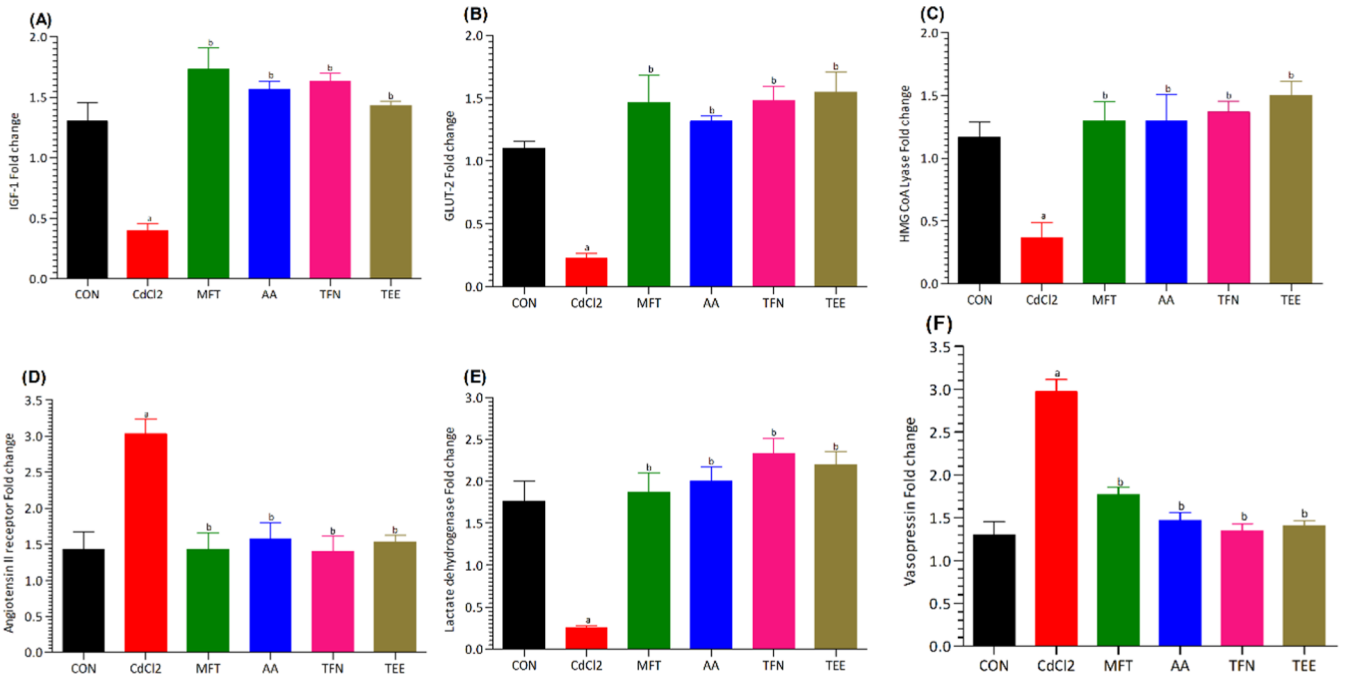

Figure 11. Impact of treatment and $CdCl_2$ on mRNA expression of (A) IGF-1, (B) GLUT2, (C) HMG-CoA lyase, (D) angiotensin II receptor, (E) lactate dehydrogenase, and (F) vasopressin. Data are presented as mean ± SD. The level of significant difference was calculated by one-way ANOVA using Bonferroni post-test. "a" represents a significant difference when compared with the control group, while "b" represents a significant difference when compared with the $CdCl_2$ group.

