



Suberosin Alleviates Thiazolidinedione-Induced Cardiomyopathy in Diabetic Rats by Inhibiting Ferroptosis via Modulation of ACSL4-LPCAT3 and PI3K-AKT Signaling Pathways

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Abstract

Thiazolidinediones are useful antidiabetic medications. However, their use is associated with adverse side effects like edema, heart failure and bone fractures. In this study, we investigated the anti-ferroptosis effects of suberosin (SBR; a prenylated coumarin) in diabetic Sprague Dawley rats. Further, we assessed the effects of co-administration of SBR (30 and 90 mg/kg/day) with thiazolidinedione (TZ at 15 mg/kg) to mitigate TZ-induced cardiomyopathy in diabetic rats. Our results showed that cardiac output, stroke volume, left ventricle systolic and diastolic pressures were aggravated in diabetic rats treated with TZ alone after 4 weeks. TZ treatments induced ferroptosis as well as marked histoarchitecture disarrangements in rat cardiomyocytes. The study found that optimizing volume overload alleviated cardiac hypertrophy and mitigated left ventricular dysfunction in diabetic rats co-treated with SBR. SBR co-administration with TZ reduced MDA levels in heart tissue and serum iron concentration (biomarkers of ferroptosis), downregulated mRNA expressions of LOX, ACSL4, LPCAT3, and promoted GPX4 activity as well as upregulated mRNA levels of AKT/PI3K/GSK3 β as compared to the group administered with TZ at 15 mg/kg. SBR co-administration also helped to retain the normal histoarchitecture of cardiomyocytes in diabetic rats. Hence, our results suggested that SBR is an effective supplement and could be prescribed to diabetic patients along with TZ but this requires further clinical trials.

Keywords Thiazolidinediones · Ferroptosis · Cardiomyopathy · AKT · GSK3 β · GPX4

Abbreviations

LPCAT3 Lysophosphatidylcholine acyltransferase 3
ACSL4 Acyl coenzyme A synthetase long-chain member 4
LOX Lysyl oxidase
GPX4 Glutathione peroxidase 4
AKT Protein kinase B

PI3K Phosphoinositide 3-kinase
GSK3 β Glycogen synthase kinase-3 beta
LVDd Left ventricular diastolic diameter
LVDs Left ventricular systolic diameter
LVEVd Left ventricular end-diastolic volume
LVEVs Left ventricular end-systolic volume
FS Fraction shortening
EF Ejection fraction

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Introduction

Diabetes mellitus is a carbohydrate metabolic disorder, usually due to impaired glucose homeostasis [1]. The 2021 global diabetes prevalence raised up to 536 million cases and this might approach 783 million in 2045 [2]. It is known that there exists a correlation between cardiovascular ailments and fatalities or illnesses in individuals diagnosed with diabetes mellitus [3, 4].

Thiazolidinediones (TZs) such as rosiglitazone and pioglitazone are currently being employed in the clinical

management of diabetes mellitus. However, there seem to be increased risks of congestive heart failure and other adverse side effects following the administration of TZs [5]. Nissen and Wolski conducted a comprehensive meta-analysis which revealed that the administration of rosiglitazone was linked to heightened risks of cardiovascular death and myocardial infarction [6]. However, subsequent studies have presented several evidences regarding the risk of acute myocardial infarction, stroke, heart failure, and death in elderly Medicare patients treated with rosiglitazone or pioglitazone. Antidiabetic therapies such as TZs that target peroxisome proliferator-activated receptor-gamma requires more comprehensive knowledge regarding their adverse effects [7, 8].

TZs have the potential to directly or indirectly cause cardiomyopathy. Research has demonstrated that rosiglitazone induces sodium reabsorption by upregulating renal tubule transporters, such as *Atp1a1*, *Npt2*, and *NHE3*, in the convoluted tubule, as well as *Nkcc2* in ascending tubes of the kidney [9]. The state of chronic volume overload is initially counteracted by cardiac hypertrophy; however, if the condition is not resolved, it ultimately results in cardiomyopathy and heart failure. At the moment, no such causative factors are known regarding TZs-induced cardiomyopathy and elevated plasma volume. Similarly, there is no experimental evidence indicating that the mitigation of TZs-induced congestive heart failure can be achieved through plasma volume release or the reduction of sodium reabsorption [10, 11]. Heart failure is distinguished by the presence of regulated cell death, autophagy, and ferroptosis. Ferroptosis is a type of programmed cell death that can be readily differentiated from other cellular mechanisms, including apoptosis, necroptosis, autophagy, and pyroptosis [12]. There is a need to explore natural products for their bioactivity for the management of side effects of TZs to lower the risk heart failure. The prenylated coumarin derivative (7-methoxy-6-(3-methylbut-2-enyl)bchromen-2-one), commonly known as suberosin (Fig. 1), is a natural product that has been previously isolated from the roots and aerial parts of *Cudrania tricuspidata*, *Ferulago carduchorum*, and *Prangos bucharica* [13, 14]. The potential of Suberosin (SBR) in the activation of trypsin, caspase- and chymotrypsin-like, and proteasome activities in a 20S proteasome activity assay has previously been reported to be dose-dependent [14]. This investigation

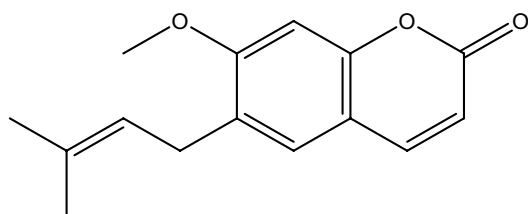


Fig. 1 Chemical structure of suberosin

targeted ferroptosis for managing TZs-induced cardiovascular conditions and to elucidate the anti-ferroptotic role of SBR when co-administered with TZs to reduce the risk of cardiomyopathy in diabetic rats.

