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THE EFFECTS OF A NEW CHROMENYL-METHYLENE-THIAZOLIDINE-2,4-DIONE IN ALLEVIATING OXIDATIVE STRESS IN A RAT MODEL OF STREPTOZOTOCIN-INDUCED DIABETES

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ABSTRACT. Type 1 diabetes mellitus (T1DM) is caused by the insulin deficiency resulting from the progressive destruction of pancreatic β cells. Thiazolidine-2,4-diones (TZDs) activate the peroxisome proliferator-activated receptor-γ (PPARγ) and enhance the actions of insulin. 5-((6-methyl-4-oxo-4*H*-chromen-3-yl)methylene)-3-(2-(4-nitrophenyl)-2-oxoethyl)-thiazolidine-2,4-dione (TZDd) is a heterocyclic derivative synthesized in our laboratory. The purpose of this study was to examine whether TZDd has hypoglycemic and antioxidant effects in diabetic rats. Its effects were compared with those of quercetin (Que), a potent antioxidant, and with pioglitazone (Pio), a well-known antidiabetic drug. Type 1 DM was induced in Wistar rats by the intraperitoneal administration of streptozotocin (STZ) (60 mg/kg). The non-diabetic and diabetic rats were treated with Que (30 mg/kg/day), pioglitazone (30 mg/kg/day), or TZDd (30 mg/kg/day), for 5 weeks. The serum levels of malondialdehyde (MDA) and protein carbonyl (PC) groups, and the superoxide dismutase (SOD) and catalase (CAT) activities in the blood were then assessed. The results indicated that the TZDd decreased the blood oxidative stress parameters in the treated diabetic rats, compared to Que and pioglitazone. In conclusion, the hypoglycemic and antioxidant effects of TZDd in diabetic rats, suggest its therapeutic properties for the clinical treatment of T1DM.

Keywords: *diabetes mellitus; oxidative stress; quercetin; pioglitazone, thiazolidine-2,4-dione*

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INTRODUCTION

The pathophysiological risk factor of insulin resistance, oxidative stress, hyperglycemia, hyperlipidemia and inflammation are involved in the development of diabetes mellitus (DM) and increase the incidence of diabetic complications [1,2].

Antioxidants from nutrients administered to diabetic animals, have been shown to improve the neuropathy and the endothelial dysfunction in DM [3-5]. Quercetin, one of the major flavonoids present in many fruits and vegetables, is known for its multiple biological effects such as potent vasodilator, free radical-scavenging and antioxidant action, anti-inflammatory, hypoglycemic and neuroprotective [6-9].

Thiazolidine-2,4-diones (TZDs) are the nuclear receptor peroxisome proliferator-activated receptor–gamma (PPAR-γ) agonists and constitute a new class of pharmacological agents used in the management of dyslipidemia and hyperglycemia. Two TZDs, rosiglitazone and pioglitazone, are insulin sensitizers used as oral hypoglycemic agents to treat patients with type 2 DM (T2DM). PPAR-γ is highly expressed in the adipose tissue, where it plays an essential role in the regulation of lipogenesis, lipid storage, insulin sensitivity, glucose metabolism and the transcriptional regulation of a number of genes involved in these metabolic processes. Pioglitazone function as insulin sensitizer and thus, enhance the insulin action and improve hyperglycemia in patients with T2DM [10]. TZDs have also been shown to decrease pancreatic β cell destruction and therefore have a potential role in the treatment and prevention of T1DM [11].

Based on our previous experience in the synthesis of new bioactive compounds bearing the thiazolidine-2,4-dione heterocycle as an important scaffold in medicinal chemistry [12-14], in the present study, we present the synthesis of a new 5-chromenyl-methylene-thiazolidine-2,4-dione derivative (TZDd). Further, we evaluated its effect on the oxidative stress in streptozotocin (STZ)-induced diabetic rats. We hypothesized that TZDd might reduce the oxidative stress and the hyperglycemia induced by T1DM, by attenuating the fasting blood glucose levels and by free radical-scavenging, too.

