doi.org/10.36721/PJPS.2026.39.3.REG.13078.1

Effect of baicalin on oxidative stress, inflammation and pancreatic cell apoptosis in streptozotocin-induced diabetic rats

Xin Wei and Geying Hu*
Geriatric /Traditional Chinese Medicine department, Daxing Hospital, Xian City, Shaanxi Province, China

Abstract: **Background**: Baicalin is a natural compound with established antioxidant and anti-inflammatory activities. Nevertheless, its specific influence on oxidative damage, inflammatory pathways, and pancreatic β-cell apoptosis in streptozotocin-induced diabetes remains insufficiently characterized. **Objectives**: We evaluated the therapeutic role of baicalin to reduce inflammation, oxidative stress and pancreatic cell apoptosis in diabetic rats. **Methods**: 18 Wistar rats were grouped into a control, diabetic and diabetic rats with administered 100 mg/kg baicalin. Antioxidant capacity (TAC), 8-hydroxy-2′-deoxyguanosine (8-OHdG), malondialdehyde and protein carbonyls were measured. Apoptosis of pancreatic cells was examined and the expression of TNF-α, IL-10, catalase, superoxide dismutase, Caspases-3/9, Bcl2 and Bax was evaluated using Real Time PCR. **Results**: Baicalin administration significantly improved body weight, blood insulin, glucose levels, hyperlipidemia and TAC level ($p < 0.01$), while markedly decreased malondialdehyde, protein carbonyls and 8-OHdG contents ($p<0.01$) in diabetic animals. Baicalin treatment significantly reduced the percentage of pancreatic apoptotic cells compared to diabetic control ($8.67 \pm 1.17\%$ vs. $14.46 \pm 2.39\%$; $p < 0.001$). A marked increase was found in Bax, TNF-α, Caspases expression in diabetic control, but IL-10, superoxide dismutase, catalase and Bcl2 expression was markedly decreased ($p < 0.001$). Baicalin supplementation significantly improved the expression of these genes in pancreatic tissue ($p < 0.01$). **Conclusion**: Baicalin protects pancreatic cells and restores insulin production in diabetic subjects by enhancing the antioxidant pool, attenuating oxidative stress, inflammation and pancreatic cell apoptosis in diabetic subjects.

Keywords: Apoptosis; Baicalin; Diabetes; Inflammation; Oxidative Stress; Pancreatic injury

Submitted on 08-07-2024 – Revised on 27-08-2025 – Accepted on 01-09-2025

INTRODUCTION

Diabetes mellitus is recognized as one of the most common types of chronic metabolic disorder worldwide. The prevalence of DM is rising and it has been estimated that DM will impact ~350 million people by 2030 (Whiting *et al*., 2011; Yaghini *et al*., 2011). Type II diabetes (T2D) that results from a defect in pancreatic beta-cells function can be linked to a spectrum of serious conditions such as hyperlipidemia, ketoacidosis, inflammation, hypertension, nonalcoholic fatty liver disease (NAFLD) and cardiovascular disease (CVD) (Kharroubi and Darwish 2015; Barbot *et al*., 2018). Although the pathophysiology of the diabetes development and its association with various disorders has remained unclear, it is proposed that oxidative stress caused by uncontrolled production of reactive oxygen species (ROS), depletion of cellular antioxidants pool, inflammatory reactions, cellular damage and apoptosis are the important mechanisms for pancreatic cells damage and T2D development (Oguntibeju 2019; Galicia-Garcia *et al*., 2020; Caturano *et al*., 2023). Thus, inhibiting the ROS-induced excessive oxidative stress and inflammatory reactions may be an effective strategy for preventing pancreatic cells injury and T2D management. Since oxidative stress and inflammation may play a pivotal role in pancreatic cells apoptosis, treatment with antioxidants may be effective in ameliorating/preventing oxidative stress-induced pancreatic injury and T2D development.

**Corresponding author:* e-mail: 18220060501@sina.cn

Nowadays, numerous studies have been focused on finding plant-based compounds with high antioxidant and anti-inflammatory activities for treatment of various diseases. Baicalin is one of the interesting plant-derived biologically active compounds with high antioxidant, inflammatory and immunomodulatory activities that is present in high amounts in *Scutellaria baicalensis* (Jang *et al*., 2023). This natural compound has a therapeutic role in various diseases such as metabolic syndrome (Khazeei *et al*., 2021), inflammatory bowel disease (Wang *et al*., 2022), cardiovascular diseases (Xin *et al*., 2020), rheumatoid arthritis (Wen *et al*., 2023) and some certain kinds of cancer (Singh *et al*., 2021). Since both oxidative stress and inflammation are closely linked to pancreatic cells injury and T2D development, the antioxidant and anti-inflammatory activities of baicalin may be effective for T2D management. To support it, previous experimental studies reported that baicalin has anti-diabetic effect through improving the pancreatic β-cell function, modulating GALR2/GLUT4 activation, blood glucose and insulin resistance (Fu *et al*., 2014; Yu *et al*., 2022; Szkudelski and Szkudelska 2023). Nevertheless, there are inconsistent results regarding the potential effect of baicalin on oxidative stress, inflammation and pancreatic cells apoptosis in T2D models. Therefore, we designed this study to consider the protective effect of baicalin on inflammatory mediators, oxidative stress and apoptosis in pancreas tissue of Wistar rats with streptozotocin (STZ)-induced T2D.

MATERIALS AND METHODS

Animals and study procedure

Baicalin was provided from national institutes for food and drug control (Beijing, PR China). All experimental procedures involving animals were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines. Male Wistar rats (aged 8 weeks old and weighing 150-200 g) were included in the study. All rats were placed in typical laboratory conditions with 12 h dark/12 h light cycle, humidity of 55 ± 5%, temperature of 20 ± 2 ºC and free access to adequate food and drinking water). For DM induction, a single dose of streptozotocin (50 mg/kg BW; ZellBio Company, German) was injected intravenously. 48 hours after the injection, rats with blood glucose above 250 mg/dl were considered as diabetic model (Nouri *et al*., 2022). Then diabetic rats were subdivided into two experimental groups, including diabetic control (n=6) and baicalin (n=6) (100 mg/kg once daily via gavage for 5 weeks). Rats in normal group (n=6) were fed with standard diet and water during the same period. Fig. 1 summarized the study design.

Collection of tissue and blood samples

At the endpoint, all rats were anesthetized and sacrificed following intravenous injection of 30 mg/kg ketamine hydrochloride. Serum samples were collected by centrifugation of blood specimens at 3,500 rpm for 10 minutes at 4ºC for biochemical marker estimation. In order to investigate and compare the study genes in pancreatic tissues, as well as assessing the levels of oxidant/antioxidant parameters, pancreatic tissues were isolated and washed with PBS (phosphate buffer saline). Tissue specimens were immediately stored at -80°C for further gene expression molecular and biomarker analysis.

Lipid concentration measurement

Plasma concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) were measured with specific enzymatic assay kits (Biomaghreb, Tunisia) as per manufacturer's instructions. Parameters related to coronary heart disease, including atherogenic coefficient (AC) and cardiac risk ratio (CRR) were estimated by the method described by Feriani *et al*., (2021). The amount of insulin in blood samples was determined by the Insulin Rat ELISA Kit (Thermo Fisher Scientific).

