Original Article

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Serum Lipids, Lipoproteins, Lipid Cardiovascular Indices and Peroxisome Proliferator-Activated Receptor-Gamma Among Non-Diabetic and Streptozotocin-Induced Diabetic Rats with Various Interventions

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Abstract

Type 2 diabetes (T2DM) is a universal serious chronic disease with many complications. The oral antidiabetic drug of the thiazolidinediones (TZDs) including Pioglitazone) PGZ), and dietary supplements like chromium picolinate (Cr-PL) and chromium glucose tolerance factor (Cr-GTF) are mostly used as T2DM treatments. The study aims to determine the effects of Cr-PL, Cr-GTF, and PGZ, and their combination on serum peroxisome proliferator-activated receptors (PPAR-γ), and lipid profile including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG), as well as lipid atherogenic indices. A randomized design experiment using adult male Sprague-Dawley rats (220-300 g, n=63) was applied. Nine rats were selected randomly and assigned as a normal non-diabetic group, the other 54 rats were divided randomly into 6 main groups each with 9 rats, and each rat was injected intraperitoneally with streptozotocin (STZ) of dose = 40mg/kg of rat to induce T2DM. A dose of each of PGZ = 0.65 mg/kg (rat weight)/day, Cr-PL = 1 mg/kg (rat weight)/day, Cr-GTF = 1 mg/kg (rat weight)/day, and their combinations of (PGZ+Cr-PL) and (PGZ+Cr-GTF) were given to rats daily for 6 weeks according to each group of intervention. PPAR-γ serum levels were significantly higher in (PGZ+Cr-PL) group ($P< 0.05$) than in the Cr-PL group (244.5±0.6, 210.5±0.6, respectively).

Keywords:

Lipid profile, T2DM, Pioglitazone, PPARγ.

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Introduction

Type 2 diabetes (T2DM) is a worldwide chronic disease that has high morbidity and mortality rates because of the high number of diabetics with serious complications responsible [1]. Thiazolidinediones (TZDs) are the first drug used to treat insulin resistance (IR) syndrome in T2DM patients [2]. Pioglitazone (PGZ) is an oral antidiabetic) Tamimi L *et* al., [3] (drug of the TZDs class that significantly improves carbohydrate and fat metabolism gamma isoform of the peroxisome proliferator-activator receptor (PPARγ) [4]. The latter controls the transcription of a set of genes involved in the regulation of lipid and carbohydrate metabolism [5]. Pioglitazone treatment in T2DM has a convenient effect in not just controlling blood glucose [6], but also improving the lipid profile. Pioglitazone had been found to cause a decrease in LDL, TG, and serum fatty acids, and an increase in HDL-C [7, 8]. In T2DM, an impaired lipid profile is commonly occurred and considered a major risk factor for cardiovascular diseases [9, 10].

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Diabetic dyslipidemia usually occurred due to the disturbance of lipid metabolism is characterized by increased insulin levels, low high-density lipoprotein-cholesterol (HDL-C) levels, and increased low-density lipoprotein-cholesterol (LDL) particles and triglyceride (TG) rich remnant lipoprotein concentrations [11].

Insulin resistance, a diminished function inhibits lipolysis which increases free fatty acid (FFA) generation and lowers lipoprotein lipase activity [12]. This is almost seen mainly after eating a meal, where a chylomicron remnant that is rich in TG will be generated, caused by elevated hepatic FFA and very-low-density lipoprotein (VLDL) TG-rich particles secretion [13, 14].

These actions will cause TG-rich lipoproteins to affect HDL-C metabolism via cholesteryl ester transfer protein to make HDL-C particles that contain high TG concentrations [15]. Hydrolysis of HDL-TG particles will be with hepatic lipase to produce TG and HDL, as a result, HDL-C particles will be shrunk and become less antiatherogenic active, and easy to be filtrated from blood vessels by the kidneys [16, 17].

In a study by Moawad et al., and Moawad [18, 19], T2DM resulted in a significant elevation of TC, TG, and LDL-C levels while HDL-C level was reduced. Treatment with pioglitazone decreased serum TC, TG, and LDL, while HDL-C was increased. When adding Cr-PL to treatment, it reduced TG and LDL-C without altering TG level besides normalizing HDL-C level.

