



Chrysin ameliorates STZ-induced diabetes in rats: possible impact of modulation of TLR4/NF- κ B pathway

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Abstract

Background and purpose: Growing evidence advocates that upregulation of toll-like receptor 4 (TLR4) has been suggested as a causative influence in the development and complications of diabetes mellitus. We aimed to study the antidiabetic activity of chrysin against streptozotocin (STZ)-induced diabetes *via* down-regulation of TLR4/nuclear factor (NF- κ B)/heat shock protein 70 (HSP70) pathway as well as modulation of clusters of differentiation 4 (CD4+) in rats.

Experimental approach: Fifty rats were divided into five groups (n = 10). Group I, normal rats received a single intraperitoneal injection of buffer citrate; group II, STZ-induced diabetic rats; groups III-V, diabetic rats received glimepiride (0.5 mg/kg; p.o.) or chrysin (40 and 80 mg/kg; p.o.) respectively, for 10 days. Serum samples were extracted to determine nitric oxide (NO), malondialdehyde (MDA), and reduced glutathione (GSH), insulin, CD4+, TLR4, and NF- κ B. Pancreatic tissue samples were extracted to determine glucose transporter 2 (GLUT2). Part of the pancreas was kept in formalin for pathological studies.

Findings/Results: An elevation in blood glucose, NO, and MDA serum levels and a reduction of pancreatic GLUT2 content, insulin, and GSH serum levels were observed in diabetic rats. STZ injection, also, showed an increase in serum TLR4, NF- κ B, and HSP70 levels and a reduction in serum CD4+ levels with pancreatic cells necrosis. These biochemical and histological changes were reversed in glimepiride and chrysin groups.

Conclusion and implications: The present study proved that chrysin has a potent anti-diabetic effect through the elevation of insulin and GLUT2 levels, the reduction of oxidative stress, and the inflammatory pathways TLR4/NF- κ B/HSP70 with the regulation of CD4+.

Keywords: CD4+; Chrysin; GLUT2; HSP70; TLR4.

INTRODUCTION

Diabetes mellitus is a chronic inflammatory disease that markedly displays a deficiency of insulin secretion associated with hyperglycemia (1). It affects more than 451 million people and will reach 693 million cases in 2045, worldwide (2). There is a high incidence of diabetes in developing nations and half of the cases are undiagnosed (3). It is also triggered by long-term immune system imbalance and metabolic syndrome and enhanced by an energy-rich diet associated with obesity and a sedentary lifestyle (4).

Streptozotocin (STZ) is a famous diabetogenic drug usually used in many animal models to induce diabetes *via* targeting the

β cells of the pancreas (5) and is uptaken by glucose transporter 2 (GLUT2) as a glucose analog and falsely identified as glucose (6). GLUT2 is a transmembrane carrier protein, formed of 524 amino acids, that facilitates glucose transport across cell membranes (7). It is located in pancreatic β cells, the intestinal mucosa absorptive epithelial cells, hepatocytes, and kidneys (8). Studies have revealed that there is a strong relation between GLUT2 and diabetes pathogenesis since there was a decrease in GLUT2 levels in pancreatic β cells in diabetic animals (9).

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Diabetes mellitus is associated with inflammation that formed multiorgan dysfunction. The inflammatory cytokines such as interleukin (IL)-1 β and interferon (IFN)- γ modulate and elevate insulin signaling in diabetes (10). Also, some pro-inflammatory cytokines can cause insulin resistance in adipose tissues and the liver due to inhibition of insulin signal transduction (11). Toll-like receptor 4 (TLR4) is a transmembrane protein and is considered an important inflammatory factor that activates the innate system *via* increasing signaling of nuclear factor kappa B (NF- κ B) that leads to enhancement of activated β cells and some inflammatory cytokines (12). Additionally, under stressful conditions as diabetes, changes in heat shock proteins (HSPs) expression occur related to hyperglycemia (13).

Nowadays, researchers are targeting natural products in the management and treatment of many diseases. One of the most important flavones-rich natural products is chrysin (5,7-dihydroxyflavone). It is an abundant flavonoid in *Oroxylum* and *Passiflora* genus, Chamomile aerial parts as well as honey and propolis (14). It has been established that chrysin is rich in antioxidant enzymes and has many pharmacological actions including antidiabetic, anti-inflammatory, and antioxidant properties as well as cardiac and hepatic protective effects (15). Suppression of inflammation that activates β -cells and insulin secretion are the basic mechanisms of the antidiabetic drugs (16). TLR4 and HSPs are interesting biomarkers for diabetes in experimental studies and clinical trials. The current study aimed to evaluate a novel underlying mechanism of chrysin as an antidiabetic drug against STZ-induced diabetes *via* down-regulation of TLR4/NF- κ B/HSP70 pathway as well as modulation of clusters of differentiation 4 (CD4+) in rats.