Detection of apoptotic cells

In order to detect pancreatic cells apoptosis, we determined DNA fragmentation as a hallmark of dying cells using TUNEL assay Kit (abcam, FITC, ab66108) as per manufacturer's instructions.

Analysis of oxidative stress biomarkers

In order to estimate the level of antioxidants, protein, lipid and DNA oxidation in the pancreas samples, we measured total antioxidant capacity (TAC), protein carbonyl (PC), malondialdehyde (MDA) and 8-hydroxy-2′-deoxyguanosine (8-OHdG), respectively. Pancreatic tissues homogenates were obtained according to a previously described method (Sparmann, Jaschke *et al*. 1997). The homogenates were centrifuged (10,000 rpm/4°C/15 min) and the supernatants were kept at -80ºC for further biomarkers analysis. The Bradford method was applied to estimate the level of total proteins in tissue homogenates (Bradford, 1976).

The concentrations of TAC and MDA were measured by the ferric reducing of antioxidant power (FRAP) method (Benzie and Strain 1999) and colorimetric kit (ZB-0156-R9648, Germany), respectively. Protein carbonyls were measured in tissue homogenates using the method previously described by Dalle-Donne *et al*., (2003). 8-OHdG level was determined using 8-hydroxy-2′-deoxyguanosine assay kit (Ann Arbor, MI, USA).

Analysis of gene expression

In brief, total RNAs were isolated from the homogenized pancreatic tissues by TRIzol™ Reagent (Thermo Fisher Scientific, Germany; Cat NO: 15596026) according to the manufacturer's instruction. cDNA was synthesized from extracted RNAs (2 µg) using Revert Aid First Strand Synthesis Kit (Sigma-Aldrich, USA). Ampliqon SYBR® Green 2x Master Mix on a Rotor-Gene 6000 (Thermo Fisher Scientific Inc.) was applied in order to examine the expression of candidate genes.

The list of all primers and their sequence is summarized in table 1. The homogeneity of gene expression was confirmed by Wilcoxon-Mann-Whitney test. The obtained quantification cycle threshold (Ct) in experimental in relation to control group was expressed on a logarithm scale. Genes with a fold-change ≥ 1.6-fold and significant change expression were considered as a differentially expressed gene.

Statistical analysis

Statistical analyses were performed using SPSS software (version 22). Data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied for multiple group comparisons. Exact p-values are reported where available; otherwise, p-value thresholds are indicated. Ninety-five percent confidence intervals (95% CIs) were calculated for all mean differences. The sample size (n=6 per group) was determined based on prior studies using similar STZ-induced diabetic rat models, which indicated that this size achieves >80% power to detect a 20% difference in primary oxidative stress markers at α=0.05. A p-value <0.05 was considered statistically significant.

RESULTS

Baicalin effect on weight

Diabetic animals exhibited 24.05% weight loss compared to their initial weight ($p < 0.01$), while rats in normal group showed the highest weight gain of 40.86% as compared to their initial weight at the 0th week. Baicalin administration for 5 weeks caused a marked increase in weight gain of 20.25% compared to their initial weight ($p < 0.01$). At the 5th week, diabetic animals exhibited lower mean weight than rats in control group (142.37 ± 6.97 g vs. 247.29 ± 7.12 g; $p<0.001$). The weight in the baicalin treatment group was significantly improved when compared to the diabetic controls (Fig. 2A; $p<0.01$).

Effect of baicalin on blood insulin and glucose levels

Fasting blood glucose (FBG) level was in the normal range in the control group, but it was higher than 400 mg/dl all the time in the DM group. FBG value exhibited a downward trend in the baicalin treated rats. At the 5th week, the FBG of rats was markedly decreased in the baicalin treatment groups as compared to the DM group (Fig. 2B, $p < 0.01$). The FBG in the baicalin group was close to that in the control group. A significant decline in insulin levels was noted in diabetic control compared to normal and baicalin groups at the 5th week (Fig. 2C, $p < 0.001$). Baicalin supplementation significantly improved insulin value in diabetic rats when compared to the DM rats ($P<0.01$).

Effect of baicalin on lipid profiles

Relative to the control rats, the diabetic animals had a marked increase in the levels of plasma TG, LDL, TC and significant decrease in HDL level (Table 2, $p < 0.001$). Similarly, diabetic rats had significantly increased AC and CRR values compared to the normal rats ($p<0.001$; Table 2). Baicalin treatment for 5 weeks significantly restored the hyperlipidemia, as well as atherosclerosis risk factors in diabetic rats.

Baicalin effect on oxidative stress parameters

Concentrations of oxidative stress biomarkers in pancreatic tissue of experimental animals are illustrated in fig. 3. Significant reduction was noted in the level of FRAP in pancreatic tissue of diabetic control rats compared to the control ($p < 0.001$). Baicalin administration significantly increased pancreatic FRAP content when compared to diabetic controls (Fig. 3A; $p < 0.01$). Accordingly, pancreatic MDA level was significantly higher in diabetic control rats than normal and baicalin groups (Fig. 3B; $p < 0.001$). The levels PC (Fig. 3C) and of 8-OHdG (Fig. 3D) were significantly higher in diabetic rats than the control group ($p < 0.001$). However, baicalin treatment markedly decreased the PC and 8-OHdG contents in pancreas tissues when compared to diabetic control ($p < 0.001$). There was no significant difference in the mean levels of PC and 8-OHdG between control and baicalin groups.

Baicalin effect on percentage of pancreatic apoptotic cells

Fig. 4A shows apoptosis of pancreatic cells' by TUNEL assay. There was a significant difference in the percentage of apoptotic cells between groups ($p < 0.001$). Diabetic control rats exhibited a marked increase of pancreatic apoptotic cells as compared to the normal group (14.46 ± 2.39% vs. 5.47 ± 1.22%; Fig. 4B; $p < 0.001$). Baicalin treatment significantly reduced the percentage of pancreatic apoptotic cells compared to diabetic control rats (8.67 ± 1.17% vs. 14.46 ± 2.39%; $p < 0.001$).

Baicalin effect on gene expression

The expression of study genes in the pancreatic tissues of all experimental groups is shown in table 3 and fig.5. Diabetic control rats exhibited significantly up-regulation of *Bax*, *Caspase-3*, *Caspase-9*, *TNF-α* and down-regulation of *Bcl2*, *IL-10*, *SOD* and *CAT* compared to the normal group ($p < 0.001$). Baicalin administration significantly downregulated *Bax* (2.11-fold), *Caspase-3* (2.01-fold), *Caspase-9* (1.64-fold), *TNF-α* (2.07-fold) and upregulated *Bcl2* (1.56-fold), *IL-10* (2.04-fold), *SOD* (1.95-fold), *CAT* (1.78-fold) compared to diabetic control rats ($p < 0.05$).