RESULTS AND DISCUSSION

Chemistry

A new *N*-substituted 5-chromenyl-methylene-thiazolidine-2,4-dione derivative (Figure 1) was synthesized in our laboratory, following a procedure described previously [15].

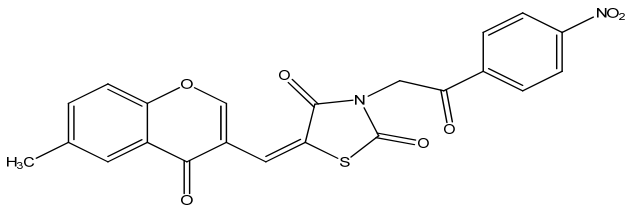

Figure 1. The new chromenyl-methylene-thiazolidine-2,4-dione derivative

Body Weight and Blood Glucose Level in Experimental Groups

The body weight and blood glucose levels of all the groups are shown in Table 1. The body weight decreased significantly ($P<0.05$) in the diabetic rats. The administration of quercetin, pioglitazone or TZDd produced no significant change in the body weight of the non-diabetic or diabetic animals, as compared to the respective controls. The insulin treatment had significant ($P<0.05$) effect on the body weight of diabetic rats, as compared to their respective control. The blood glucose levels were significantly increased in all STZ administered animals. The treatment with quercetin, pioglitazone or insulin produced a significant reduction in the blood glucose levels after 5 weeks, as compared to their respective control groups. The treatment of diabetic rats with TZDd (**DT** group) had cumulative effects in significantly reducing the blood glucose levels in diabetic rats.

Table 1. Body weight and blood glucose level in the experimental groups

Groups	Body weight (g)		Fasting blood glucose level (mg/dl)	
	Intial	Final	Initial	Final
CC	318.2±26.9	331±29.11	81.7±4.54	86.8±3.99
CQ	299.5±45.43	314.5±44.84	86.8±3.19	89.7±2.26
CP	288.4±24.74	307.1±26.03	85.2±2.86	90.5±2.95
CI	291.8±27.21	303±23.05	87.1±4.30	89.4±3.30
CT	298.5±31.1	312.3±30.56	87.3±3.68	90.9±1.85
DC	317.1±26.04	274.5±25.87^{a}	449.4±10.28^{aaa}	457.2±9.22
DQ	303.5±12.13	298.6±16.08	437.8±11.97^{aaa}	247.3±12.2^{bbb}
DP	316.9±13.4	311.4±11.09	441.9±9.26^{aaa}	341.9±9.26^{bbb}
DI	298.1±43.63	264.5±24.43^{a}	439.7±8.98^{aaa}	122.2±8.71^{bbb}
DT	306.1±37.78	299.6±38.94	445.6±6.83^{aaa}	303.5±12.13^{bbb}

CC=control+CMC, CQ=control+quercetin, CP= control+ pioglitazone,
CI=control+insulin, CT=control+TZDd; DS=diabetes+CMC, DQ=diabetes+quercetin,
DP=diabetes+pioglitazone, DI=diabetes+insulin;
DT= diabetes+TZDd.
Results are mean± SD of 10 rats per each group. Statistically significant differences are indicated by the symbols: $^{a}P<0.05$, $^{aaa}P<0.001$ vs. **CC** group; $^{b}P<0.05$, $^{bbb}P<0.001$ vs. **DC** group.