Materials and Methods

Chemicals and Reagents

Thiazolidinedione (CAS 2295-31-0) utilized in the current investigation was procured from Sigma Aldrich while SBR (CAS 581-31-7) was obtained from Cayman Chemical. The rest of the chemicals were of high analytical purity and were used without any further purification.

Streptozotocin-Induced Diabetes Mellitus in Rats

Diabetes was induced in Sprague Dawley rats by intraperitoneal injection (1 ml/kg of 50 mg/kg body weight (bw)) of citrate buffered streptozotocin (STZ). The STZ-treated rats were observed to have developed and achieved stabilization of diabetes for seven days. The control group were administered citrate buffer (at pH of 4.5). After 1 week, the plasma glucose levels of the rats were determined. The study selected rats whose fasting plasma glucose levels ranged between 330–380 mg/dl [15].

Assessment of Suberosin Toxicity

The subacute and acute toxicity of SBR was determined in Sprague Dawley rats with and without diabetes (150–200 g) following the OECD guideline 423 [16].

Experimental Design

Thirty Sprague Dawley male rats (~100 g; 4 weeks old) were placed in clean steel cages at the animal house, following the approved animal handling and feeding protocol guidelines. Thirty rats were randomly allocated into six groups ($n=5$). The cage temperature (25 °C) and humidity (60–70%) were optimized, and the rats were provided with feeds and water ad libitum. The experiment was conducted for 4 weeks and all doses were administered orally. SBR (30 and 90 mg/kg) and TZ (30 mg/kg) were given by oral gavage. For TZ, it was dissolved in deionized water and administered at the standard dosage (30 mg/kg for people) daily [17]. SBR was dissolved in deionized water and doses were selected after acute and sub-acute toxicity. All rats were treated for 4 weeks, under the following experimental groups.

NC-G1 group: Normal control rats received only water and feeds.

DC-G2 group: Diabetic control rats were received only water and feeds.

SBR-DC-G3 group: Diabetic rats were only received SBR at 90 mg/kg.

TZ-G4 group: TZ was administered orally at 30 mg/kg/day to diabetic rats.

SBR + TZ-DC-G5 group: Diabetic rats were received SBR at 30 mg/kg/day and TZ (30 mg/kg/day) via oral gavage.

SBR + TZ-DC-G6 group: Diabetic rats were co-administered SBR (90 mg/kg/day) and TZ (30 mg/kg/day) via oral gavage.

Volume Analysis of Blood Plasma

Following anesthesia, 0.1 mL of Evans Blue dye (1 mg/mL) was injected via the portal vein. Subsequently, blood was collected from the vena cava after 3 min. The plasma volume was assessed by dividing the aggregate quantity of administered dye by the plasma dye concentration obtained through spectrophotometry [18].

Analysis of Sodium

The ARCHITECT c4000 Clinical Chemistry Analyze (Illinois, USA) was utilized to measure the concentrations of urinary sodium. The daily excretion of sodium was determined by multiplying the concentration of sodium with the volume of urine and subsequently dividing the product by the individual's body weight [18].

Iron Assay

Iron concentration was determined by iron kit (abcam, ab239715). To prepare the serum, collected whole blood in a covered test tube was placed on ice. The blood samples were vortexed to remove coagulants and retain the sample at ambient temperature, typically ranging from 15 to 30 °C. They were centrifuged at 1000–2000×g for 10 min and the serum was transferred into a pristine polypropylene tube.

Malondialdehyde Assay

The MDA concentrations were determined utilizing the same techniques as those utilized in the previous study conducted by Ohkawa et al. [19]. Following centrifugation of the reaction mixture at 4000×g for 10 min, the absorbance of the supernatant was measured at 532 nm. The study presented the data in terms of nanomolar concentrations per gram of tissue [19].

Assessment of Echocardiography

The rats were anesthetized with isoflurane. Acquisition of echocardiographic images was achieved using a Vevo 770 microimaging system with a 25-MHz probe (Visual Sonics, Toronto, ON, Canada). The left ventricle (LV) was imaged using parasternal short-axis views at the papillary muscle level, and M-mode recordings were collected. The study conducted diastolic measurements at the location of the greatest cavity dimension, while systolic measurements were taken at the point of minimal cavity dimension, utilizing the leading edge method [20]. To evaluate the systolic function of the left ventricle, the fractional shortening (FS%) and ejection fraction (EF%) were calculated [21].

cDNA Synthesis

After assessment of the amount and integrity of the entire RNA sample from heart tissue, a solution was made by combining 5 µg of total RNA with 10 µL volume, along with 1 µL of oligo (dT) primer and 1 µL of random hexamer primer. Subsequently, the blend was subjected to incubation at a temperature of 70 °C for 300 s, expeditiously cooled on ice, and augmented with 0.5 µL of RNase inhibitor (40 U/µL), 1 µL of 10 mM deoxyribonucleoside triphosphates, 4 µL of quintuple first-strand buffer and 1 µL of reverse transcriptase (Moloney Murine Leukemia Virus) [22].