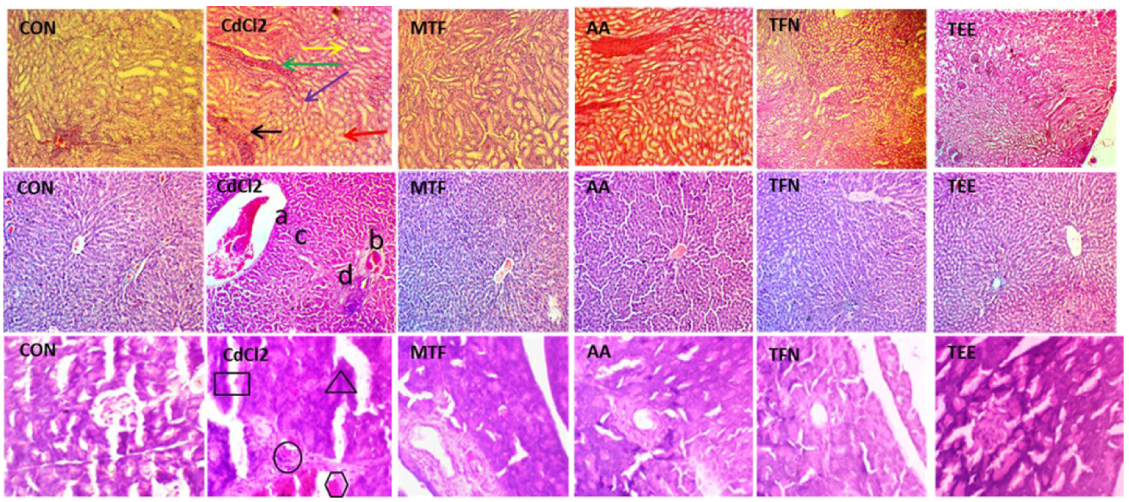

Figure 12. Impact of treatment and $CdCl_2$ on kidney, liver, and pancreas histopathology: kidney histopathology: tubular vacuolization and dilation (yellow arrow), pyknotic tubular nucleus (black arrow), infiltration of leukocytes and mononuclear cells (red arrow), hemorrhage, and congestion (green arrow). Liver histopathology: blood sinusoids and congestion (a), infiltration of inflammatory cells (b), cytoplasmic vacuolation (c), and hepatic cell necrosis (d). Pancreas histopathology: blood sinusoids (circle shape), infiltration of inflammatory cells (rectangle shape), fatty changes (hexagonal shape), and apoptotic changes (triangle shape).

expression, but to a lesser extent compared to TEE in the TEE group (Figure 11F).

Effect of Treatment on Histopathology of Vital Organs. Histopathological examination revealed that the CON group exhibited a normal architecture of hepatic tissues with well-defined nuclei, chromatin material, intact parenchyma, and organized hepatic cords. In contrast, the $CdCl_2$ group showed disrupted hepatic cords, inflammation, cytoplasmic fat droplet deposition, and infiltration of inflammatory mediators. However, treatment with the taxifolin-enriched extract and taxifolin demonstrated improvements in histopathological features, including the restoration of normal sinusoidal spaces, prominent nuclei, and the absence of hepatocyte inflammation (Figure 12). The CON group displayed a normal appearance and a healthy distribution of Islets of Langerhans within the pancreas parenchyma. In the $CdCl_2$ group, there was a significant decrease in the number of Islets of Langerhans, indicating necrotic changes and impaired insulin production. However, in the TEE group, the number of healthy Islets of Langerhans was markedly restored compared to that in the TFN, MTF, and AA groups, demonstrating the restorative function of the pancreas. Microscopic examination of the

https://doi.org/10.1021/acsomega.3c08989
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kidneys in the $CdCl_2$ group revealed vacuolar degenerations and glomerular lesions. Kidney histopathological analysis further indicated thickening of the basement membrane, tubular lesions, mononuclear cell and leukocyte infiltration, edema, and granular deposit formation due to $CdCl_2$ administration. These pathological hallmarks were mitigated and improved in the TEE and TFN groups compared to those in other treatment groups and the $CdCl_2$ group (Figure 12).