Biochemical Parameters of Oxidative Stress in the Experimental Groups

As a measurement of the oxidative stress, we determined the malondialdehyde (MDA) and protein carbonyl content (PCC) levels in the serum of STZ-induced diabetic rats after 6 weeks of diabetes (Figure 2 a and b). The data in figures 2a and 2b demonstrate that the serum levels of MDA and PCC increased significantly ($P<0.05$) after 6 weeks of diabetes (**DC** group). The diabetic rats treated with quercetin **(DQ** group) and pioglitazone (**DP** group), for 5 weeks, exhibited a significant decrease ($P<0.05$) of the levels of MDA and PCC in their serum, when compared to the diabetic control rats treated with CMC (**DC** group). The administration of insulin (**DI** group) in diabetic rats for 5 weeks reduced non-significantly ($P>0.05$) the serum levels of MDA and PCC, compared to the diabetic control rats treated with CMC (**DC** group). The diabetic rats treated with TZDd for 5 weeks (**DT** group) showed significantly reduced MDA and PC levels ($P<0.05$) in the serum, as compared with the diabetic control rats treated with CMC (**DC** group). The results showed that TZDd decreased the MDA and PCC levels in the serum in a manner similar to quercetin and pioglitazone.

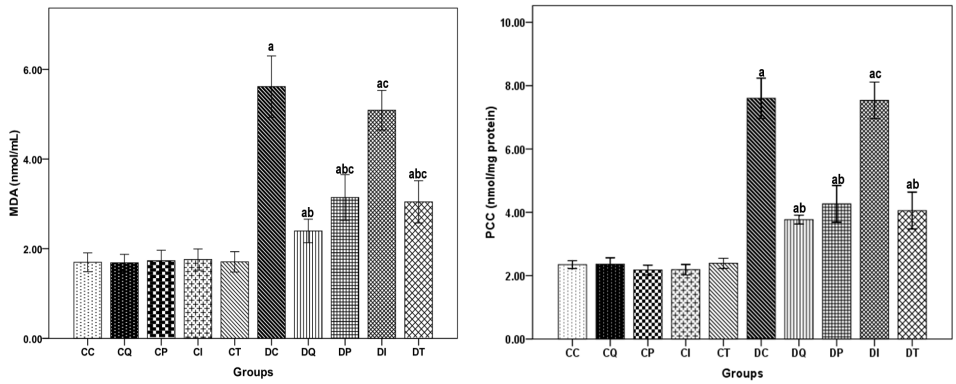

Figure 2. The effects of quercetin, pioglitazone, insulin and TZDd on (a) lipid peroxidation (MDA) (nmol/mL) and (b) protein oxidation (protein carbonyl content) (PCC) (nmol/mg protein) levels in the serum of non-diabetic and diabetic rats. Results are the means ± SD for ten animals each group defined as in the caption of Fig. 2. Statistically significant differences are indicated by the symbols: $^{a}P < 0.05$ vs. **CC** group, $^{b}P < 0.05$ vs. **DC** group and $^{c}P < 0.05$ vs. **DQ** group.

Figure 3 shows the effects of quercetin, pioglitazone, insulin and TZDd on the activities of superoxide dismutase (SOD) (Fig. 3a) and catalase (CAT) (Fig. 3b) in the blood of the non-diabetic control rats and

diabetic rats. The activities of SOD and CAT were significantly lowered (P<0.05) 6 weeks after the STZ administration (**DC** group). The quercetin and pioglitazone treatment for 5 weeks significantly increased the SOD and CAT activities in the blood of the diabetic rats (**DQ** and **DP** groups), when compared to the diabetic control rats treated with CMC (**DC** group). The diabetic rats treated with insulin (**DI** group) for 5 weeks, exhibited a non-significant increase (P>0.05) of the SOD and CAT activities in the blood, compared with the diabetic control rats treated with CMC (**DC** group). The SOD and CAT activities significantly increased (P<0.05) in the blood of the diabetic rats treated with TZDd (**DT** group), when compared to the diabetic control rats treated with CMC (**DC** group).