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

The experimental procedure involved combination of 0.5 µL of both forward and reverse primers (Table 1) for the genes PI3K, AKT, GSK3β, LPCAT3, ACSL4, LOX, GPX4, and β-actin (utilized as a house-keeping gene), with cDNA (1 µL), SYBR Green qPCR Mix 2X (10 µL), and nuclease-free water (8 µL), resulting into a 20 µL reaction volume in a 0.2 mL thin-walled PCR microtube. The study was carried out following the QuantiTect Rev. Transcription Kit protocol utilizing the Rotor-Gene® Q qPCR (QIAGEN®) instrument. The Rotor-Gene Software was employed to conduct the cycling process, which consisted of an initial holding phase at 95 °C for 10 min, followed by 40 cycles of denaturation (at 95 °C for 10 s), annealing (at 60 °C for 15 s), and extension (at 72 °C for 20 s). The process was concluded with a final extension step at 72 °C for 10 min. Following this, secondary incubation was performed at a temperature of 95 °C for one minute. Furthermore, a gradual temperature increase ranging from 54 to 95 °C was implemented for approximately 3–4 min to execute the melting process. Following the amplification of each PCR product, a melting curve analysis was performed which yielded a single peak [23, 24].

Table 1 Primer sequences used for the qRT-PCR

Gene name	Primers 5'–3'	Accession number
Akt	Forward: 5'-GCACCGTGTGACCATGAACG -3' Reverse: 5'- AGTAAGCGTGTGGGCAACCT -3'	XM_006240631.3
PI3K	Forward: 5'-TTACGGCGGCATGGGAATCT-3' Reverse: 5'-CCAGCTTTCCTGAGTGCCT-3'	XM_017595947.2
GSK3 β	Forward: 5'- CCTCGGGACGAGGAAGAAGAG -3' Reverse: 5'-ACGTCATTGACAACGGCCTC-3'	NM_001014198.2
LPCAT3	Forward: 5'- AGCCTTAACAAGTTGGCGACG-3' Reverse: 5'- AGTGGTAGAACTGGTGGCCG-3'	NM_001012189.1
GPX4	Forward: 5'- GCCGTCTGAGCCGCTTATTG-3' Reverse: 5'- TGCGAATTCGTGCATGGAGC-3'	NM_017165.4
ACSL4	Forward: 5'-ACAGAATCATGCGGTGCTGGA-3' Reverse: 5'- ACAGCAAATAAGAGGAGCGCCA-3'	XM_006257315.4
LOX	Forward: 5'- TGCCTGGCCAGTTCAGCATA-3' Reverse: 5'- ATCCAGCAGGTCGTAGTGGC-3'	XM_006254715.4
β -actin	Forward: 5'-CGCGAGTACAACCTTCTTGCGAG-3' Reverse: 5'- CGCAGCGATATCGTCATCCA-3'	NM_031144.3

Histological Analysis

The heart tissues underwent standard paraffin processing, involving a series of steps such as dehydration (in 70%, 95%, and absolute alcohol), clearance in xylene, and infiltration at 70 °C for 2 h each. Subsequently, the tissues were subjected to paraffin wax embedding and thereafter sliced into sections of 5 μ m thickness. The specimens were subjected to hematoxylin and eosin staining before histopathological examination [25].

Statistical Analysis

Obtained data were subjected to one-way or two-way analysis of variance and unpaired two-tailed test followed by Tukey's posthoc test in GraphPad Prism for Windows (v5, GraphPad Software, USA). The means were considered to be statistically significant where $p < 0.05$.

Results

Toxicity Assessment Results

The outcomes of the acute toxicity test on SBR indicated that its median lethal dose (LD₅₀) is > 2000 mg/kg, hence it is safe for use in rats. For SBR subacute toxicity in rats at 30 mg/kg and 90 mg/kg, the complete blood chemistry, liver, and kidney function tests, and histology results indicated that it is safe after 4 weeks of administration. During the experiment, the rats were observed daily but only slight alterations in behavior were noted and could not be attributed directly to the toxicity of SBR.

SBR Mitigates TZ-Induced Volume Expansion and Ventricle Dysfunction

In this, rats were co-administered with SBR (at 30 mg/kg and 90 mg/kg/day) and TZ (at 15 mg/kg/day) to mitigate volume expansion and investigated whether it is a causative factor in TZ-induced heart weight gain and observed heart function by electrocardiographic measurements (Figure S1). Following 4 weeks of co-treatment with SBR and TZ, FS%, EF%, left ventricular systolic diameter (LVDs), left ventricular diastolic diameter (LVDd), left ventricular end-systolic volume (LVEVs), and left ventricular end-diastolic volume (LVEVd), stroke volume, and cardiac output were optimized more than in only TZ-treated rats (Fig. 2). Co-administration of SBR with TZ retarded TZ-mediated reduction of hematocrit, plasma volume/BW, urine sodium/BW, and HW/BW than in the TZ-DC-G4 group (Fig. 3).