Thus, results prove the benefits of co-administration of Cr-PL with pioglitazone on the lipid profile of diabetic rats. Management of T2DM relies mainly on variable use [20] or a combination of diet, oral antidiabetic agents, insulin, or analogs [21]. However, depending on conventional drug therapy faces serious limitations due to adverse effects [22], reduced efficacy, non-adherence of diabetic patients, and increased cost [23].

Chromium supplementation lipid-lowering effects are explained by its antioxidant properties that have positive effects on OS in patients with T2DM [24]. It was found that Cr has a good impact on blood lipids metabolism (cholesterol, LDL, HDL, TG) as it is reported that Cr treatment decreased levels of all lipids except for LDL-C [25-27].

A recent meta-analysis on T2DM patients aimed to study the effect of Cr on patients' lipid profile proved that Cr-supplementation has significant positive effects on lipid profile by lowering TC, TG, and elevating HDL, although Cr failed to affect LDL-C levels [28, 29]. This study aims to determine the effects of Cr-PL, Cr-GTF, and PGZ, and their combination on lipid profile; triacylglycerol (TG), total cholesterol (TC), low-density lipoproteins- cholesterol (LDS: -C), high-density lipoproteins- cholesterol (HDL-C), including the atherogenic index of plasma (AIP), atherogenic coefficient (AC), and cardiac risk ratio (CRR).

Materials and Methods

Design and Animal Experiment

A randomized controlled design study was conducted between January and March 2021. Adult male Sprague-Dawley rats (220-300 g) were used as animal models because of their high sensitivity to developing T2DM. Rats (n=63) were obtained from the University of Petra, Amman, Jordan, then housed in cages in a well-ventilated and temperature-controlled room (25°C) with a 12-h dark/12-h light cycle.

The rats were accessed to tap water and fed a practical natural diet that contained (g/kg): ground yellow corn, 560; soybean meal, 300; DL-methionine, 3; choline chloride, 2; mineral mix, 40; vitamin mix, 20 and corn oil, 75. The study was approved by the Institutional Animal Ethics Committee following the recommended guidelines for animal care and use [30].

Type 2 Diabetes Induction

After the acclimatization period, nine animals were randomly chosen to assign a healthy non-diabetic group (G0) while the remainder of rats (n=54) were selected for the T2DM induction procedure by intraperitoneal injection with streptozotocin (STZ) of dose = 40mg/kg of rat [31-33].

Streptozotocin 95 % was obtained from Cayman-USA, (188883-66-4) and stored at 4 °C. STZ was freshly and immediately prepared before injection using 10% buffer citrate with a pH of 4.5. Streptozotocin doses are calculated according to dose and rats' weights. Rats fasted for 4 hours before injection while 10% sucrose drinking water was given to rats for 24 hr. after injection.

Inclusion criteria were following the blood glucose level after T2DM induction with fasting blood glucose (12 hr.) ≥ 170 mg/dl and non-fasting blood glucose ≥ 250 mg/dl but not exceeding 300 mg/dl. Blood glucose levels were measured using the blood glucose meter model Glucocard-S (Arkray-Japan).

Constant levels of blood glucose after 1 week of STZ injections indicated the inclusivity of rats in the experimental intervention stage. After induction and inclusive selection, the rats (n=54) were randomly sorted into 6 different groups (1 - 6) each of 9 rats with individual labeling of rats according to the type of intervention as shown in figure 1.

Intervention Stage

According to the weekly weights of rats, an assigned dose of each of Cr-PL, Cr-GTF, and PGZ, and their combinations (figure 1) were administered at 10 am daily to each rat in all the groups: 2, 3, 4, 5, and 6 using gastric gavage, while group 1 was kept as a control group without any intervention. The intervention stage

began at the end of the T2DM induction period and lasted for 6-weeks.