DISCUSSION

Over the past 5 decades, research has established the role of inorganic metals in disrupting insulin and glucose homeostasis in target tissues. Inorganic metals, such as Cd, have been shown to induce metabolic disorders that impair β-cell function and contribute to the development of T2DM. The deleterious effects of Cd on metabolic pathways, particularly carbohydrate and lipid metabolism, have garnered increasing attention due to their potential role in the development of metabolic disorders such as diabetes and cardiovascular diseases. Cd exposure has been linked to impaired carbohydrate metabolism, which is characterized by insulin resistance and glucose intolerance. Cd disrupts insulin signaling pathways, reducing the sensitivity of target tissues to insulin, and thereby impairing glucose uptake. This leads to elevated blood glucose levels, a hallmark of DM. Additionally, Cd interferes with glucose homeostasis by modulating genes involved in glycometabolism such as GLUT2 and IGF-1. These disruptions in glucose metabolism can set the stage for the development of T2DM. Furthermore, Cd exerts a detrimental effect on lipid metabolism. It disrupts the balance between HDL and LDL cholesterol, leading to dyslipidemia characterized by elevated LDL cholesterol and TGs, along with decreased HDL cholesterol levels. Cd-induced oxidative stress and inflammation further exacerbate lipid dysregulation, contributing to the progression of atherosclerosis and cardiovascular diseases. Cd's association with metabolic impairment, particularly in carbohydrate and lipid metabolism, highlights its potential role as a significant risk factor for metabolic disorders. Understanding the mechanisms underlying Cd's disruptive effects on these metabolic pathways is crucial for developing strategies to mitigate its adverse health outcomes. The study's findings suggest that TEE may hold promise in counteracting Cd-induced metabolic disturbances, offering hope for potential therapeutic interventions in individuals exposed to environmental Cd contamination.

In the present study, our focus is on Cd-induced impairment of carbohydrate metabolism and the development of DM. Cd was selected for this study due to its high presence in soil, its uptake by plants, and its resulting dietary consumption, which raises serious public health concerns. Additionally, cigarette smoke is another source of this toxic metal as smokers have been found to have higher serum levels of Cd. Recent research has indicated that Cd plays a role in metabolic disruption through the modulation of gene expression, oxidative stress, and inflammatory cytokines. Human clinical studies and animal studies have demonstrated that Cd has the ability to accumulate in the pancreas and induce pancreatotoxic effects. In recent decades, the role of Cd in the development of DM and insulin resistance has been reported, affecting processes such as glycogenesis, glucose filtration, and gluconeogenesis. Therefore, in the current study, a diabetic rat model induced by Cd consumption was established to investigate the effects of Cd on the carbohydrate metabolism and diabetes-related complications.

P. roxburghii is rich in taxifolin, a bioactive compound known for its diverse pharmacological activities. In our current study, we prepared a taxifolin-enriched extract from *P. roxburghii* and investigated its protective potential against $CdCl_2$-induced metabolic impairments and DM. In our rat model, $CdCl_2$ administration led to increased body weight, fasting, and random blood sugar levels during the experimental period. In our current study, TEE reduced body weight, fasting, and random blood sugar levels, consistent with its reported actions. Studies by Gao et al.,[25] Zhao et al.[26] have documented the antidiabetic effects of TFN through the modulation of glucose metabolism via the PI3K/AKT antioxidant signaling pathways. In our study, treatment with TEE reduced blood glucose levels in the glucose tolerance test, aligning with the previously reported actions of taxifolin.[26]

The ITT is considered the gold standard for investigating glucocorticoid depletion. Following an overnight fast, insulin is administered, and blood samples are collected at 30, 60, 90, and 120 min intervals to assess blood glucose levels. The rate of plasma glucose disappearance, denoted as kITT, is then estimated to determine insulin sensitivity. In the current study, $CdCl_2$ administration resulted in an increase in serum insulin levels due to impaired glucose metabolism. However, treatment with TEE and TFN reduced serum insulin levels and increased insulin sensitivity in the treatment groups. This aligns with the findings of Lee et al.,[27] who reported the therapeutic effect of TFN in mitigating insulin resistance and enhancing insulin tolerance in a model of insulin resistance induced by free fatty acids. Consistent with this work, our study demonstrated that TEE treatment reduced insulin resistance and improved insulin sensitivity in $CdCl_2$-induced metabolic impairments. The PTT is an indirect method for quantifying gluconeogenesis, designed to assess the conversion rate of pyruvate to glucose. It serves as a gold standard assay for estimating glucose production. Animals fasted for 6−8 h before receiving intraperitoneal pyruvate administration, and the AUC is calculated to determine glucose production from pyruvate. Excessive conversion of pyruvate to glucose can contribute to glucose intolerance. Treatment with TEE in our study exhibited antihyperglycemic effects by regulating blood glucose levels and improving insulin sensitivity, consistent with previous research. It has been reported that an extract enriched with taxifolin from *Hydnocarpus alpina* produced robust glycemic control and increased insulin secretion through a signaling pathway dependent on K^+-ATP channels.[21]