DISCUSSION

It is well elucidated that oxidative stress and pro-inflammatory mediators are major causes of pancreatic cells apoptosis, dysfunction and DM development (Watson and Loweth 2009 Eguchi *et al*. 2021). For this reason, a growing number of studies are seeking the best antioxidant to inhibit oxidative stress-induced pancreatic cells apoptosis and dysfunction and subsequently DM management (Dinic *et al*., 2022). Some studies proposed that Baicalin administration alleviates oxidative stress and cells apoptosis through compensation of cellular antioxidant pools and suppressing of inflammatory reactions (Ma*et al*., 2021 Liang *et al*., 2023). We therefore designed the current study to consider the protective role of baicalin administration on oxidative stress-induced pancreatic cells in T2D rats. In our study, T2D was closely linked to weight loss, hyperglycemia, hyperlipidemia and increased CRR and AC values, indicating T2D is a potential risk factor for not only pancreatic injury, but also for CVD.

We also found that T2D was strongly associated with depletion of TAC contents and downregulation of SOD and CAT antioxidant enzymes in pancreatic tissue, whereas MDA, PC and 8-OHdG levels were elevated. Our findings align with previous studies demonstrating that excessive oxidative stress leads to lipid peroxidation, protein oxidation and DNA damage, ultimately triggering apoptosis in diabetic pancreatic tissue (Srivastava *et al*., 2019; Tripathi *et al*., 2019; Rai *et al*., 2020).

Table 1: Sequence of primers used for RT-PCR

Genes	Forward	Reverse
TNF-α	5′-GCCCAGACCCTCACACTC-3′	5′-CCACTCCAGCTGCTCCTCT-3′
IL-10	5′- CACTGCTGTCCGTTCTCC-3′	5′- GCTCTTCCTTCTTACCCTCA-3′
CAT	5′-CTTCTGGAGTCTTTGTCCAG-3′	5′-CCTGGTCAGTCTTGTAATGG-3′
SOD	5′-TTCGTTTCCTGCGGCGGCTT-3′	5′-TTCAGCACGCACACGGCCTT-3′
Caspase-3	5′- GTGGAACTGACGATGATATGGC-3′	5′-CGCAAAGTGACTGGATGAACC-3′
Caspase-9	5′-CGACCATGATCGAGGATATTCAG	5′-TGCCTCCCTCGAGTCTCA-3′
Bax	5′- CGGCGAATTGGAGATGAACTGG-3′	5′- CTAGCAAAGTAGAAGAGGGCAACC-3′
Bcl2	5′- ATCCACGCTGTTTTGACC-3′	5′- CCGGACACGCTGAACTTGT-3′
GAPDH	5′- GATGAATTTGGGATCATCATCAGG-3′	5′- CCATGGCCACTAGACACAAC-3′

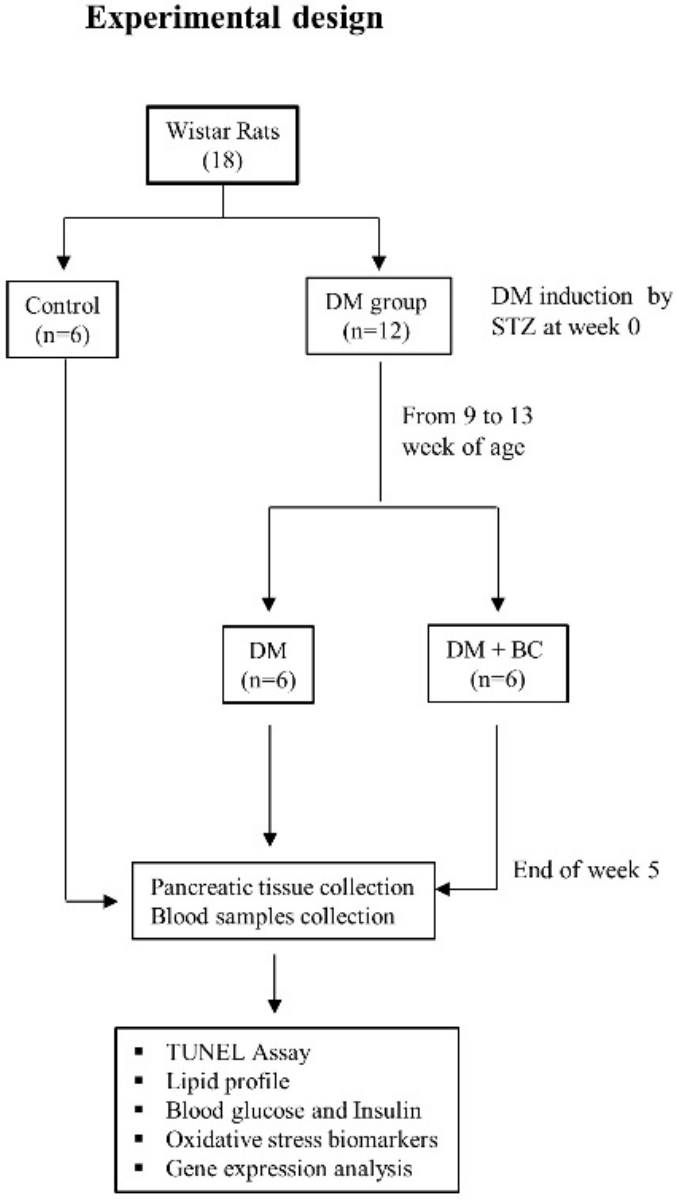

Fig. 1: The experimental design of the study. Schematic representation of the experimental workflow.
Male Wistar rats (n=18) were divided into three groups: (i) Control (n=6), (ii) Diabetic Mellitus (DM, n=6; induced by a single intravenous dose of streptozotocin, 50 mg/kg), and (iii) DM + Baicalin treatment (n=6; 100 mg/kg/day, gavage for 5 weeks). At the end of the study, body weight, blood samples, lipid profiles, oxidative stress markers, gene expression, and histological analyses were performed.