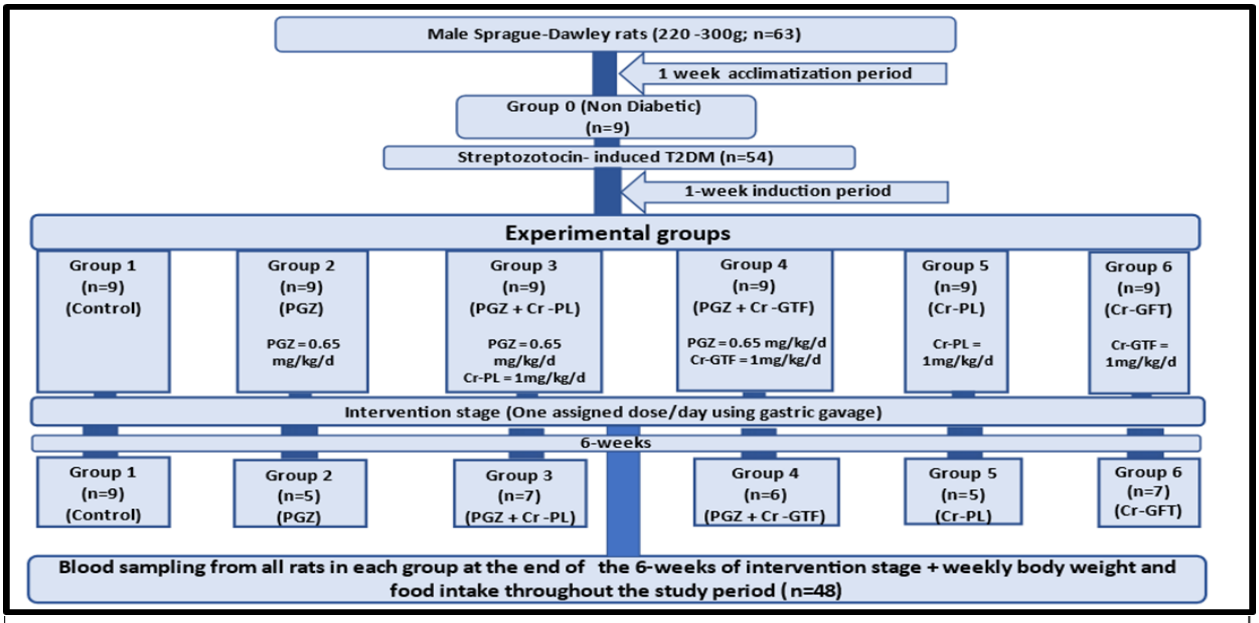


Figure 1: The experimental stages of non-diabetic and streptozotocin-induced diabetic rats with various interventions for 6 weeks

Doses Calculation

Pioglitazone was used in its HCl conjugated derivative, oral crystalline powder sample of pioglitazone HCl was obtained as a raw material from Dar Al Dawa, Amman, Jordan (Batch no. BWP200007-CIPLA LTD.). Chromium-PL and Cr-GTF were obtained from Now Foods, USA. (Batch no. 20129861 and 3172406) respectively. Pioglitazone HCL and Chromium powder samples dissolved freshly in CMC 10% as a vehicle, and doses volumes were calculated per rat accordingly. The pioglitazone HCl dose was calculated by considering the maximum single dose of 45mg/70kg adult human/day, which equals 0.65mg/kg (rat weight) /day [34]. Cr-PL and Cr-GTF daily dose was calculated referring to the Cr maximum safe dose of 1mg/kg(rat weight)/day which was used safely and efficiently in previous animal research [35]. Daily doses of PGZ, Cr-PL, and Cr-GTF, and their combinations were administered to rats using the gastric gavage at (8:00-10:00 am) to ensure the delivery of an accurate dose into the digestive system of rats. Accordingly, the 5 experimental groups of rats were sorted and administered by Cr-PL, Cr-GTF, and PGZ, and their combinations were as follows:

Group 0: Healthy nondiabetic group (no intervention)
Group 1: Control diabetic group (no intervention)
Group 2: PGZ = 0.65 mg/kg/day
Group 3: PGZ = 0.65 mg/kg/day + Cr-PL = 1 mg/kg/day
Group 4: PGZ = 0.65 mg/kg/day + Cr-GTF = 1 mg/kg/day
Group 5: Cr-PL = 1 mg/kg/day
Group 6: Cr-GTF = 1 mg/kg/day

Blood and Serum Sampling

At the end of the 6-weeks intervention stage, rats (n= 48) were fasting for 8 hr. and then anesthetized using isoflurane. Blood was collected via the left ventricle using a 19–21-gauge needle slowly for blood sampling per rat. Samples were then divided into the different aliquots and then transferred into properly labeled 3 mL EDTA Eppendorf tubes.