Co-administration of SBR with TZ Alleviates Biomarkers of Ferroptosis (Total Iron Concentration and Lipid Peroxidation)

SBR co-administration with TZ reinstated the levels of oxidation by-product malondialdehyde (MDA) and optimized total iron concentration ($p < 0.001$) than in TZ-only treated group after 4 weeks of treatments. Groups treated with TZ for 4 weeks showed significantly higher total iron and MDA levels (Fig. 4).

SBR Modulates TZ-Induced Mediators of Ferroptosis (ACSL4-LPCAT3 and PI3K-AKT Signaling Pathways)

The mRNA levels of ferroptosis mediators LOX, LPCAT3, ACSL4, and GPX4, and AKT, PI3K, and GSK-3 β were determined by RT-qPCR following a 4-week administration

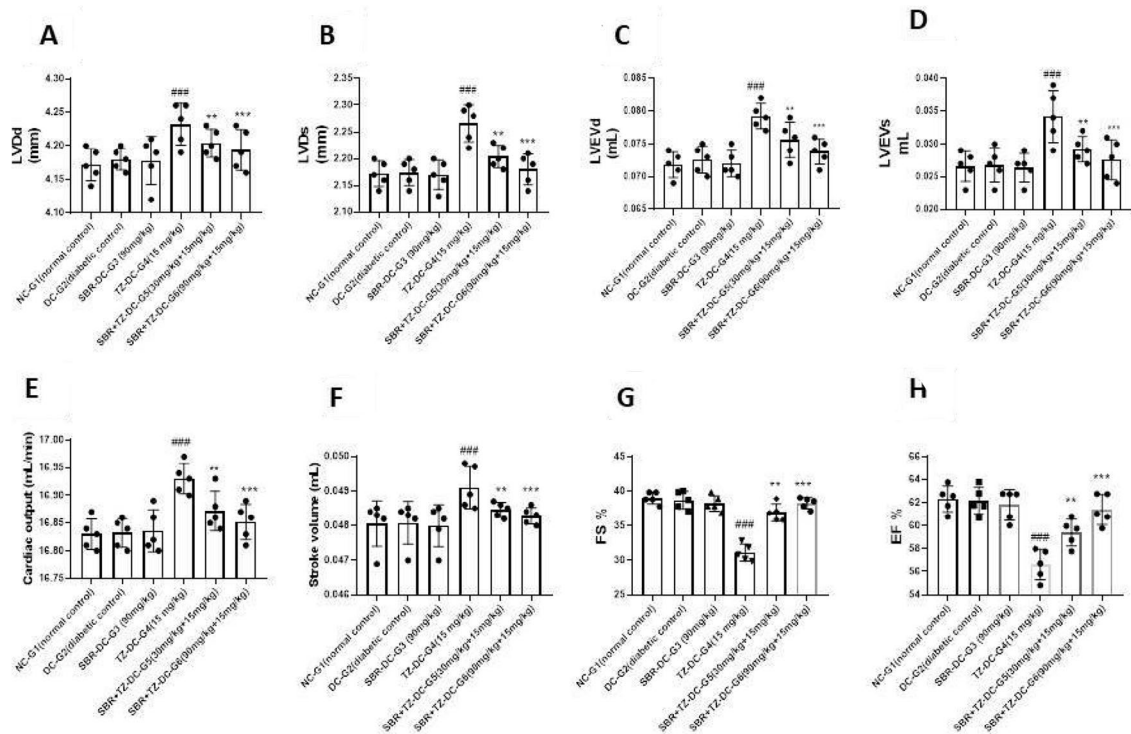


Fig. 2 Effect of co-treatment of SBR with TZ on **A** LVDd, **B** LVDs, **C** LVEVd, **D** LVEVs, **E** stroke volume, **F** cardiac output, **G** Fraction shortening (FS), and **H** Ejection fraction (EF) of rats. Values

are stated as means $\pm$ standard deviations of replicates, ** $p < 0.01$, *** $p < 0.001$ compared with the TZ-DC-G4 group. ### $p < 0.001$ compared with NC-G1 and DC-G2