MATERIALS AND METHODS

Animals

Fifty albino Wistar male rats, weighing 120-140 g, were purchased from the National Research Centre (NRC; Cairo, Egypt). Throughout the experimental period, the animal had free access to food and water as well as kept in a quiet area with a maintained temperature of

20 \pm 1 $^{\circ}$ C on a 12/12-h light/dark cycle. The experimental protocol was approved by the Ethics and Animal Care Committee of the National Research Centre (Ethic No. NRC-MREC) and following the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

Drugs and kits

Chrysin (purity 97%) was purchased from Sigma Aldrich Chemical Co., USA; glimepiride, Sanofi-Aventis, Egypt; STZ, Sigma Aldrich Chemical Co., USA; nitric oxide (NO), MDA, and GSH, Biodiagnostic, Egypt; insulin, GLUT2, CD4+, TLR4, and NF- κ B ELISA kits were from NOVA, Beijing, China; and HSP70 ELISA kit procured from Cusabio, China.

Experimental design

STZ was dissolved in 0.1 M citrate buffer (pH 4.5) 50 mM sodium citrate solution in a dose of 50 mg/kg (intraperitoneally) to induce diabetes (17); then rats were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Blood samples were taken 48 h after injection of STZ to ensure hyperglycemia, and the fasting plasma glucose level of rats was determined using glucose strips. Rats with plasma glucose concentrations of more than 300 mg/dL were considered diabetic and included in the experiments (18). On the other hand, rats that were not hyperglycemic were excluded from the study (about 10%).

Fifty rats were divided into five groups, 10 each including group I, normal rats received a single intraperitoneal injection of buffer citrate; group II, STZ-induced diabetic rats; groups III-V, diabetic rats received glimepiride (0.5 mg/kg; p.o.) (19) or chrysin (40 and 80 mg/kg; p.o.), respectively, for 10 days. (20).

Determination of blood glucose level

Collection of blood samples was done *via* tail vein of all rats, and quickly fixed on the glucose strips. Then, blood glucose levels were quantified using One Touch SureStep Meter, LifeScan, Calif, USA. Blood glucose levels were evaluated in groups at the first ten days of the experiment.

Estimation of oxidative stress biomarkers

At finalizing the experiment, blood samples were collected *via* the retro-orbital plexus of each fasting rat anesthetized with a low dose of ketamine (20 mg/kg) for 12 h, with the aid of a glass capillary tube. Blood samples were centrifuged using a cooling centrifuge (Laborezentrifugen, 2k15, Sigma, Germany) at 3000 rpm for 15 min (21). Serum samples were harvested and kept at -20 °C until analyzed. GSH, NO, and MDA was determined according to the method described previously (22-24).

Determination of GSH

GSH assessment is based on the reduction of 5,5-dithiobis-2-nitrobenzoic acid to produce a yellow compound. Serum was deproteinized with 2 mol/L perchloric acid, centrifuged for 10 min at 1000 g and the supernatant was neutralized with 2 mol/L potassium hydroxide. The reaction medium contained 100 mmol/L phosphate buffer (pH 7.2), 2 mmol/L nicotinamide dinucleotide phosphate acid, 0.2 U/mL glutathione reductase, 70 µmol/L 5,5-dithiobis (2-nitrobenzoic acid). To determine GSH the supernatant was neutralized with 2 mol/L potassium hydroxide, reacted with 70 µmol/l 5,5-dithiobis (2-nitro benzoic acid), and read at 420 nm.

Determination of nitric oxide

The serum content of NO was detected by measuring the nitrate/nitrite (NO_x), the stable degradation products of NO. Thus, the stable end-products of NO, nitrite (NO₂⁻), and nitrate (NO₃⁻), were used as indicators of NO production. NO_x was measured after the reduction of nitrate to nitrite by copperized cadmium granules (Cd) in glycine buffer at pH 9.7. Quantitation of NO₂⁻ was based on the Griess reaction, in which a chromophore with strong absorbance at 540 nm is formed by the reaction of nitrite with a mixture of naphthyl ethylenediamine and sulphanilamide.