The fresh blood of 0.3 ml sample volume for HbA1c and FBG analysis was withdrawn and then stored at 4 °C till analysis (≤ 24 hrs.), while the remainder of the blood was allowed to clot by leaving it undisturbed at room temperature. Then, samples were centrifuged at 300 rpm for 15 min and serum was stored at −80 °C until analysis. The fasting blood samples that were collected at the end of the 6th week of the study were processed by routine experimental protocols.

Body Weight, Water Intake, and Food Intake

Body weights, water, and food intake were measured once a week throughout the study and at the end of the 6th week. All biochemical experimental procedures and analyses were performed by the researcher at Aurum Biotech Laboratories, Amman, Jordan.

Biochemical Analyses

Standard biochemical kits for the analysis of the biochemical variables are purchased and stored properly. The analysis was applied according to the kit's procedures.

Lipid profile Analysis

Lipid profile analysis was done using serum samples by Snibe Bioassays 240 Plus Analyzer. Before starting analysis procedures, stored frozen serum was centrifuged for approximately 20 minutes at 3000 rpm

within 30 minutes after thawing the sample. The supernatants were carefully collected and assayed immediately with the avoidance of repeated freeze/thaw cycles. A 10 µL of serum plus 1ml of reagent was mixed and incubated at 37 °C for 10 min. Absorbance is then read against blank at 450 nm. All reagents were placed at room temperature (20-25 °C) before starting the analysis. Lipid profile relevant variables were analyzed and then used to calculate the cardiovascular indices including the atherogenic index of plasma (AIP), atherogenic coefficient (AC), and cardiac risk ratio (CRR) using the following formulas: AIP= [log (Triglyceride/HDL-C)], AC= [TC-HDL-C/HDL-C], CRR= (TC/HDL-C)

Statistical Analysis

Data were analyzed using statistical analysis software (SAS version 9; SAS Institute Inc., Cary, NC, USA). The test of homogeneity of variances was done using Levene's test, while the test of normality was done using Kolmogorov-Smirnova and Shapiro-Wilk tests.

Statistical significance was assessed by Kruskal-Wallis Test and adjusted by Bonferroni correction. Data were presented as means with standard errors of the mean (SEM), and the probability of P< 0.05 was accepted as being statistically significant. Pearson's correlation test is used to determine the correlation level between all different variables.

Results

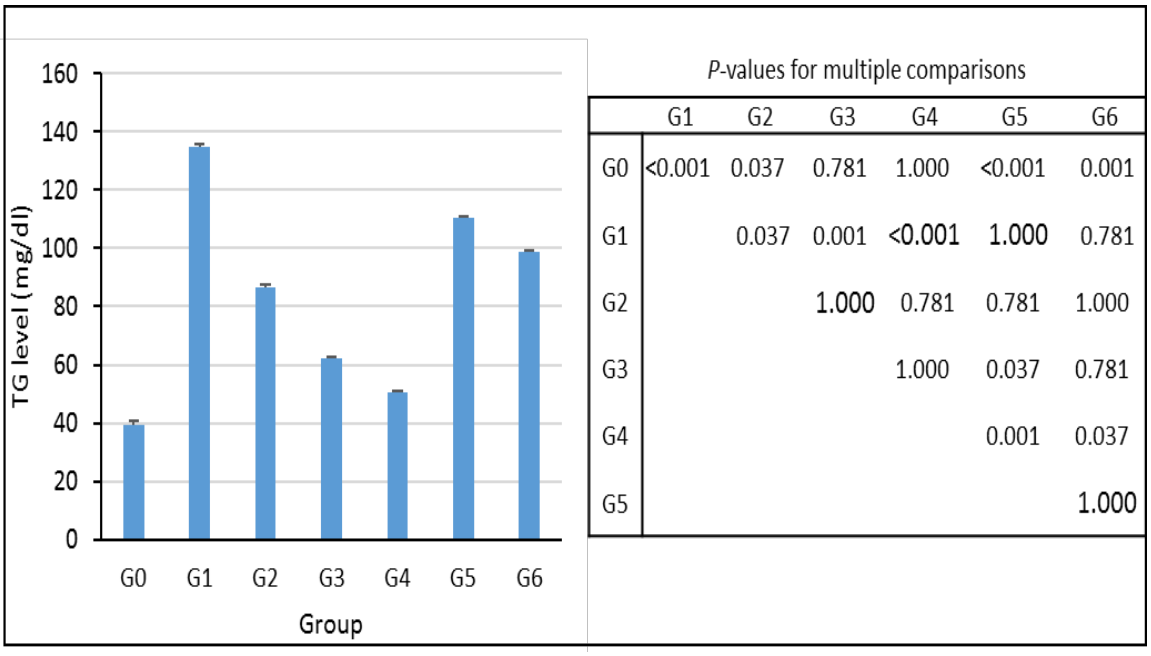
Serum lipids, lipoproteins, and cardiovascular indices of non-diabetic and streptozotocin-induced diabetic rats of healthy non-diabetic group G (0), control diabetic group G (1), PGZ treated group G (2), PGZ +Cr-PL treated group G (3), PGZ +Cr-GTF treated group G (4), and Cr-PL G (5), Cr-GTF G (6) with various interventions for 6 weeks showed valuable different results as mentioned in table 1. Results in Table 1 and Figure 2 presented significant (P<0.05) higher levels of TG (mg/dl) in G(1) (134.8±001.1), G(2) (86.78±00.61), G(5) (110.4±00.63), and G(6) (98.61±00.53) than that of G(0) (39.50±01.09).