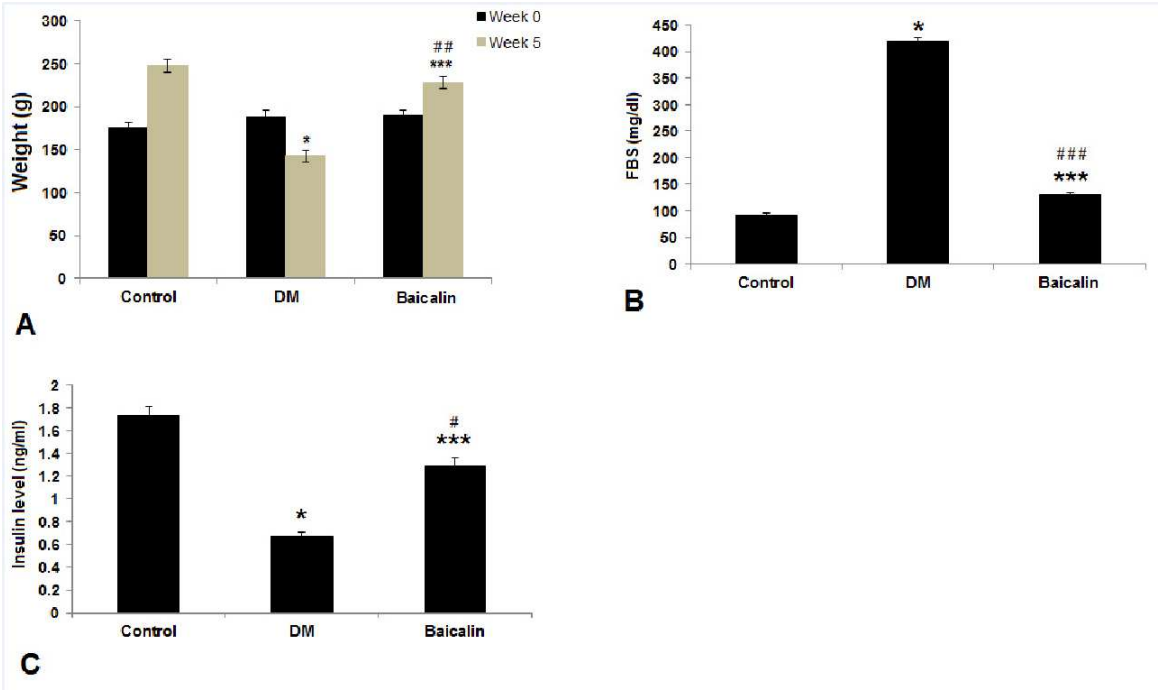

Fig. 2: Effect of baicalin on body weight, fasting blood glucose, and serum insulin levels
(A) Change in body weight (%) from baseline to week 5. (B) Fasting blood glucose (FBG, mg/dl). (C) Serum insulin concentration (µIU/ml). Data are expressed as mean ± SD (n=6 rats per group). Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. Control; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs. DM group. DM: Diabetic mellitus.

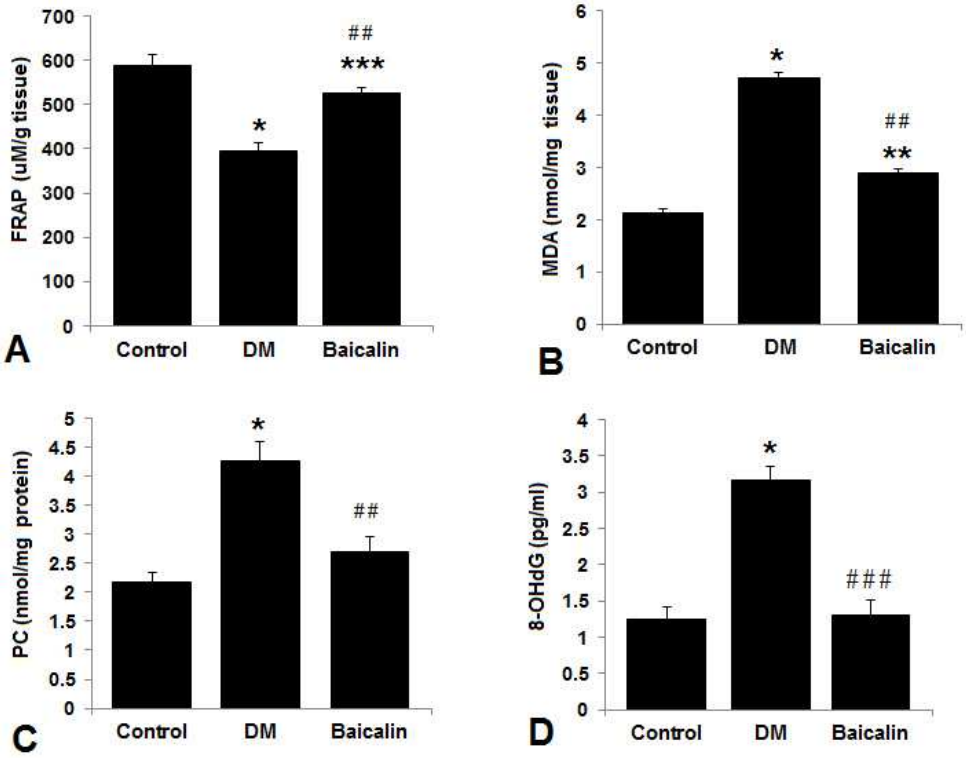

Fig. 3: Effect of baicalin on oxidative stress biomarkers in pancreatic tissue.
(A) Total antioxidant capacity (TAC, FRAP assay). (B) Malondialdehyde (MDA, nmol/mg protein). (C) Protein carbonyls (PC, nmol/mg protein). (D) 8-hydroxy-2′-deoxyguanosine (8-OHdG, ng/mg protein). Data are expressed as mean ± SD (n=6 rats per group). One-way ANOVA with Tukey's test was applied. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. Control; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs. DM group.

Table 2: Weight and serum lipid profile pattern in different groups

	Control	DM	Baicalin	p-value
HDL (mg/dl)	43.44 ± 3.17	31.27 ± 2.15***	39.24 ± 2.96$^{**\#}$	<0.001
LDL (mg/dl)	31.59 ± 3.91	42.31 ± 2.03***	32.67 ± 3.55$^{**\#\#}$	<0.001
TC (mg/dl)	44.19 ± 3.47	52.07 ± 1.67***	42.61 ± 4.29$^{**\#\#}$	<0.001
TG (mg/dl)	37.32 ± 3.25	43.35 ± 2.45***	35.31 ± 4.38$^{**\#\#}$	<0.001
CRR	1.02 ± 0.01	1.67 ± 0.06***	1.29 ± 0.04$^{**\#}$	<0.001
AC	0.02 ± 0.008	0.67 ± 0.04***	0.09 ± 0.006$^{**\#}$	<0.001

Values are expressed as mean ± SD (n=6 per group). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. *p<0.05, **p<0.01, ***p<0.001 vs. Control group; $^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 vs. DM group. AC: Atherogenic coefficient; CRR: Cardiac risk ratio; DM: Diabetic mellitus; HDL: High density lipoproteins; LDL: Low density lipoproteins; TC: Total cholesterol; TG: Triglycerides