$CdCl_2$ administration increased the levels of α-amylase and α-glucosidase in the treatment group, resulting in elevated postprandial hyperglycemia. Both enzymes catalyze the digestion of carbohydrates by cleaving α1-4 glyosidic linkages, leading to increased levels of monosaccharides.[28] In the present study, treatment with TEE and TFN decreased the levels of α-amylase and α-glucosidase in the serum, resulting in controlled postprandial sugar levels, which supports the previously reported antidiabetic effects of TFN. Su et al.[29] documented the inhibitory effect of TFN on these enzymes in both *in vitro* and *in vivo* experimental studies. Similar to previous findings, TFN treatment in our study normalized blood glucose levels, hexokinase activity, and insulin secretion.[21] In the present study, $CdCl_2$ decreased the levels of phosphofructokinases. However, treatment with TEE normalized the levels of phosphofructokinases, similar to the findings of Pérez Gutiérrez,[30] who reported that TEE treatment increased the decreased levels of phosphofructokinases in a diabetes model. Like previous

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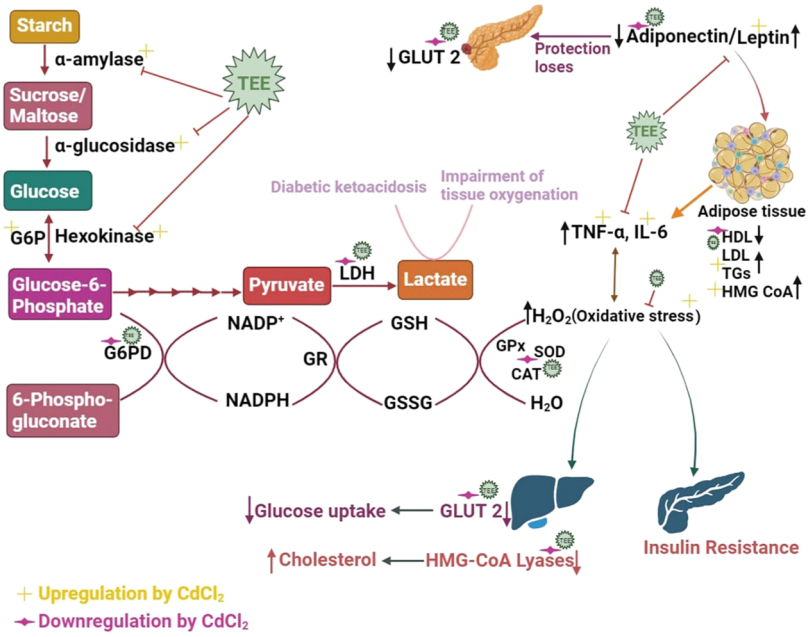

Figure 13. Schematic representation illustrates the intricate mechanism of action through which taxifolin-enriched extract (TEE) modulates the biochemical pathways affected by cadmium.