In Addition, significantly (p<0.05) higher levels found in G(5) compared to G(3), and in G(5) and G(6) compared to G(4). Significantly (p< 0.05) lower levels found in G(2), G(3), and G(4) comparing to G(1). Different but non-significant levels were detected between other groups (figure 2).

Measurements of TC (mg/dl) (table 1 and figure 3) found to be significantly (P<0.05) higher in G(1) (106.2±001.0), G(2) (70.83±00.87), G(5) (89.44±00.19), and G(6) (80.94±00.41) than that of G(0) (54.89±00.26). Also, significantly higher measurements were found in G(5) compared to G(3), G(5), and G(6) compared to G(4). Significantly lower levels of TC are found in G(2), G(3), and G(4) compared to G(1).

Other non-significant measurements were detected between other groups. Concerning LDL-C (mg/dl), levels of G(1), G(5), and G(6) were significantly (P< 0.05) higher than G(0) (67.12±00.79, 55.73±00.26, 41.20±00.44 vs. 20.88±00.49, respectively) (Table 1).

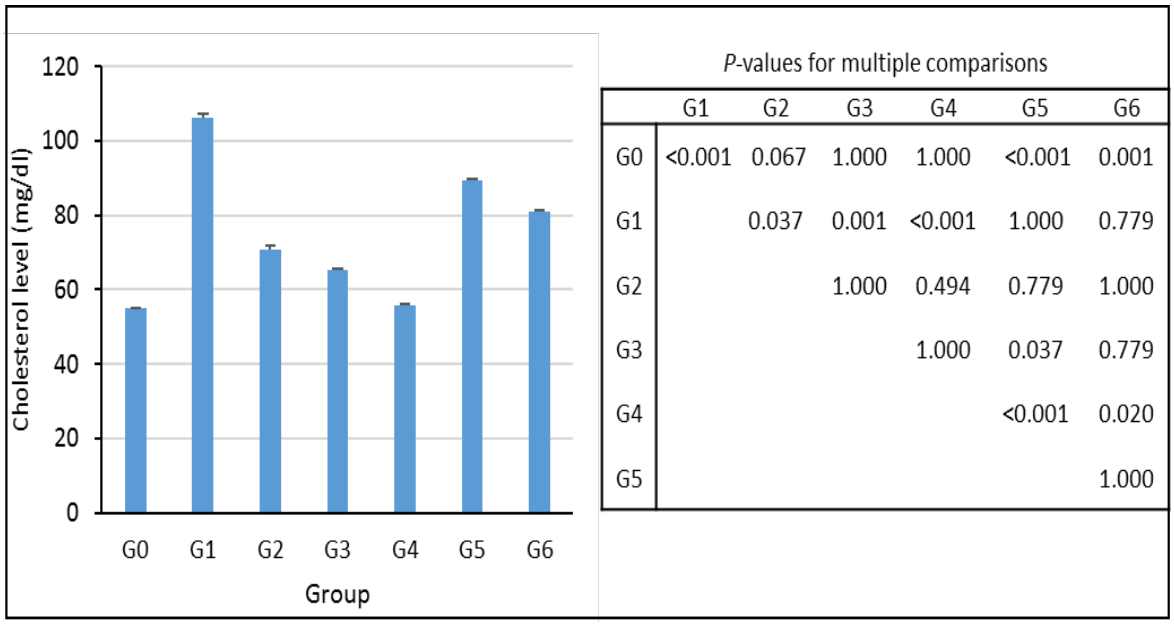
Also as in Figure 4, significantly higher levels were found in G(5) compared to (3), and for G(5) and G(6) compared to G (4), while significantly lower levels were found in G(2), G(3), and G(4) compared to G(1). Other non-significant results were found in G(2), G(3), and G(4) compared to G(0) and within different groups.