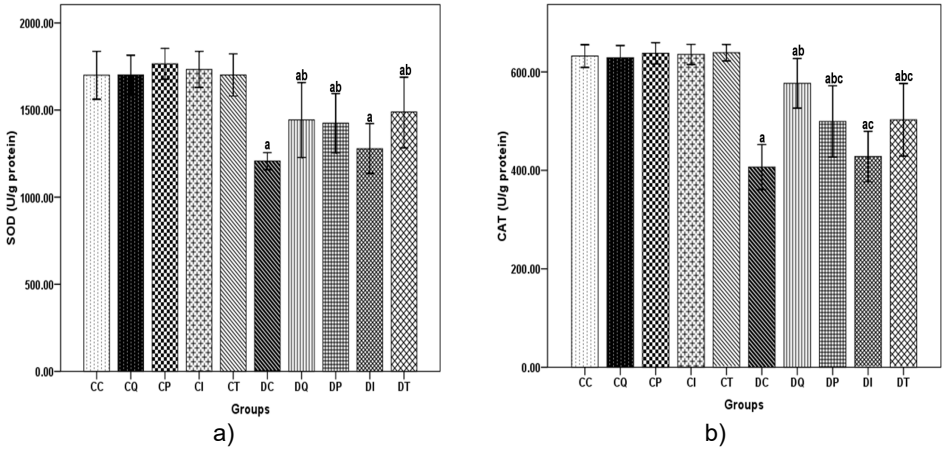

Figure 3. The effects of quercetin, pioglitazone, insulin and TZDd on the level of (a) superoxide dismutase (SOD) (U/g protein) and (b) catalase (CAT) (U/g protein) activities in the blood of non-diabetic and diabetic rats. Results are the means ± SD for ten animals each group defined as in the caption of Statistically significant differences are indicated by the symbols: $^{a}P < 0.05$ vs. **CC** group, $^{b}P < 0.05$ vs. **DC** group and $^{c}P < 0.05$ vs. **DQ** group.

Patients with T1DM suffer from hyperglycemia. The thiazolidine-2,4-diones (TZDs) represent a class of oral antidiabetic agents that are indicated for the treatment of patients with T2DM. Pioglitazone selectively stimulates PPAR-γ and modulates the transcription of genes involved in the control of glucose and lipid metabolism in the liver, adipose tissue and muscles [16,17]. In monotherapy or in combination with other oral antidiabetic drugs, it enhances the blood glucose levels, the long-term glucose control, and the lipid profiles [17]. Therefore, pioglitazone was chosen as a positive control drug in the analysis of glucose metabolism in our research.

Our results revealed that fasting blood glucose (FBG) levels significantly increased in the STZ-induced diabetic rats. This biochemical parameter was also improved in the diabetic rats treated with quercetin and pioglitazone, thereby suggesting that quercetin ameliorated the dysfunction of glucose metabolism in the STZ-induced diabetic rats. In our study, the thiazolidine-2,4-dione derivative (TZDd) effectively alleviated the glucose metabolism disorder in the STZ-induced diabetic rat model. The decrease in the FBG levels was interpreted as being due to the direct hypoglycemic effects.

The oxidative stress is involved in the development and progression of DM and its complications [18]. There are multiple likely sources of reactive oxygen species (ROS) in DM, including glucose autoxidation, glycation of proteins, consumption of NADPH through the polyol pathway, and activation of protein kinase C. ROS are targeted for removal by antioxidants and enzymes such as SOD and CAT. Hyperglycemia leads to elevated ROS, which dysregulates important metabolic pathways to promote micro- and macrovascular complications.

In our work, lipid peroxidation (MDA level) and oxidized proteins (PCC) were used as biomarkers of oxidative stress. This study found that the oxidative stress was higher in the serum of the STZ-induced diabetic rats, evidenced by the increased MDA and PCC levels and decreased antioxidant activities of SOD and CAT in the blood. The treatment of the STZ-diabetic rats with quercetin or pioglitazone reduced the MDA and the PCC levels in serum and increased the antioxidant SOD and CAT activities in the blood, suggesting the capacity of quercetin and pioglitazone to improve the antioxidant defenses in diabetic rats. Recent studies proved that quercetin and pioglitazone reduced the glycemia and decreased the oxidative stress in DM [2-5]. The SOD and CAT activities in the blood significantly increased and MDA and PCC levels in the serum significantly decreased in the diabetic rats treated with TZDd for 5 weeks. These findings suggest that the treatment with TZDd restores the antioxidant status in diabetes.