Determination of MDA

MDA, a reactive aldehyde that is a measure of lipid peroxidation, was determined using a spectrophotometer. A mixture of 0.5 mL serum, 0.5 mL physiological solution, and 0.5 mL 25%

trichloroacetic acid was centrifuged at 2,000 rpm for 20 min. One mL of protein-free supernatant was mixed with 0.25 mL 0.5% thiobarbituric acid and heated at 95 °C for 1 h. After cooling, the intensity of the pink color of the end fraction product was determined at 532 nm. Estimation of serum levels of inflammatory mediators (TLR4, NF-κβ, HSP70), insulin, and CD4+.

Serum levels of TLR4, NF-κβ, HSP70, insulin, and CD4+ were evaluated using an ELISA kit. NOVA and Cusabio kits manufacturer's instructions were followed for calculating the results. Standards and samples were pipetted into wells with immobilized antibodies specific for rat TLR4, NF-κβ, HSP70, insulin, and CD4+ and then were incubated for 30 min at 37 °C. After incubation and washing, horseradish peroxidase-conjugated streptavidin was pipetted into the wells and incubated for 30 min at 37 °C, which were washed once again. Tetramethylbenzidine (TMB) substrate solution was added to the wells and incubated for 15 min at 37 °C; color developed proportionally to the amount of TLR4, NF-κβ, HSP70, insulin, and CD4+ bound. Color development was discontinued (stop solution) and after 10 min color intensity was measured at 450 nm.

Estimation of pancreatic GLUT2 content

Animals were sacrificed by decapitation under a high dose of ketamine (60 mg/kg) (25) under light anesthesia then pancreas tissues were dissected immediately. One part of the pancreas was extracted and was used for the preparation of 20% homogenate for estimation of pancreatic GLUT2 level. Standards and samples were pipetted into wells with immobilized antibodies specific for rat GLUT2 and then were incubated for 30 min at 37°C. After incubation and washing, horseradish peroxidase-conjugated streptavidin was pipetted into the wells and incubated for 30 min at 37 °C, which were washed once again. TMB substrate solution was added to the wells and incubated for 15 min at 37 °C; color developed proportionally to the amount of GLUT2 bound, then add the stop solution and after 10 min color intensity was measured at 450 nm.

Histopathological studies

Another part of the pancreas was used. The specimens were fixed, for 72 h, in 10% neutral-buffered formalin saline. Following an overnight fixation, slices (3-4 mm) of kidney tissue were dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin wax (58-60 °C). Blocks were made and sectioned of 5 µm thickness with a microtome. The tissue sections were stained with hematoxylin and eosin (26). Images were examined using a binocular Olympus CX31 microscope.

Statistical analysis

Data are presented as mean ± SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Tukey-Kramer test for multiple comparisons. *P* values < 0.05 were considered statistically significant.

RESULTS

Effect of chrysin on blood glucose level, pancreatic GLUT2 content, and serum insulin level

STZ injection showed a significant elevation of blood glucose level by 493% ($593 \pm$

3.64 mg/dL) as compared to the normal group ($100 \pm 3.76 \text{ mg/dL}$). While glimepiride (0.5 mg/kg) and chrysin (40 and 80 mg/kg) administration significantly reduced blood glucose level (310 ± 19.74 , 277 ± 5.12 , and $105 \pm 9.01 \text{ mg/dL}$) by 48%, 53%, and 82%, respectively as compared to STZ group. Moreover, chrysin (80 mg/kg) administration showed a significant reduction of blood glucose level by 66% compared to the glimepiride group (Fig. 1).

STZ injection led to a significant reduction in pancreatic GLUT2 content (0.38 ± 0.02 vs $1.31 \pm 0.08 \text{ ng/mL}$) and insulin serum level (0.69 ± 0.02 vs $4.08 \pm 0.10 \text{ mU/L}$) by 71% and 83%, respectively as compared to normal group, while oral administration of glimepiride and chrysin (40 and 80 mg/kg) significantly elevated pancreatic GLUT2 content (0.66 ± 0.04 , 0.69 ± 0.07 , and $1.28 \pm 0.09 \text{ ng/mL}$) by 71%, 81%, and 237%, respectively, and serum insulin level (1.89 ± 0.02 , 2.09 ± 0.07 , and $3.54 \pm 0.08 \text{ mU/L}$) by 175%, 205%, and 417%, respectively as compared to STZ group. Moreover, chrysin (80 mg/kg) administration showed a significant elevation of pancreatic GLUT2 content by 94% and serum insulin level by 87% when compared to the glimepiride group (Fig. 1).