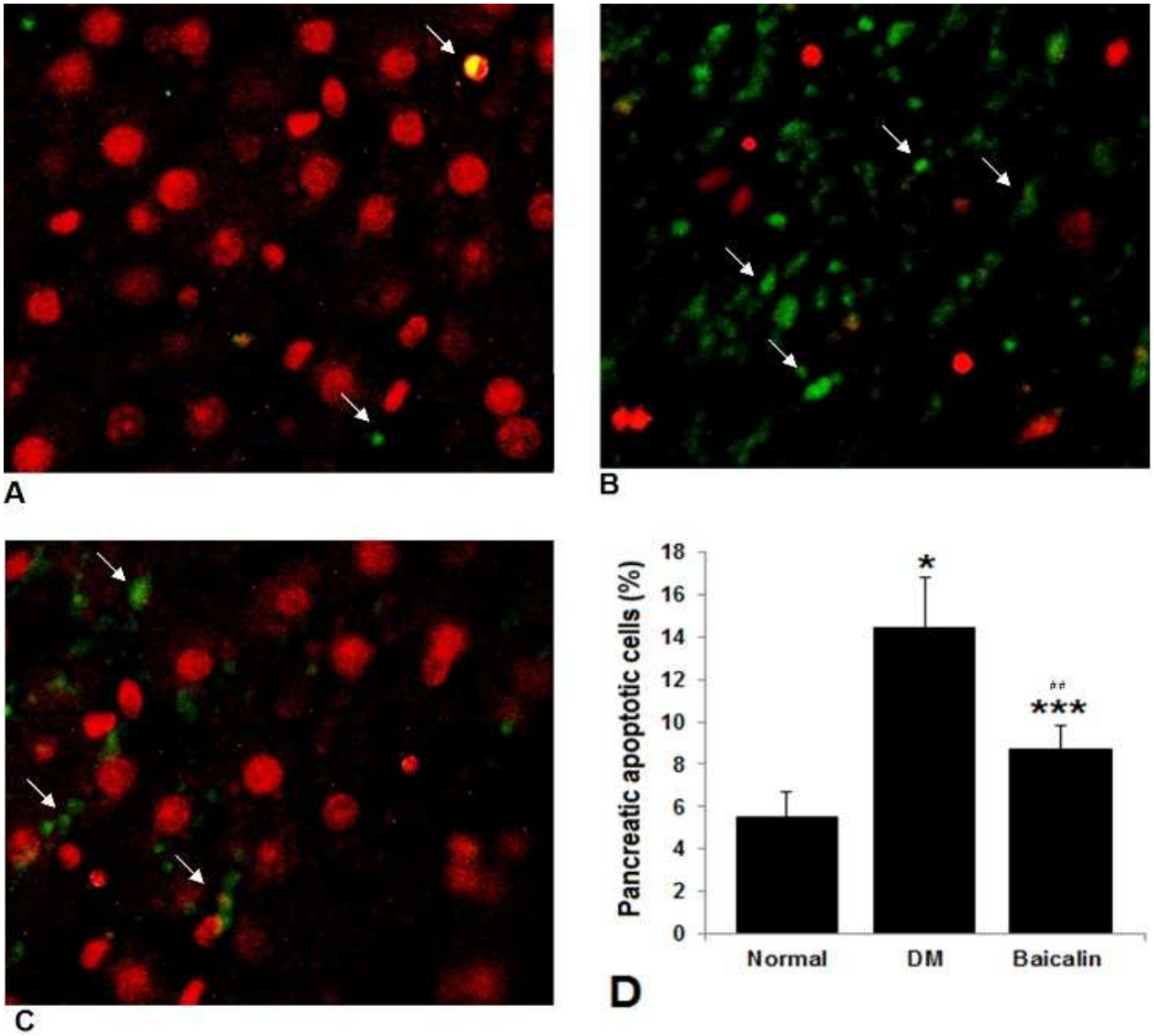

Fig. 4: Baicalin reduces apoptosis in pancreatic tissue.
(A–C) Representative TUNEL staining images of pancreatic tissue in Control, DM, and DM + Baicalin groups. Green fluorescence indicates apoptotic nuclei (FITC); blue fluorescence indicates DAPI nuclear counterstain. (D) Quantification of apoptotic pancreatic cells (%). Data are presented as mean ± SD (n=6 rats per group). Statistical analysis was performed by one-way ANOVA with Tukey's post hoc test. *p<0.05, **p<0.01, ***p<0.001 vs. Control; $^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 vs. DM group.

Table 4: Comparison of the gene expression between each group based on fold-change ratio

	CAT	IL-10	Bcl2	Casp3	Bax	TNF-α	SOD
Control vs. DM	+2.44	+2.91	+1.95	-3.74	-4.08	-4.74	+2.64
Control vs. Baicalin	+1.37	+1.42	+1.25	-1.87	-1.94	-2.28	+1.35
Baicalin vs. DM	+1.78	+2.04	+1.56	-2.01	-2.11	-2.07	+1.95

DM: Diabetic mellitus; $^{(+)}$ means overexpression, $^{(-)}$ means lower expression

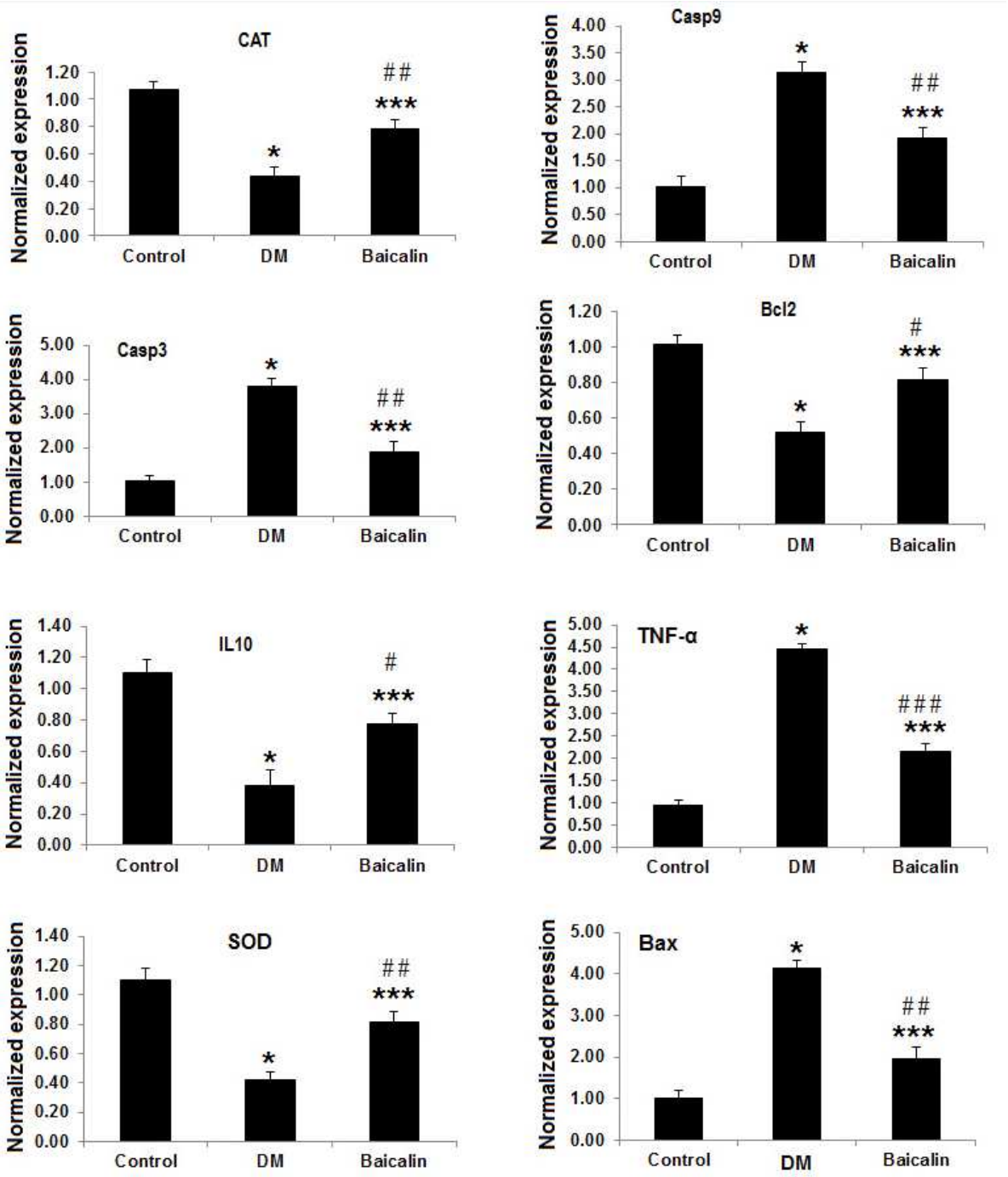

Fig. 5: Effect of baicalin on mRNA expression of apoptosis- and inflammation-related genes in pancreatic tissue. Normalized fold change in expression of Bax, Bcl2, Caspase-3, Caspase-9, TNF-α, IL-10, SOD, and CAT among experimental groups. Expression levels were normalized to GAPDH and analyzed using the ΔΔCt method. Data represent mean ± SD (n=6 rats per group). One-way ANOVA followed by Tukey's post hoc test was used. *p<0.05, **p<0.01, ***p<0.001 vs. Control; $^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 vs. DM group.