CONCLUSIONS

In summary, our findings suggest that the treatment with the new thiazolidine-2,4-dione derivative (TZDd) improved hyperglycemia and the antioxidant status in STZ-diabetic rats. Thus, the present study suggests that TZDd is able to produce positive therapeutic interaction in decreasing the oxidative stress produced by STZ-induced diabetes, by attenuating the fasting blood glucose level and by suppressing the oxidative stress.

EXPERIMENTAL SECTION

Drugs and Chemicals

Solvents were obtained from commercial sources (Sigma-Aldrich GmbH, Germany); the reagents were synthesized in our laboratory. Analytical thin layer chromatography was carried out on precoated Silica Gel 60F254 sheets using UV absorption for visualization. The melting points were taken with two melting point meters, Electrothermal and MPMH1 Schorpp, and are uncorrected. The ^{1}H NMR spectra were recorded at room temperature on a Bruker Avance NMR spectrometer operating at 400 MHz and were in accord with the assigned structures. Chemical shift values were reported relative to tetramethylsilane (TMS) as internal standard. The samples were prepared by dissolving the synthesized powder of the compounds in DMSO-d_6 as solvent. GC-MS analyses were realized with an Agilent gas chromatograph 6890 equipped with an apolar Macherey Nagel Permabond SE 52 capillary column. Elemental analysis was registered with a Vario El CHNS instrument.

Streptozotocin (STZ), pioglitazone hydrochloride (Pio), quercetin (Que) (3,3',4',5,7-pentahydroxyflavone dihydrate, >98% purity powder) were purchased from Sigma-Aldrich Chemical Company Inc., (Gillingham, Dorset, UK).

Chemistry

5-((6-Methyl-4-oxo-4*H*-chromen-3-yl)methylene)-3-(2-(4-nitrophenyl)-2-oxoethyl)-thiazolidine-2,4-dione, was obtained according to a technique previously described [15].

5-((6-methyl-4-oxo-4H-chromen-3-yl)methylene)-3-(2-(4-nitrophenyl)-2-oxoethyl)thiazolidine-2,4-dione. Yield 86 %. Light-brown powder, mp: 262 ^{0}C. ^{1}H NMR (DMSO-d_6, 500 MHz,ppm): δ 3.04 (s, 3H, -CH_3); 5.20 (s, 2H, -CH_2-); 7.08 (d, 2H, phenyl); 7.60 (d, 1H, C8-chromone-H); 7.68 (dd, 1H, C7-chromone-H); 7.68 (s,1H, C=CH); 7.92 (s, 1H, C5-Chromone-H); 8.12 (d, 2H, phenyl); 8.91 (s, 1H, C2-chromone-H). Anal. Calcd. (%) for $C_{22}H_{14}N_2O_7S$ (450.42): C, 58.66; H, 3.13; N, 6.22; S, 7.12. Found: C, 58.65; H, 3.13; N, 6.23; S, 7.11. MS (EI, 70 eV): m/z: 451 [M+1].

Animals

All protocols were approved by the Ethical Committee on Animal Welfare of "Iuliu Haţieganu" University (No. 44/13.03.2017), in accordance