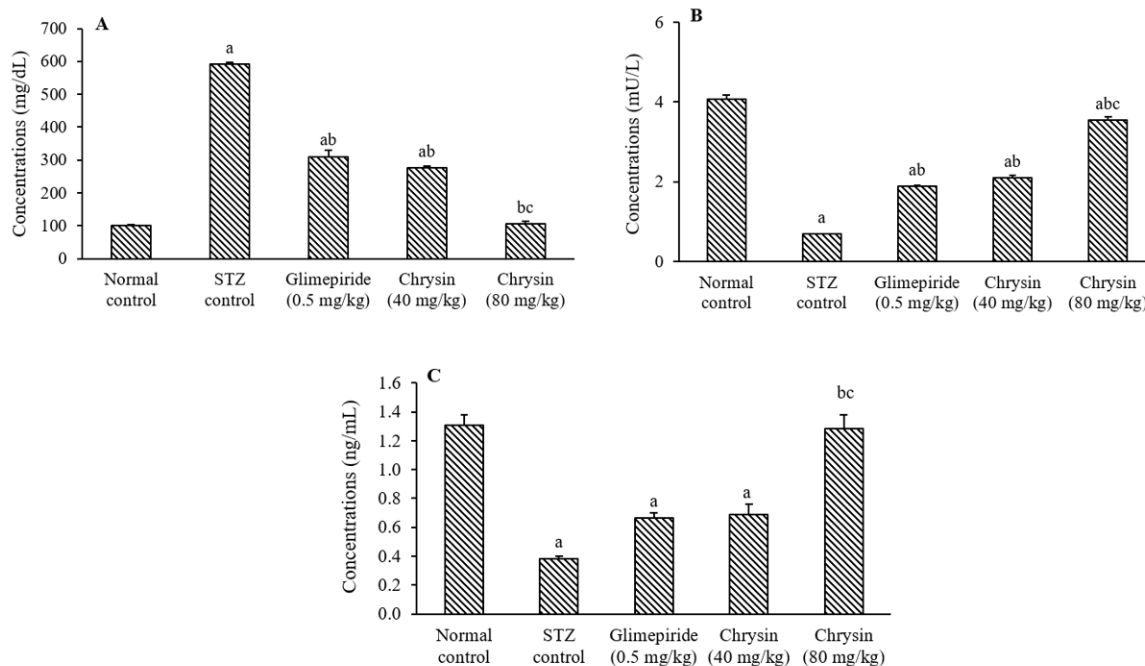


Fig. 1. Effect of chrysin (40 and 80 mg/kg) on (A) blood glucose level, (B) serum insulin level, and (C) pancreatic glucose transporter 2 level. Data are expressed as mean ± SEM. ^a*P* < 0.05 Indicates significant differences in comparison with the normal control (saline), ^b*P* < 0.05 versus STZ control, and ^c*P* < 0.05 against glimepiride. STZ, Streptozotocin.

Table 1. Effect of chrysin (40 and 80 mg/kg) on serum levels of nitric oxide, malondialdehyde, and reduced-glutathione. Data are expressed as mean \pm SEM.

Factors	Normal control	Streptozotocin control	Glimepiride (0.5 mg/kg)	Chrysin (40 mg/kg)	Chrysin (80 mg/kg)
Nitric oxide ($\mu\text{mol/L}$)	59.16 \pm 6.97	160.00 \pm 0.93 ^a	112.69 \pm 2.89 ^{ab}	119.33 \pm 0.92 ^{ab}	94.96 \pm 1.92 ^{abc}
Malondialdehyde (nmol/L)	9.60 \pm 0.54	35.33 \pm 0.91 ^a	26.10 \pm 0.43 ^{ab}	26.00 \pm 0.55 ^{ab}	23.90 \pm 0.08 ^{ab}
Glutathione (mg/dL)	19.97 \pm 1.29	6.92 \pm 0.10 ^a	12.40 \pm 0.31 ^{ab}	11.73 \pm 0.64 ^{ab}	13.07 \pm 0.67 ^{ab}

^a $P < 0.05$ Indicates significant differences in comparison with the normal control (saline), ^b $P < 0.05$ versus streptozotocin control, and ^c $P < 0.05$ against glimepiride.