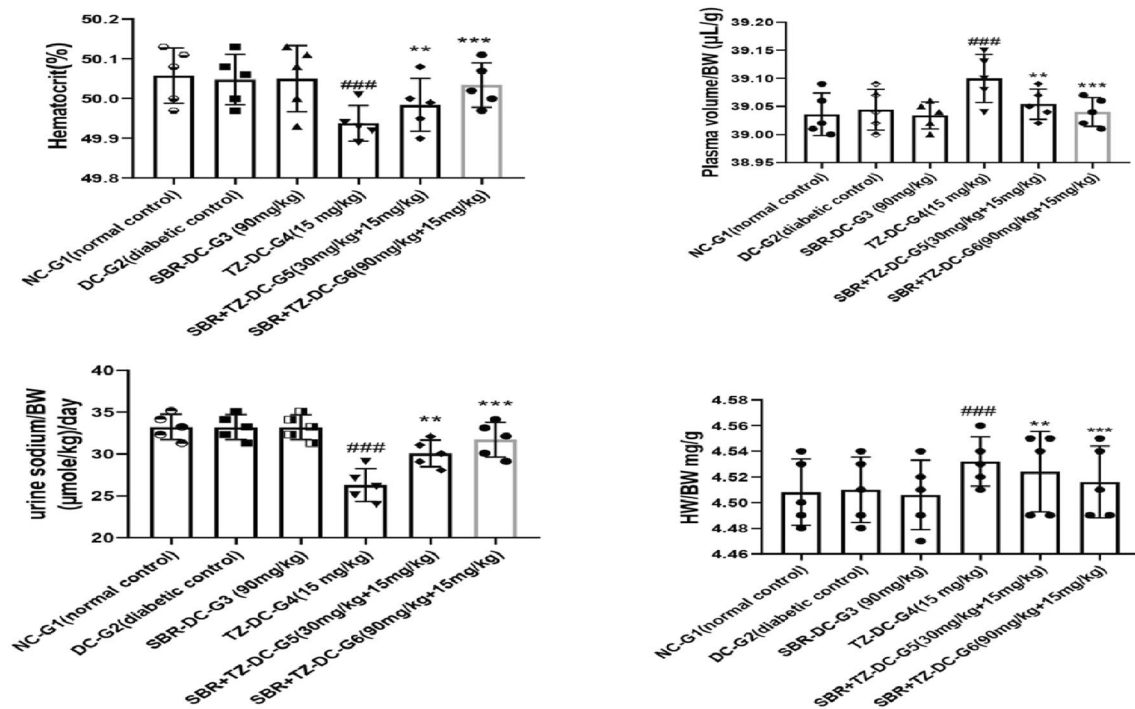


Fig. 3 Effects of SBR co-treatments with TZ improved hematocrit, Heart weight/body weight (HW/BW), plasma volume/BW, and urine sodium/BW. Values are stated as means $\pm$ standard deviations of repli-

cates, ** $p < 0.01$, *** $p < 0.001$, * $p < 0.05$ compared with the TZ-DC-G4 group. ### $p < 0.001$ compared with control (NG1-G1 & DC-G2)

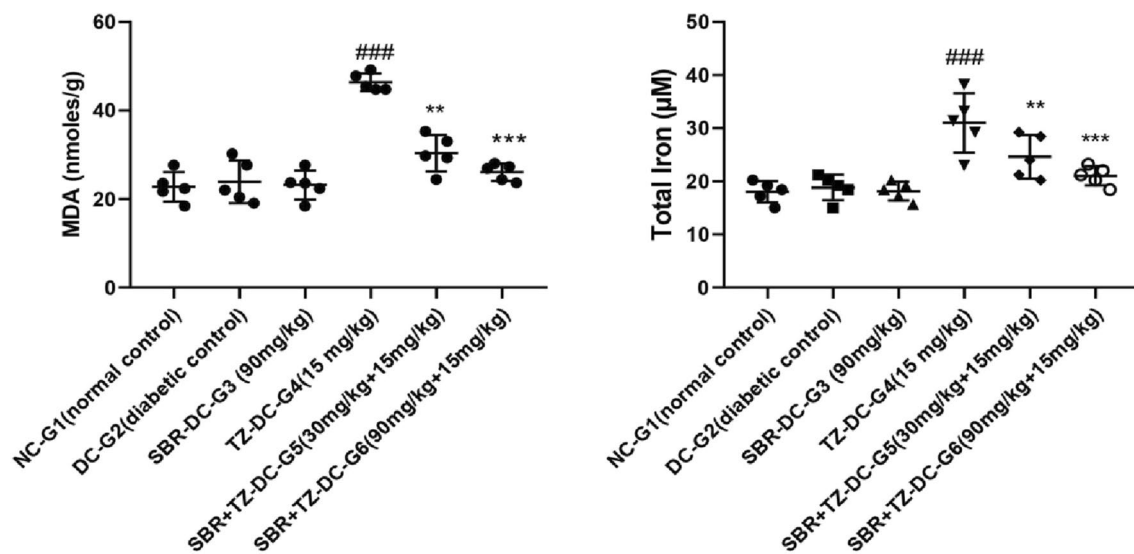


Fig. 4 The co-treatments of SBR with TZ reinstated total iron concentration in serum and MDA levels in heart tissues as compared with TZ-only treated rats. Values are means $\pm$ standard deviations of

replicates. $**p < 0.01$, $***p < 0.001$, $**p < 0.05$ compared with the TZ-DC-G4 group. $###p < 0.001$ compared with the control (NC-G1 & DC-G2)

of TZ with or without SBR. Administration of TZ only resulted in significant upregulation of mRNA levels of ACSL4, LOX and LPCAT3, and simultaneous downregulation of mRNA expressions of AKT, PI3K, and GSK3 β ($p < 0.01$, $p < 0.001$). The findings of our study indicate a statistically significant decrease in ferroptotic inducers ($p < 0.01$, $p < 0.001$) in groups that were co-administered SBR and TZ (SBR + TZ-DC-G5 and SBR + TZ-DC G6) as compared to the TZ-DC-G4 group (Fig. 5).