Numerous studies reported that DM is significantly associated with decreased levels of different antioxidants such as glutathione, SOD, CAT and glutathione peroxidase (GPX), while enhanced levels of MDA, PC, 8-OHdG and reactive oxygen species (ROS) in different organs (Asmat *et al*., 2016 Ghasemi-Dehnoo *et al*. 2020 Darenskaya *et al*., 2021).

These findings mean that diabetes may be closely linked to pancreatic antioxidants depletion and increased cells susceptibility to free radicals, which consequently causes pancreatic cells apoptosis and dysfunction leading to diabetes development. To support this hypothesis, we found higher rate of pancreatic cells apoptosis, as well as overexpression of apoptosis-related genes (Bax, Caspsaes-3, -9) in pancreas tissue of diabetic rats. Besides, diabetes was significantly linked to upregulation of pro-inflammatory mediator TNF-α, while anti-inflammatory mediator IL-10 was downregulated in pancreatic tissue. A study reported upregulation of apoptosis proteins (Bax and Caspase-3) and inflammatory cytokines (TNF-α and IL-6) in pancreatic tissues of diabetic rats (Tomita 2016). These data explain the essential role of inflammatory mediators and oxidative stress as the main mediators of pancreatic cells apoptosis and injury which subsequently leads to T2D development.

Baicalin's ability to enhance antioxidant defenses and reduce pro-apoptotic signaling supports its potential as a protective agent against diabetic pancreatic injury (Ramakrishna *et al*., 2024). These results corroborate recent reports suggesting that targeting oxidative stress pathways can attenuate β-cell dysfunction and improve metabolic outcomes in diabetes (Yadav *et al*., 2024; Prajapati *et al*., 2025). The observed improvements in insulin secretion, glucose regulation and lipid metabolism in baicalin-treated diabetic rats appear to play a central role in reducing oxidative stress and apoptosis in pancreatic tissues. Chronic hyperglycemia and dyslipidemia are known to trigger excessive production of ROS, contributing to oxidative damage of cellular macromolecules and impairing pancreatic β-cell viability and function. In this study, baicalin significantly reduced fasting blood glucose and improved insulin levels, which likely mitigated glucotoxicity-a key driver of oxidative stress. Additionally, the correction of hyperlipidemia, including reductions in TG, LDL-C, TC and atherogenic indices, may have alleviated lipotoxicity, further supporting β-cell survival. These metabolic improvements were closely associated with decreased oxidative damage markers (MDA, PC, 8-OHdG), increased antioxidant capacity and reduced β-cell apoptosis, as evidenced by both TUNEL assay and modulation of pro- and anti-apoptotic gene expression. Collectively, these findings suggest that baicalin's protective effects on the pancreas are at least partially mediated through its ability to normalize metabolic disturbances that otherwise exacerbate oxidative injury and apoptotic pathways in diabetes. Our findings support the idea that antioxidant therapy could protect pancreatic cells against oxidative stress in T2D. Here, we examined the protective role of baicalin administration on hyperglycemia, hyperlipidemia and inflammatory reactions, oxidative stress, antioxidants depletion, apoptosis of pancreatic cells and expression of apoptosis-related genes in pancreas tissue of diabetic rats. We found that treatment with baicalin not only decreased hyperlipidemia and improved the weight of diabetic animals, but also restored the insulin level that was associated with reduced blood glucose. Baicalin was also correlated to a marked increase of TAC value, as well as SOD and CAT upregulation in pancreas tissue. Furthermore, baicalin administration markedly decreased MDA, PC and 8-OHdG levels in pancreatic tissue, indicating its high ability in protecting pancreatic cells against lipid peroxidation, DNA and protein oxidation in diabetic animals. Interestingly, it was found that baicalin administration significantly enhanced the expression of anti-inflammatory mediator IL-10 and decreased the expression of pro-inflammatory cytokine TNFα in pancreatic tissue. Additionally, it balanced the expression of apoptotic genes in the pancreas tissue of diabetic animals. Although the levels of biomarkers of oxidative stress and expression of apoptotic genes in the pancreatic tissue of diabetic rats treated with baicalin were still somewhat high compared to normal group, baicalin supplementation significantly restored these abnormalities compared to non-treated diabetic rats. These results indicate that baicalin has protective effect on pancreatic cells by mitigating oxidative stress and cells apoptosis. Many studies observed that baicalin administration protects different organs by reducing free radicals generation, inflammation, oxidative stress and cell apoptosis. For instance, Zhao *et al*. (2021) reported that treatment of pancreatic cells with baicalin increases their survival rate by enhancing the cellular antioxidants pool, ameliorating inflammation, oxidative stress and pancreatic cells apoptosis.

The protective effects of baicalin observed in our study may be attributed to its multifaceted role in regulating oxidative stress and inflammation. Mechanistically, baicalin appears to enhance endogenous antioxidant defense systems by upregulating SOD and CAT gene expression, which are critical enzymes in neutralizing ROS and reducing oxidative injury to lipids, proteins and DNA. This is supported by the observed decrease in MDA, PC and 8-OHdG levels in baicalin-treated rats. Furthermore, baicalin's anti-inflammatory effect may be mediated via inhibition of the NF-κB signaling pathway, which controls the transcription of pro-inflammatory cytokines. The significant downregulation of TNF-α and upregulation of IL-10 observed in our study supports this anti-inflammatory mechanism. These actions collectively contribute to reduced pancreatic apoptosis and improved