Effect of chrysin on serum levels of NO, MDA, and GSH

STZ injection significantly elevated NO and MDA levels (160.00 \pm 0.93 $\mu\text{mol/L}$ and 35.33 \pm 0.91 nmol/L) by 170% and 268%, respectively as compared to the normal group (59.16 \pm 6.97 $\mu\text{mol/L}$ and 9.60 \pm 0.54 nmol/L). While glimepiride and chrysin (40 and 80 mg/kg) administration showed a significant reduction of NO serum level (112.69 \pm 2.89, 119.33 \pm 0.92, and 94.96 \pm 1.92 $\mu\text{mol/L}$) by 30%, 25% and 41% and MDA serum level (26.10 \pm 0.43, 26.00 \pm 0.55, and 23.90 \pm 0.08 nmol/L) by 26%, 26%, and 32%, respectively as compared to STZ group. Besides, chrysin (80 mg/kg) administration diminished NO serum level by 16% as compared to glimepiride (Table 1).

Serum level of GSH was significantly reduced in STZ group (6.92 \pm 0.10 mg/dL) by 65%, as compared to normal group (19.97 \pm 1.29 mg/dL), while the treatment with glimepiride and chrysin (40 and 80 mg/kg) showed a significant elevation of GSH (12.40 \pm 0.31, 11.73 \pm 0.64, and 13.07 \pm 0.67 mg/dL) by 79%, 70%, and 89%, respectively when compared to STZ rats (Table 1).

Effect of chrysin on serum levels of TLR4, NF- κ B, and HSP70

STZ injection induced a significant elevation in TLR4 (2568 \pm 120.74 vs 602 \pm 93.40 pg/mL) and NF- κ B serum levels (5910 \pm 28.84 vs 4682 \pm 81.11 pg/mL) by 327% and 26%, respectively as compared to the normal group. Glimepiride and chrysin (80 mg/kg)

administration indicated a significant reduction of TLR4 serum levels (1140 \pm 45.61 and 680 \pm 71.55 pg/mL) by 56% and 74%, while glimepiride and both doses of chrysin (40 and 80 mg/kg) administration showed a significant reduction of NF- κ B (5313 \pm 5.00, 5535 \pm 28.8, 1 and 4935 \pm 14.00 pg/mL) by 10%, 6%, and 17%, respectively as compared to STZ group. Moreover, chrysin (80 mg/kg) administration showed a significant reduction of TLR4 serum level by 40.35% and NF- κ B by 7% as compared to the glimepiride group (Fig. 2).

HSP70 was significantly elevated in the STZ group (3473 \pm 31 vs 1180 \pm 106 pg/mL) by 194% as compared to the normal group. While the treatment with glimepiride and chrysin (40 and 80 mg/kg) showed a significant decrease of HSP70 (2458 \pm 9, 2445 \pm 2, and 1390 \pm 8 pg/mL) by 29%, 30%, and 60%, respectively as compared to STZ group. Moreover, chrysin (80 mg/kg) showed a significant reduction of HSP70 serum level by 43% compared to the glimepiride group (Fig. 2).

Effect of chrysin on serum CD4+ level

STZ injection showed a significant reduction CD4+ serum levels (1.62 \pm 0.05 vs 3.32 \pm 0.15 U/mL) by 51%, as compared to the normal group, while oral administration of glimepiride and chrysin (40 and 80 mg/kg) demonstrated a significant elevation of CD4+ serum level by (2.26 \pm 0.06, 2.33 \pm 0.02, and 3.13 \pm 0.19 U/mL) 40%, 44% and 94% as compared to STZ group. Moreover, chrysin (80 mg/kg) showed a significant elevation of CD4+ serum level by 38% as compared to the glimepiride group (Fig. 3).