with the Romanian Ministry of Health and complying with. The investigation conformed to the Guidelines in the Use of Animals in Toxicology. Type 1 diabetes mellitus (T1DM) was induced in Wistar rats (280-380 g), purchased from the Experimental Animal House ("Iuliu Haţieganu" University of Medicine and Pharmacy, Faculty of Medicine, Cluj-Napoca, Romania), by a single intraperitoneal (i.p.) injection of 60 mg/kg streptozotocin (STZ) dissolved in sodium citrate buffer (0.1 M, pH 4.5) as previously described [2-4]. The animals were given standard rat pellets diet and water *ad libitum*. The control group received a single i.p. injection with an equal volume of citrate sodium buffer, used to dissolve STZ. 96 H after the STZ administration, T1DM was confirmed by measuring the fasting blood glucose (FBG) concentration. Rats that had a FBG level higher than 250 mg/dL were included in the study as diabetic rats. One week after the STZ administration, the FBG level was measured and the treatment started. Blood glucose was measured from the retro-orbital venous plexus of the overnight fasting animals using the ACCU-CHEK Sensor System from Roche Diagnostics GmbH (Mannheim, Germany).

The rats were randomly divided into ten experimental groups (n = 10): the first group (control+CMC, **CC**)-non-diabetic control rats treated with carboxymethylcellulose (CMC); the second group (control + quercetin, **CQ**)-non-diabetic control rats treated with quercetin; the third group (control + pioglitazone, **CP**)-non-diabetic control rats treated with pioglitazone; the fourth group (control + insulin, **CI**)-non-diabetic control rats treated with insulin; the fifth group (control+TZDd, **CT**)-non-diabetic control rats treated with TZDd; the sixth group (diabetes + CMC, **DC**)-diabetic rats treated with CMC; the seventh group (diabetes + quercetin, **DQ**)-diabetic rats treated with quercetin; the eighth group (diabetes + pioglitazone, **DP**)-diabetic rats treated with pioglitazone; the ninth group (diabetes + insulin, **DI**)-diabetic rats treated with insulin; the tenth group (diabetes +TZDd, **DT**)-diabetic rats treated with TZDd.

One week after the STZ administration, the rats were treated with Que, Pio or TZDd (30 mg/kg body weight) daily by oral gavage for 5 weeks. The insulin (10 UI/kg body weight) was administered intraperitoneal (i.p.), daily, for 5 weeks.The rats from the groups: CC and DC received equal volumes of vehicle (CMC).

The FBG level was measured in all the experimental animals at the beginning of the experiment, 96 h after the STZ administration, 7 days after the STZ administration and at the end of the experiment. Upon the termination of the experiment at the end of 6 weeks, the animals were anesthetized with an i.p. injection of sodium pentobarbital (60 mg/rat) and sacrificed by cervical dislocation.

Measurement of the Biochemical Parameters of Oxidative Stres

The venous blood samples were collected from the rats' retro-orbital sinuses. Immediately after sampling, the blood was centrifuged to separate the serum. The serum was frozen with liquid nitrogen and stored in a −80 °C refrigerator, until the biochemical assays.

Measurement of MDA production. Serum malondialdehyde (MDA) level, an end product of lipid peroxidation, was determined using the Conti method [19]. The MDA level was expressed as nanomole per milliliter (nmol MDA/mL).

Protein Carbonyl Content Measurement. Protein carbonyl content (PCC), a marker of oxidized proteins, was measured spectrophotometrically using the fluorimetric method with 2,4-dinitrophenyl-hydrazine (DNPH) [20]. The levels of the PCC were expressed as nanomole per milligram of protein (nmol/mg protein).

Activities of antioxidant enzymes in the erythrocytes were estimated.

Superoxide dismutase (SOD) activity was determined by the method of Flohe et al., [21]. The SOD activity was expressed as units per gram of protein (U/g protein).

Catalase (CAT) activity was determined by the method of Pippenger et al. [22]. The CAT activity was also expressed as units per gram of protein (U/g protein).

Statistical Analysis

Results are expressed as means ± SD (n = 10). Statistical analysis was performed using SPSS software package version 17.0. One-way analysis of variance (ANOVA) was used to compare multiple data sets and when the *P* value obtained from ANOVA was significant ($P<0.05$), Tukey's test was applied to test for differences among groups. $P < 0.05$ was taken to indicate a significant difference between group means.

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