biochemical homeostasis in diabetic rats. These findings are consistent with previous reports demonstrating the antioxidative and anti-inflammatory potential of baicalin in other diabetic and inflammatory models (Singh *et al*., 2021). Previous studies suggest that the protective effects of baicalin may be mediated through modulation of key intracellular signaling pathways. The Nrf2 (nuclear factor erythroid 2–related factor 2) pathway is a major regulator of antioxidant defense by inducing the expression of antioxidant enzymes such as SOD and CAT. Upregulation of these enzymes in our study may reflect baicalin's ability to activate Nrf2 signaling, thereby enhancing the cellular antioxidant pool and reducing oxidative damage. Additionally, baicalin has been reported to inhibit the MAPK (mitogen-activated protein kinase) pathway, which is often involved in stress-induced apoptosis, as well as the TLR4 (Toll-like receptor 4) signaling cascade, a key upstream mediator of NF-κB–driven inflammation. The observed reduction in pro-inflammatory cytokine TNF-α and increase in anti-inflammatory IL-10 in our study may therefore be partly due to suppression of TLR4/NF-κB signaling. These mechanisms collectively suggest that baicalin's antioxidant, anti-inflammatory and anti-apoptotic effects may be linked to coordinated regulation of Nrf2, MAPK and TLR4 signaling pathways. A recent study has reported that baicalin administration not only enhanced SOD and GPX activities, but also it ameliorated levels of IL-1β, IL-6 and TNF-α, as well as decreased cardiac cells apoptosis in rat model with Myocardial Ischemia (Li *et al*. 2022). He *et al*., (2017) demonstrated that baicalin supplementation improved liver injury through inhibiting pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β and cellular apoptosis in rats. These findings collectively indicate that depletion of cellular antioxidant pools, inflammatory reactions, oxidative stress and dysregulation of apoptosis-related genes are probably the main underlying mechanisms of pancreatic cells injury in T2D. Baicalin treatment reversed these adverse effects. Our findings support the hypothesis that the adverse effects of T2D are mediated through the oxidative stress, depletion of pancreatic antioxidant pools and pancreas cell apoptosis. Therefore, antioxidant therapy with baicalin is useful to restore the pancreatic cells and consequently insulin production in T2D.

Therefore, the present findings highlight baicalin's potential as a complementary therapeutic agent for diabetes management, given its ability to improve glycemic control, correct lipid abnormalities and reduce oxidative stress and apoptosis in pancreatic β-cells. By targeting multiple pathogenic pathways, baicalin may help preserve β-cell function and delay disease progression, which is crucial in both type 1 and type 2 diabetes. Although our study was limited to a 5-week treatment period, the sustained antioxidant and anti-inflammatory effects observed suggest that longer-term baicalin administration could offer continued pancreatic protection and metabolic benefits. Nevertheless, further long-term preclinical studies and well-designed clinical trials are essential to validate these effects and assess safety, dosage and efficacy in human patients.

This study has several limitations that should be considered when interpreting the findings. First, the sample size was relatively small (n=6 per group), which, while consistent with prior studies in STZ-induced diabetic rat models, may limit the statistical power to detect smaller effect sizes. Second, the intervention period was relatively short (5 weeks) and longer-term studies are necessary to determine whether the protective effects of baicalin on pancreatic cells are sustained over time. Third, we did not perform a dose–response analysis; therefore, it remains unclear whether lower or higher doses of baicalin would produce similar or greater benefits. Finally, our study was conducted in an animal model and the results may not fully translate to human physiology without further preclinical and clinical validation.

Our findings indicate that baicalin supplementation ameliorates oxidative stress, inflammation and apoptosis in pancreatic tissue of streptozotocin-induced diabetic rats, leading to improvements in insulin secretion, glucose homeostasis and lipid profiles. These results suggest a potential protective role of baicalin in preserving pancreatic function under diabetic conditions. However, given the limited sample size, short duration of treatment and use of an animal model, these findings should be interpreted with caution. Further long-term preclinical studies and well-designed clinical trials are warranted to confirm the therapeutic potential and safety of baicalin in diabetes management.

Acknowledgments
The authors of this manuscript wish to express their thanks and appreciation to Geriatric/Traditional Chinese Medicine Department, Daxing Hospital for its technical support.

Authors' contributions
GH designed the experiments; XW performed experiments and collected data; GH and XW discussed the results and strategy; GH Supervised, directed and managed the study; GH and XW Final approved of the version to be published. GH contributed to manuscript editing and submitting.

Funding
This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement
The datasets used to support the outcomes of this research are available from the corresponding author upon request.

Ethical approval
The study protocol was reviewed and approved by the Animal Care and Use Committee of Xi'an Daxing Hospital, Xi'an, China (Approval No. LW2024-034).

Conflict of interest

The authors declare that they have no conflicts of interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Asmat U, Abad K and Ismail K (2016). Diabetes mellitus and oxidative stress-A concise review. *Saudi. Pharm. J*., **24**(5): 547-553.

Barbot M, Ceccato F and Scaroni C (2018). Diabetes mellitus secondary to Cushing's disease. *Front. Endocrinol. (Lausanne)*, **9**: 284.

Benzie IF and Strain J (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods. enzymol*., **299**: 15-27.

Bradford N (1976). A rapid and sensitive method for the quantitation microgram quantities of a protein isolated from red cell membranes. *Anal. Biochem*., **72**(248): e254.

Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R (2003). Protein carbonyl groups as biomarkers of oxidative stress. *Clin. Chim. Acta.,* **329**(1-2): 23-38.

Caturano A, D'Angelo M, Mormone A, Russo V, Mollica MP, Salvatore T, Galiero R, Rinaldi L, Vetrano E, Marfella R, Monda M, Giordano A and Sasso FC (2023). Oxidative stress in type 2 diabetes: Impacts from pathogenesis to lifestyle modifications. *Curr. Issues. Mol. Biol*., **45**(8): 6651-6666.

Darenskaya MA, Kolesnikova LI and Kolesnikov SI (2021). Oxidative stress: Pathogenetic role in diabetes mellitus and its complications and therapeutic approaches to correction. *Bull. Exp. Biol. Med*., **171**(2): 179-189.

Dinic S, Arambasic Jovanovic J, Uskokovic A, Mihailovic M, Grdovic N, Tolic N, Rajic J, Dordevic M and Vidakovic M (2022). Oxidative stress-mediated beta cell death and dysfunction as a target for diabetes management. *Front. Endocrinol. (Lausanne)*, **13**: 1006376.

Eguchi N, Vaziri ND, Dafoe DC and Ichii H (2021). The role of oxidative stress in pancreatic β cell dysfunction in diabetes *Int. J. Mol. Sci*., **22**(4): 1509.

Feriani A, Bizzarri M, Tir M, Aldawood N, Alobaid H, Allagui MS, Dahmash W, Tlili N, Mnafgui K, Alwasel S and Harrath AH (2021). High-fat diet-induced aggravation of cardiovascular impairment in permethrin-treated Wistar rats. *Ecotoxicol. Environ. Saf*., **222**: 112461.

Fu Y, Luo J, Jia Z, Zhen W, Zhou K, Gilbert E and Liu D (2014). Baicalein protects against type 2 diabetes via promoting islet β-cell function in obese diabetic mice. *Int. J. Endocrinol*., **2014**: 846742.

Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, Ostolaza H and Martín C (2020). Pathophysiology of type 2 diabetes mellitus. *Int. J. Mol. Sci*., **21**(17).

Ghasemi-Dehnoo M, Amini-Khoei H, Lorigooini Z and Rafieian-Kopaei M (2020). Oxidative stress and antioxidants in diabetes mellitus. *Asian Pac. J. Trop. Med.,* **13**(10): 431-438.

He P, Wu Y, Shun J, Liang Y, Cheng M and Wang Y (2017). Baicalin ameliorates liver injury induced by chronic plus binge ethanol feeding by modulating oxidative stress and inflammation via CYP2E1 and NRF2 in mice. *Oxid Med. Cell. Longev*., **2017**: 4820414.