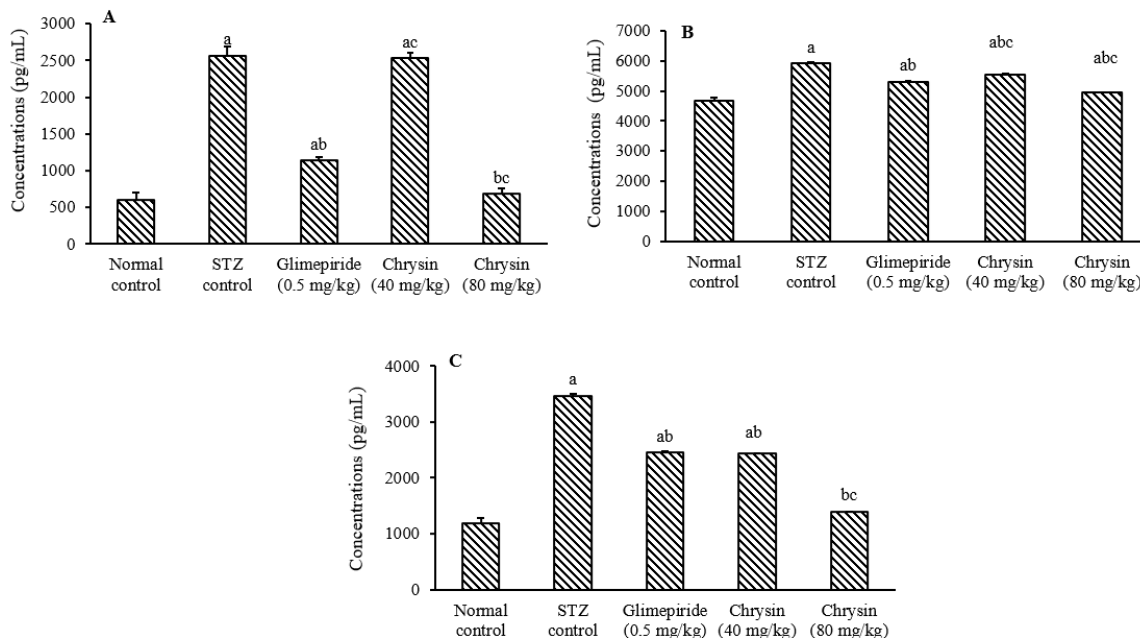


Fig. 2. Effect of chrysin (40 and 80 mg/kg) on serum levels of (A) toll-like receptor 4, (B) nuclear factor-κβ, and (C) heat shock protein 70. Data are expressed as mean ± SEM. ^a*P* < 0.05 Indicates significant differences in comparison with the normal control (saline), ^b*P* < 0.05 versus STZ control, and ^c*P* < 0.05 against glimepiride. STZ, Streptozotocin.

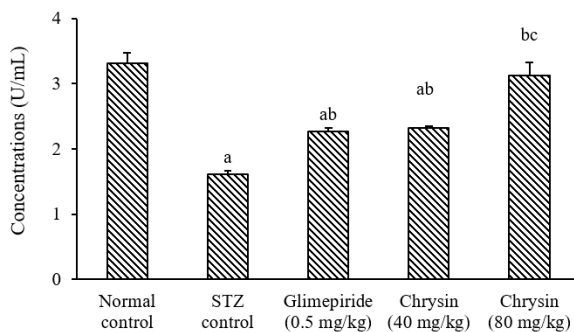


Fig. 3. Effect of chrysin (40 and 80 mg/kg) on serum CD4+ level. Data are expressed as mean ± SEM. ^a*P* < 0.05 Indicates significant differences in comparison with the normal control (saline), ^b*P* < 0.05 versus STZ control, and ^c*P* < 0.05 against glimepiride. STZ, Streptozotocin.

Histopathological results

The normal control group pancreatic section showed regularly shaped pancreatic islets with normal acini tissues and islets of Langerhans (Fig. 4A), while the STZ group pancreatic section showed pancreatic islets with irregular, not well-defined, necrotic islets of Langerhans cells (Fig. 4B). Glimepiride group pancreatic section showed regularly arranged pancreatic

islets with normal acini tissues and normal islets of Langerhans (Fig. 4C). Chrysin (40 mg/kg) group pancreatic section showed regularly and arranged pancreatic islets, with almost normal acini tissues and islets of Langerhans (Fig. 4D). Chrysin (80 mg/kg) group pancreatic section showed regularly arranged pancreatic islets with normal acini tissues and islets of Langerhans (Fig. 4E).