findings, TFN treatment mitigated the Cd-induced increase in G6P levels markedly.[21] Taxifolin's robust antioxidant and anti-inflammatory potential has been established in the literature.[31−33] Similarly, in the current experimental design, TFN reduced oxidative stress induced by $CdCl_2$ through the modulation of first-line antioxidant enzymes and decreased inflammation by modulating key inflammatory cytokines IL-6 and TNF-α.[34,35] $CdCl_2$ is an obesogenic factor, as its administration led to increased levels of fatty acids, cholesterol, TGs, and FFAs, consistent with the literature.[36] However, treatment with TEE mitigated hyperlipidemia by modulating hepatic lipoprotein synthesis and other multiple lipid-lowering pathways.[37] Cd produced hepatotoxicity in the current study's treatment groups, as evidenced by elevated levels of AST and ALT due to Cd_2^+ binding with sulfhydryl groups in mitochondria, leading to oxidative stress, mitochondrial dysfunction, and ischemia to hepatocytes. Cd also induced the activation of Kupffer cells and mediated inflammatory responses through the activation of inflammatory cytokines. TEE exhibited a hepatoprotective effect and decreased the levels of ALT and AST to within the normal physiological range. Cd also induced nephrotoxicity in the treatment group, leading to elevated levels of blood urea nitrogen and serum creatinine, similar to previous studies reporting the nephrotoxic effects of this metal.[38,39] TEE, due to its nephron-protective effect, modulated the levels of blood urea nitrogen and serum creatinine.[40] Gene expression analysis of critical genes linked to glucose metabolism, insulin tolerance, and sensitivity was also conducted. $CdCl_2$ administration downregulated the expression of IGF-1, a crucial factor regulating insulin release. TEE treatment regulated glycometabolism, mitochondrial function, and IGF-1 expression, similar to previous reports.[41] $CdCl_2$ treatment downregulated the expression of GLUT2 in adipocytes, impairing carbohydrate metabolism and glucose tolerance, consistent with previous findings.[42] However, TFN upregulated the mRNA expression of these key genes that regulate glucose and insulin homeostasis. mRNA expression of lactate dehydrogenase (LDH) was upregulated in the $CdCl_2$ group, resulting in oxidative stress, as reported in recent work.[43] However, TEE treatment mitigated the expression of LDH. $CdCl_2$ treatment upregulated the expression of angiotensin II receptors, leading to insulin insensitivity, impaired glucose transport in skeletal muscles, and abnormal pancreatic blood flow, which can result in the development of diabetes. TEE treatment notably decreased the expression of angiotensin II receptors, acting as an inhibitor and showing promise in the treatment of DM. HMG-CoA lyase is responsible for the breakdown of lipids and proteins to generate glucose monomers. $CdCl_2$ treatment impaired the expression of HMG-CoA and vasopressin, resulting in an impairment of carbohydrate metabolism. However, taxifolin extract treatment remarkably recovered the mRNA expression of these key glucogenic genes.

The mechanism of action underlying the protective effects of TEE against Cd-induced metabolic disruptions involves multifaceted interactions at the molecular level. TEE primarily targets the disrupted pathways in carbohydrate and lipid metabolism. TEE mitigates Cd-induced impairments by modulating key signaling pathways, including the antioxidant signaling cascade, thus, enhancing insulin sensitivity and glucose metabolism. Furthermore, TEE exerts regulatory effects on gene expression, notably upregulating critical genes involved in glycometabolism such as GLUT2 and IGF-1, which are downregulated by Cd. The extract's antioxidant and anti-inflammatory potential contributes to the restoration of metabolic homeostasis by counteracting Cd-induced oxidative stress and inflammation. TEE's ability to normalize enzymatic activities, regulate lipid profiles, and protect against Cd-induced hepatotoxicity and nephrotoxicity further underscores its comprehensive impact on mitigating the adverse effects of cadmium on metabolic pathways. A schematic representation of the mechanism of action of TEE against Cd-induced metabolic impairment is depicted in Figure 13.

While this study provides valuable insights into the protective effects of TEE against Cd-induced metabolic impairment using an experimental animal model, it is crucial to mention the certain limitations inherent in this experimental approach. First,

https://doi.org/10.1021/acsomega.3c08989
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extrapolating the findings from animal models to human contexts requires caution due to potential species differences in metabolic responses. Additionally, the controlled environment of animal studies may not fully capture the complexity of human metabolic disorders and impairments. Moreover, the study focuses on a specific bioactive compound, i.e., taxifolin, that is enriched in taxifolin extract rather than the broader spectrum of potential interactions within a natural environment. Addressing these limitations through a discussion of potential implications emphasizes the need for cautious interpretation and encourages future studies, potentially involving human clinical trials, to validate the observed effects in a more translational context. This discussion contributes to a more comprehensive evaluation of the study, providing a balanced understanding of its significance and potential applications.