Anti-ferroptosis Activity of SBR Suppressed TZ-Induced Necrosis and Disarrangement of Cardiac Tissues

The heart histoarchitecture showed that there was significant damage in rats that were administered with TZ only (Fig. 6D). In addition, marked necrosis, wavy arrangements and hypereosinophilia were evident. Conversely, the normal histoarchitecture of the heart was observed in control rats (Fig. 6A–C). Administration of SBR at low and high doses resulted in the restoration of normal histoarchitecture in rats when co-administered with TZ (Fig. 6E–F).

Discussion

Investigation of the toxicity of natural products is important before their use in the development of drugs. In this study, SBR had $LD_{50} > 2000$ mg/kg, hence it is safe in rats. Though data on the toxicity of this compound is scarce, an extract of *Ferulago carduchorum* containing SBR was

previously reported to be safe as evidenced by its LD_{50} being greater than 2000 mg/kg [26].

Regarding the effects of SBR on TZ-induced cardiomyopathy, investigation of diverse pathophysiological mechanisms and gene regulations was done for 4 weeks. Echocardiography is a widely recognized and minimally invasive diagnostic technique utilized in clinical settings to precisely assess myocardial dysfunction, cardiac anatomy, and hemodynamic function [27]. A previous study stated that the cause of TZ-induced cardiac hypertrophy was either an elevation in plasma volume or a modification in cardiac nutrient preference [18]. The current study depicted that releasing volume overload after administration of SBR (at 30 mg/kg/day and 90 mg/kg/day) for 4 weeks reduced TZ-induced cardiac hypertrophy, and left ventricle dysfunction by regulating target factors (FS%, EF%), left ventricular systolic diameter, and diastolic diameter, end-systolic volume, and end-diastolic volume). These effects observed following TZ treatments could be attributed to volumetric expansion which led to adverse effects on cardiac tissues. Co-administration of SBR with TZ regulated ventricle function by reducing systolic and diastolic diameter, stroke volume, as well as end stroke volume of the left ventricle. Other physiological parameters (including plasma volume/BW, heart weight/BW, hematocrit, and urine sodium/ BW) were improved after SBR co-administration with TZ. The results we obtained are similar to a previous study that examined the therapeutic effect of osthol (a coumarin derivative isolated from *Cnidium monnieri* fruits) in treating cardiac hypertrophy where rats treated with osthol exhibited a significant effect

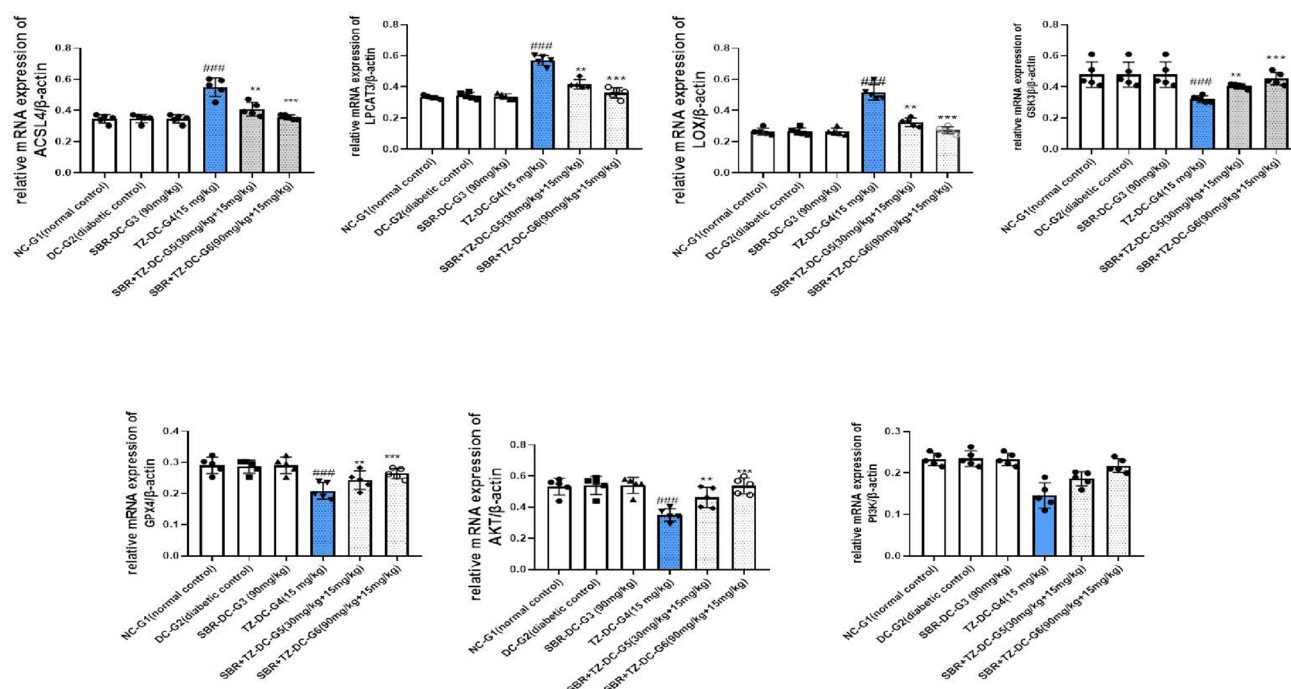


Fig. 5 Effects of co-administration of SBR with TZ on relative mRNA expressions of mediators of ferroptosis (downregulated; LPCAT3, LOX, ACSL4: promoted; GPX4) and upregulated; P13K, AKT, GSK3 β signaling pathways). Values are stated as