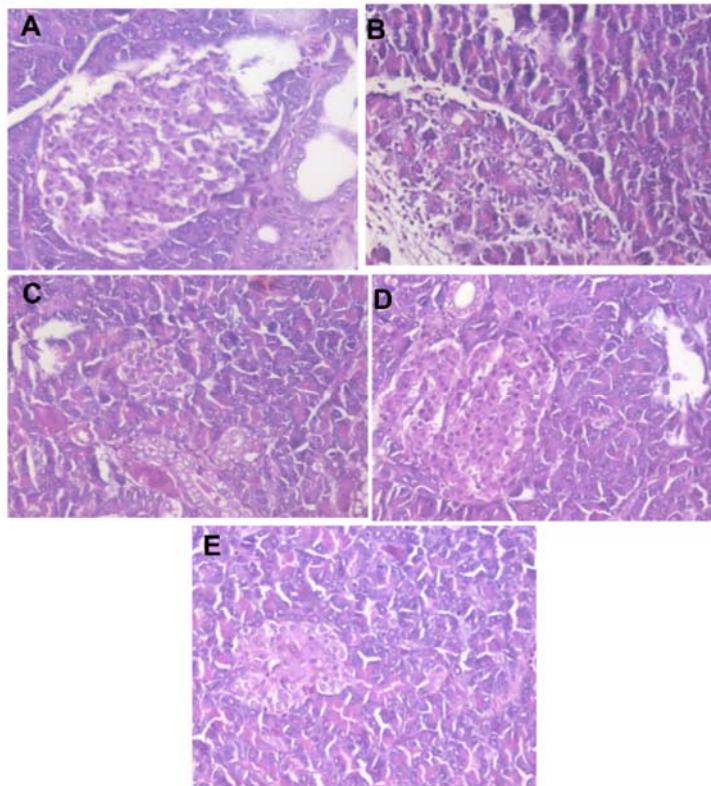


Fig. 4. Evaluation of the effect of chrysin on pancreatic tissue using hematoxylin and eosin staining method. (A) The pancreatic section from the normal control group showed pancreatic islets were shaped regularly and arranged evenly, with normal islets of Langerhans and normal acini tissues, duct; (B) pancreatic section from streptozotocin group (positive control) showed pancreatic islets with irregular islets of Langerhans cells, not well defined, necrosis of cells and normal acini tissues; (C) pancreatic section from glimepiride group showed pancreatic islets were shaped regularly and arranged evenly, with normal islets of Langerhans and normal acini tissues; (D) pancreatic section from chrysin (40 mg/kg) group showed pancreatic islets were shaped regularly and arranged evenly, with almost normal islets of Langerhans and normal acini tissues and Blood vessel; and (E) pancreatic section from chrysin (80 mg/kg) group showed pancreatic islets were shaped regularly and arranged evenly, with normal islets of Langerhans and normal acini tissues. Magnification: $\times 400$.

DISCUSSION

Diabetes mellitus is a chronic illness depicted by hyperglycemia and defect in insulin secretion. Oxidative stress is one of development the major determinants for diabetes (27). It contributes to insulin deficiency (28) and causes insulin signals inhibition through adipocytokine (29). Hyperlipoproteinemia and hyperglycemia are usually accompanied by diabetes caused by STZ due to chronic oxidative stress (30,31). In addition, neurologic complications (32) and nephropathy are also included in this disease (33). In the present study, STZ revealed a significant increase in blood glucose level and marked reduction of insulin level when compared with the control group. STZ also

showed a marked increase in NO and MDA and a significant increase in GSH levels. Same results were obtained previously (27,31) On the contrary, treatment with chrysin, at both administered doses, or glimepiride exhibited a reversal of these elevated blood glucose levels and reduced insulin serum level induced by STZ. Chrysin exhibited an antidiabetic effect similar to the effect of metformin, as it reduced the blood glucose level and elevated insulin level when administered to diabetic rats (12). Moreover, both doses of chrysin or glimepiride administration caused a reduction in NO and MDA and an increase in GSH level in serum compared to the STZ group. These results suggest chrysin's ability to modify oxidative stress (20) and its reduction of blood glucose level (12).