The findings of this study, highlighting the protective effects of TEE against Cd-induced metabolic disruptions and impairments, have significant implications for human health and the broader population. Given the ubiquitous nature of Cd exposure through soil, dietary intake, and sources such as cigarette smoke, our study underscores the potential relevance of TEE as a therapeutic intervention for individuals exposed to Cd contamination. Considering the established links between Cd and metabolic disorders, the study's outcomes suggest that TEE may offer a promising avenue for mitigating the adverse health effects associated with Cd exposure. Further studies of TEE's efficacy in human clinical studies could contribute to the development of preventive strategies and interventions for populations at risk. The study's comprehensive insights into the molecular mechanisms involved in TEE's protective effects also lay the groundwork for future research aimed at understanding and addressing the broader implications of Cd-induced metabolic impairments on public health.

CONCLUSIONS

Our comprehensive study provides valuable insights into the deleterious effects of Cd on various metabolic pathways, with a particular focus on carbohydrate and lipid metabolism. Cd, a pervasive environmental pollutant, has been shown to induce a cascade of metabolic disturbances, ultimately resulting in insulin resistance, impaired glucose tolerance, and disruptions in lipid profiles. These findings underscore the pressing need to understand the intricate interplay between environmental toxins and metabolic health, especially in the context of the alarming global rise in the number of DM cases. Cd exposure, which can occur through dietary sources, pesticides, and even cigarette smoke, poses a substantial risk to metabolic well-being. Our study elucidates how $CdCl_2$ induces not only carbohydrate metabolism dysfunction but also oxidative stress and inflammation, which further exacerbate the metabolic disruptions. These findings emphasize the multifaceted nature of Cd's impact on health and underscore the urgency of addressing environmental pollutants as contributors to the DM epidemic. On a promising note, our research highlights the therapeutic potential of TEE from *P. roxburghii*. TFN emerged as a compelling candidate for mitigating the detrimental effects of Cd exposure. It effectively ameliorates $CdCl_2$-induced insulin resistance, improves glucose tolerance, and modulates lipid profiles. Importantly, TFN also demonstrates antioxidant and anti-inflammatory properties, counteracting oxidative stress and inflammation induced by Cd. As we look to the future, our study opens up exciting avenues for further investigation. Long-term studies will be essential to assessing the sustained benefits of TFN treatment, and clinical trials may pave the way for novel interventions in populations exposed to Cd and similar environmental toxins. Additionally, future research should delve into the intricate molecular pathways through which TFN exerts its protective effects. This deeper understanding may lead to the development of targeted strategies for diabetes prevention and management, offering hope for individuals at risk due to environmental pollutant exposure. In summary, our study underscores the critical need to address the impact of environmental pollutants such as Cd on metabolic health. It also highlights taxifolin's potential as a natural therapeutic agent to mitigate the health risks associated with Cd exposure, ultimately contributing to the global effort to combat DM and its associated complications. Future research is necessary to delve deeper into the underlying mechanisms and optimize the dosage and duration of taxifolin-enriched extract treatment for the most effective therapeutic outcomes. The findings of this study shed light on potential interventions to counteract metabolic disruptions caused by environmental pollutants like cadmium.

ASSOCIATED CONTENT

Data Availability Statement

All data generated and/or analyzed during this study are included in this published article.

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Notes

This study was ethically approved by the Institutional Review Board (GCUF/ERC/35) of Government College University Faisalabad (GCUF).

The authors declare no competing financial interest.

https://doi.org/10.1021/acsomega.3c08989
ACS Omega 2024, 9, 4057−4072

■ ACKNOWLEDGMENTS

The authors acknowledge and extend their appreciation to the Researchers Supporting Project Number (RSP2023R124), King Saud University, Riyadh, Saudi Arabia.

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https://doi.org/10.1021/acsomega.3c08989
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