means $\pm$ standard deviations of replicates, ** $p < 0.01$, *** $p < 0.001$ compared with the TZ-DC-G4 group. ### $p < 0.001$ compared with control (NC-G1 & DC-G2)

on both systolic and diastolic blood pressure as well as heart weight index [28].

Administration of TZ led to the activation of sodium reabsorption by inducing the upregulation of different renal tubule transporters, specifically in the proximal convoluted tubule and the thick ascending limb. It has already been shown that activation of renal peroxisome proliferator-activated receptor gamma results in upregulation of the epithelial sodium channel (ENaCg), a subunit of the sodium transporter situated in the collecting duct [2]. The augmentation of renal sodium transporters leads to increased sodium reabsorption which in turn results in volume expansion. Cardiac hypertrophy serves as an initial compensatory mechanism to counterbalance the state of chronic volume overload. If such a medical condition persists, it can ultimately lead to the development of cardiomyopathy and subsequent heart failure [29]. Rats administered with TZ only were observed to have elevated plasma volume/BW, HW/BW and urine sodium. This effect may be attributed to increased collagen breakdown and a reduction in lysine residue glycation of collagen in the left ventricle. These findings suggest a positive correlation between effective diabetes management and the amelioration of cardiac hypertrophy [30].

In the present study, administration of SBR (at 30 mg/kg and 90 mg/kg) improved plasma volume/BW and HW/BW, urine sodium, serum total iron and MDA levels.

Ferroptosis is a form of controlled cellular demise that is primarily facilitated by iron-dependent lipid peroxidation [31]. The manifestation of ferroptosis is distinguished by anomalous configuration of mitochondria as well as elevated iron and lipid hydroperoxide concentrations [32]. Such high levels of iron stimulates the production of reactive oxygen species (ROS) through Fenton reaction and expedites the process of lipid peroxidation, as evidenced by previous research [33]. Liang et al. [34] found that the coumarin derivative (skimm) restored alterations in plasma volume and heart weight in diabetic rats by preventing ferroptosis and autophagy, and reducing oxidative stress [34]. The induction of ferroptosis is multifactorial (includes specific small molecules or conditions that hinder the synthesis of glutathione or the efficacy of the glutathione peroxidase 4 (GPX4) enzyme) and is liable on glutathione [35]. Subsequent studies have indicated that altered iron metabolism, decreased levels of glutathione, inactivation of GPX4, and increased peroxidation of polyunsaturated fatty acids (PUFAs) by reactive oxygen species are crucial factors in the onset and advancement of ferroptosis [32, 36]. Participation of acyl-CoA synthetase long-chain family member 4 (ACSL4) is essential for ferroptosis because it aids the creation of PUFAs [37]. Lipoygenases (LOXs) are a class of enzymatic proteins that contain iron and catalyze the deoxygenation of PUFAs, and thus are involved in ferroptosis