The current results also revealed that insulin deficiency in STZ-induced diabetic rats induced inflammatory cytokines that led to downregulation of GLUT2 expression which is evidenced by low GLUT2 content. Acting as a toxic glucose analog, STZ destroyed part of β -cells islets *via* targeting pancreatic β -cells *via* GLUT2 transporter, leading to a significant reduction of insulin level (34). The reduced sensitivity of insulin was attributed to stimulation of proinflammatory tumor necrosis factor-alpha (TNF- α) and IL-6 that interfered with the insulin receptor substrate regular phosphorylation, reducing inositol triphosphate kinase (PI3K/AKT) signaling pathway and provoking GLUTs expression failure (35). GLUT2 disappearance is a marker of the β cell glucose-unresponsiveness that is associated with diabetes and the combination of high glucose and mechanical stress decreased GLUT2 and GLUT4 expression at the plasma membrane (36). In addition, our results showed an increase in insulin and GLUT2 levels in chrysin or glimepiride groups. Chrysin acts as an insulin sensitizer (19) and may be due to 11 β -hydroxysteroid dehydrogenase type 1 suppression, which led to a reduction in cortisol level with insulin sensitivity elevation (37).

Hyperglycemia triggers inflammation that is evidenced by activation of TLR4 which in turn activates the expression of NF- κ B leading to the production of high levels of ROS and inflammation (38). In the current study, TLR4 and NF- κ B levels were increased in STZ-induced diabetic rats as shown in another previous study (39). The inflammation produced by STZ, in this study, led to an elevation of TLR4 and NF- κ B levels that were down-regulated with chrysin or glimepiride treatments. The antioxidant activity of chrysin was attributed to the downregulation of TLR4 in diabetic rats (38). Moreover, hyperglycemia activates the HSP pathway that plays a role in a cascade of events as oxidative stress, production of inflammatory mediators, and inducing finally cell injury (40). HSPs are highly expressed in the areas of inflammation, to refold denatured peptides caused by ROS (41). A previous study proposed that HSP70 generated inflammation that worsened the diabetic condition, which in turn provokes

overexpression of TNF- α *via* a TLR4/NF- κ B pathway (42). In the current work, STZ-induced diabetes augmented the production of HSP70 which was proved previously (31). For the first time, we evaluated the effect of chrysin or glimepiride on the expression of HSP70 levels. There was a significant reduction of HSP70 levels by the treatment with both doses of chrysin or glimepiride, so these results reflect a potent anti-diabetic effect of chrysin *via* inhibiting the TLR4/NF- κ B/HSP70 pathway (42).

In addition, the most important finding of the present study is the level of CD4+ that was reduced in diabetic rats, while an administration of chrysin restored its level to normal, especially with the high dose; also, glimepiride modulated the reduction of CD4+ induced by STZ. Diabetes mellitus causes an imbalance between TH1/TH2 defeating the immunological injuries as evidenced by a reduction in CD4+ in STZ diabetic rats (43). In healthy individuals, high CD4+ and CD8+ are observed and their counts were decreased in diabetic patients (44). Our results were supported by the histopathological investigations which showed that chrysin or glimepiride reversed the pancreatic cell necrosis induced by STZ. STZ induced degenerative changes in β -cells in the pancreas of the diabetic mice (45). Finally, chrysin high dose exhibited antidiabetic effect more potent than glimepiride through the elevation of insulin, GLUT2, CD4+ secretion and the reduction of inflammatory markers TLR4, NF- κ B, and HSP70. In addition, TLR4, HSP70, and CD4+ are considered attractive biomarkers for scientific research and clinical studies.

CONCLUSION

Treatment of diabetic rats with chrysin restored all oxidative stress and proinflammatory biomarkers to nearly the normal level as well as suppression of TLR4/NF- κ B/HSP70 pathway. It also modulated insulin, GLUT2, and CD4+ levels. Finally, it can be concluded that daily intake of chrysin attenuates the oxidative and inflammatory disorders associated with STZ-induced diabetes, which may be attributed to antioxidant and anti-inflammatory effects. Our

findings showed that chrysin exerts a powerful antioxidant property evidenced by inhibition of oxidative stress. In addition, chrysin exerted a strong anti-inflammatory effect mainly by inhibiting pro-inflammatory cytokines which significantly appeared in results obtained as well as downregulation of TLR4/NF- κ B/HSP70 pathway. So, chrysin seems to be a promising agent in diabetes therapy as it showed better effect when compared to the widely-used glimepiride.

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Conflicts of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

A. Salama designed the study, provided materials, data analysis, interpretation, wrote the article in final form; A. Salama, G.F. Asaad, and A. Shaheen provided the materials and performed the experimental parts. A. Shaheen drafted the manuscript. G.F. Asaad reviewed this manuscript, wrote according to journal style, and approved its submission. The final version of the manuscript was approved by all authors.

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