Jang JY, Im E and Kim ND (2023). Therapeutic potential of bioactive components from *Scutellaria baicalensis* Georgi in inflammatory bowel disease and colorectal cancer: A review. *Int. J. Mol. Sci*., **24**(3): 1-21.

Kharroubi AT and Darwish HM (2015). Diabetes mellitus: The epidemic of the century. *World. J. Diabetes*., **6**: 850-867.

Khazeei Tabari,MA, Iranpanah A, Bahramsoltani R and Rahimi R (2021). Flavonoids as promising antiviral agents against SARS-CoV-2 infection: A mechanistic review. *Molecules*., **26**(13): 1-36.

Li J, Yang Y, Wang H, Ma D, Wang H, Chu L, Zhang Y and Gao Y (2022). Baicalein ameliorates myocardial ischemia through reduction of oxidative stress, inflammation and apoptosis via TLR4/MyD88/MAPK(S)/NF-κB pathway and regulation of Ca(2+) homeostasis by L-type Ca(2+) channels. *Front. Pharmacol.,* **13**: 842723.

Liang J, Zhou Y, Cheng X, Chen J, Cao H, Guo X, Zhang C, Zhuang Y and Hu G (2023). Baicalin attenuates H(2)O(2)-induced oxidative stress by regulating the AMPK/Nrf2 signaling pathway in IPEC-J2 Cells. *Int. J. Mol. Sci*., **24**(11): 9435.

Ma L, Wu F, Shao Q, Chen G, Xu L and Lu F (2021). Baicalin alleviates oxidative stress and inflammation in diabetic nephropathy via Nrf2 and MAPK signaling pathway. *Drug. Des. Devel. Ther.,* **15**: 3207-3221.

Nouri A, Farzanegi P and Azarbayjani MA (2022). Effects of resveratrol supplementation and exercise on apoptosis, lipid profile and expression of farnesoid X receptor, liver X receptor and sirtuin 1 genes in the liver of type 1 diabetic rats. *Med. Lab. J.,* **16**(4): 39-46.

Oguntibeju OO (2019). Type 2 diabetes mellitus, oxidative stress and inflammation: Examining the links. *Int. J. Physiol. Pathophysiol. Pharmacol.,* **11**(3): 45-63.

Prajapati C, Rai SN, Singh AK, Chopade BA, Singh Y, Singh SK, Haque S, Prieto MA and Ashraf GM (2025). An update of fungal endophyte diversity and strategies for augmenting therapeutic potential of their potent metabolites: Recent advancement. *Appl. Biochem. Biotechnol.,* **197**(5): 2799-2866.

Rai SN, Singh C, Singh A, Singh MP and Singh BK (2020). Mitochondrial dysfunction: A potential therapeutic

target to treat Alzheimer's disease. *Mol. Neurobiol*., **57**(7): 3075-3088.

Ramakrishna K, Karuturi P, Siakabinga Q, Krishnamurthy S, Singh S, Kumari S, Kumar GS, Sobhia ME and Rai SN (2024). Indole-3 carbinol and diindolylmethane mitigated beta-amyloid-induced neurotoxicity and acetylcholinesterase enzyme activity: *In-silico*, *in-vitro* and network pharmacology study. *Diseases*., **12**(8): 1-14.

Singh S, Meena A and Luqman S (2021). Baicalin mediated regulation of key signaling pathways in cancer. *Pharmacol. Res*., **164**: 105387.

Srivastava P, Tripathi PN, Sharma P, Rai SN, Singh SP, Srivastava RK, Shankar S and Shrivastava SK (2019). Design and development of some phenyl benzoxazole derivatives as a potent acetylcholinesterase inhibitor with antioxidant property to enhance learning and memory. *Eur. J. Med. Chem*., **163**: 116-135.

Sparmann G, Jaschke A, Loehr M, Liebe S and Emmrich J (1997). Tissue homogenization as a key step in extracting RNA from human and rat pancreatic tissue. *Biotechniques*., **22**(3): 408-410, 412.

Szkudelski T and Szkudelska K (2023). The anti-diabetic potential of baicalin: Evidence from rodent studies. *Int. J. Mol. Sci*., **25**(1): 1-20.

Tomita T (2016). Apoptosis in pancreatic β-islet cells in Type 2 diabetes. *Bosn. J. Basic Med. Sci.,* **16**(3): 162-179.

Tripathi PN, Lodhi A, Rai SN, Nandi NK, Dumoga S, Yadav P, Tiwari AK, Singh SK, El-Shorbagi AA and Chaudhary S (2024). Review of pharmacotherapeutic targets in Alzheimer's disease and its management using traditional medicinal plants. Degener *Neurol. Neuromuscul. Dis*., **14**: 47-74.

Tripathi PN, Srivastava P, Sharma P, Tripathi MK, Seth A, Tripathi A, Rai SN, Singh SP and Shrivastava SK (2019). Biphenyl-3-oxo-1,2,4-triazine linked piperazine derivatives as potential cholinesterase inhibitors with anti-oxidant property to improve the learning and memory. *Bioorg. Chem*., **85**: 82-96.

Wang L, Feng T, Su Z, Pi C, Wei Y and Zhao L (2022). Latest research progress on anticancer effect of baicalin and its aglycone baicalein. *Arch. Pharm. Res*., **45**(8): 535-557.

Watson D and Loweth AC (2009). Oxidative and nitrosative stress in beta-cell apoptosis: Their contribution to beta-cell loss in type 1 diabetes mellitus. *Br. J. Biomed. Sci*., **66**(4): 208-215.

Wen Y, Wang Y, Zhao C, Zhao B and Wang J (2023). The Pharmacological Efficacy of Baicalin in Inflammatory Diseases. *Int. J. Mol. Sci*., **24**(11): 1-16.

Whiting DR, Guariguata L, Weil C and Shaw J (2011). IDF diabetes atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes. Res. Clin. Pract*., **94**: 311-321.

Xin L, Gao J, Lin H, Qu Y, Shang C, Wang Y, Lu Y and Cui X (2020). Regulatory mechanisms of baicalin in cardiovascular diseases: A review. *Front. Pharmacol*., **11**: 583200.

Yadav AK, Singh NK, Singh A, Ashish A, Singh S, Rai SN, Singh SK and Singh R (2024). Investigation of serum pro-inflammatory markers and trace elements among short stature in eastern Uttar Pradesh and Bihar populations. *J. Inflamm. Res*., **17**: 6063-6073.

Yaghini N, Mahmoodi M, Asadikaram GR, Khoramdelazad H and Kazemi Arababadi M (2011). Serum levels of interleukin 10 (IL-10) in patients with type 2 diabetes. *Iran. Red. Crescent. Med. J.,* **13**: 752.

Yu M, Han S, Wang M, Han L, Huang Y, Bo P, Fang P and Zhang Z (2022). Baicalin protects against insulin resistance and metabolic dysfunction through activation of GALR2/GLUT4 signaling. *Phytomedicine*., **95**: 153869.

Zhao ZF, Zhang Y, Sun Y, Zhang CH and Liu MW (2021). Protective effects of baicalin on caerulein-induced AR42J pancreatic acinar cells by attenuating oxidative stress through miR-136-5p downregulation. *Sci. Prog*., **104**(2): 368504211026118.