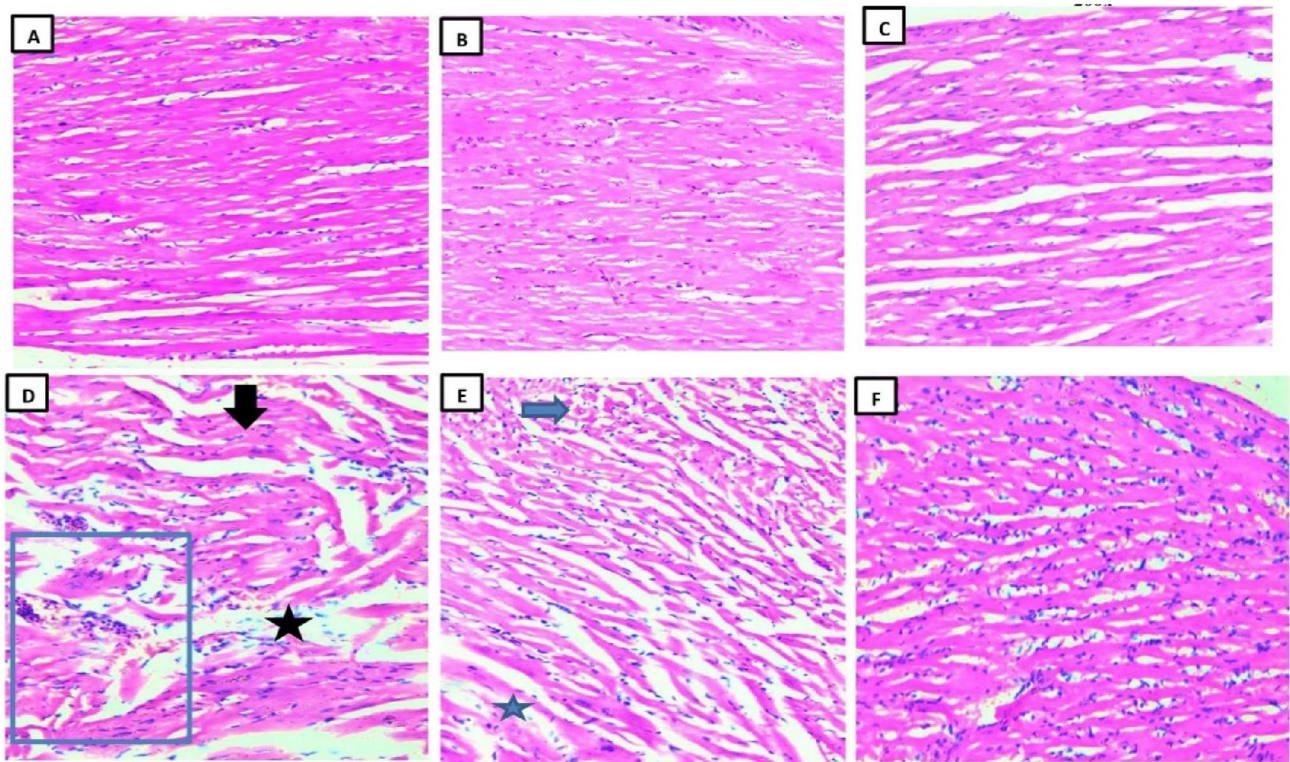


Fig. 6 **A** Normal histological appearance of cardiac tissue of the control group, Bar: 50 μ m; **B** ($\times 200$): cardiac tissue of ventricle of a diabetic control rat showing normal histoarchitecture. Bar: 50 μ m; **C**: ($\times 200$) SBR treatment alone (90 mg/kg) shows normal arrangement of cardiac tissues **D** ($\times 200$) Cardiac tissue of the rat received TZ (15 mg/kg) shows wavy arrangements (black arrow), hypereosinophilia (blue box), and necrosis (black star). Bar: 50 μ m; **D** ($\times 200$)

Ventricle histomicrograph of rats received cotreatments of SBR + TZ (30 mg/kg + 15 mg/kg) exhibited reduced myocardial disarrangements and marked less necrosis (blue arrow) and cloudy swelling in cardiomyocytes (blue star), and **E** ($\times 200$) Ventricle histomicrograph of rats received co-treatments of SBR + TZ (90 mg/kg + 15 mg/kg) are expressed normal arrangements of cardiomyocytes

[38] and sn2-15-hydroperoxy-eicasotetraenoyl-phosphatidylethanolamines (sn2-15-HpETE-PE) generated by 15-LOX are regarded as a marker of ferroptosis [39]. However, the mechanisms of ferroptosis in the process of lipid peroxidation takes place on esterified PUFAs-PLs rather than on free PUFAs [40, 41]. The present findings were showed TZ treatment induced ferroptosis, which was attributed to the upregulated mRNA expression of LOX, ACSL4, and LPCAT3 and downregulated GPX4 expression. However, co-administration of SBR with TZ was found to optimize relative mRNA expressions. A similar study demonstrated that psoralidin (a prenylated coumestan/coumarin) had anti-ferroptosis effect in vitro and exhibited inhibitory effects on ferroptosis, thereby facilitating their anti-diabetic properties. Since coumarin derivatives that promote ferroptosis are known to effectively manage cancer and liver fibrosis [42], this support our findings.

Further, our results demonstrated that upregulated AKT-PI3K signaling and downregulated ferroptosis inducer pathway upon co-administration of SBR with TZ. The study conducted by de Oliveira et al. [43] revealed that carnosic

acid (a coumarin derivative like SBR in this study) had a mitigating effect on methylglyoxalin-induced ferroptosis in neuroblastoma cells. This was accomplished through activation of the PI3K/Akt/Nrf2 signaling pathway and the modulation of antioxidant enzymes by the Nrf2 transcription factor, which could be the case in the present anti-autophagy effect of SBR.

Conclusion

The onset of cardiomyopathy in rats with diabetes was caused by TZ. However, co-administration of SBR with TZ was found to be effective in reducing the risk of cardiomyopathy. This was achieved by suppressing ferroptosis and maintaining physiological parameters related to heart function, such as left ventricle systolic and diastolic diameter volume, stroke volume, and cardiac output. Though this study used a fewer number of experimental animals ($n = 30$), SBR proved to be a potential supplement that could be prescribed along with TZ to diabetic patients so as to reduce the

risk of cardiomyopathy. However, this will require further clinical trials.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s12012-023-09804-7>.

Author Contributions SI: Investigation, writing of original draft; FJ: supervision, validation and writing; IK: data analysis; TO: review and editing.

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Data Availability The data is available upon request from the authors.

Declarations

Conflict of interest The author declares no competing interests.

Ethical Approval The animal handling and treatment experimental protocols underwent review and monitoring by the ethical committee of the Government College University, Faisalabad, Pakistan. These protocols followed the approved protocol of the European Union of animal care and Experimentation (CEE Council 86/609). The research was conducted in adherence to the ARRIVE guidelines.

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