P-values for multiple comparisons

	G1	G2	G3	G4	G5	G6
G0	<0.001	0.037	0.781	1.000	<0.001	0.001
G1		0.037	0.001	<0.001	1.000	0.781
G2			1.000	0.781	0.781	1.000
G3				1.000	0.037	0.781
G4					0.001	0.037
G5						1.000

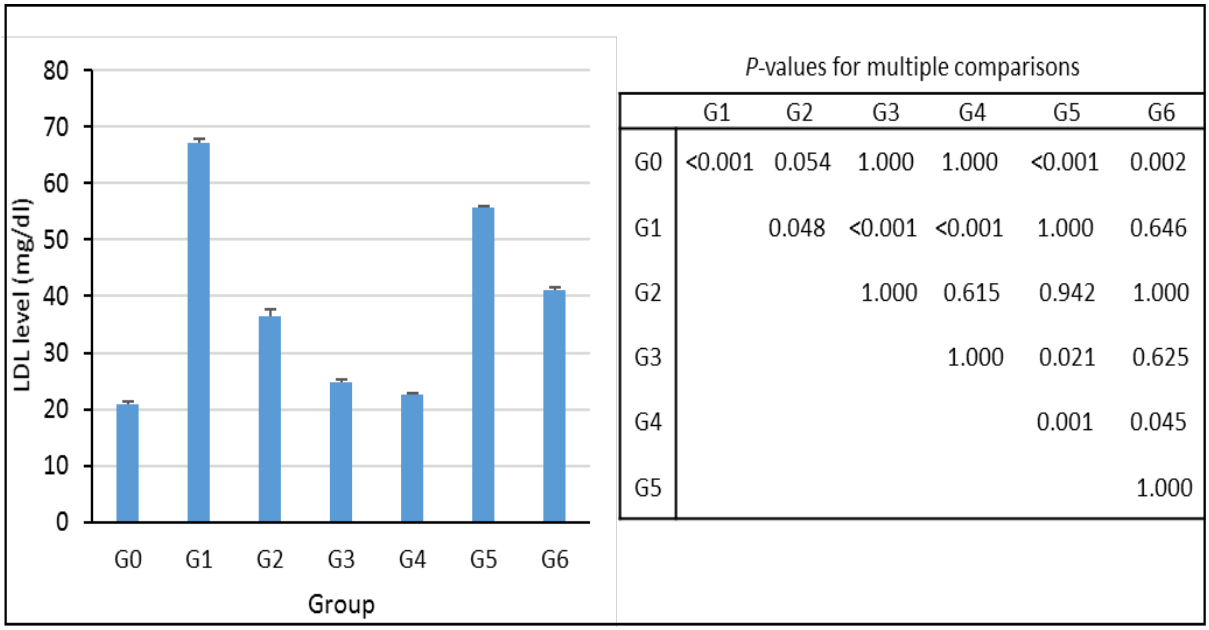
Figure 2: Significant differences in triglyceride (TG) levels (mg/dl) among non-diabetic and streptozotocin-induced diabetic rats with various interventions for 6 weeks. (Oral intervention groups: G(1): no intervention; G(2): PGZ=0.65 mg/kg/day; G(3): PGZ=0.65 mg/kg/day+ Cr-PL =1 mg/kg/day; G(4): PGZ=0.65 mg/kg/day+ Cr-GTF=1 mg/kg/day; G(5): Cr-PL=1 mg/kg/day; G(6): Cr-GTF=1 mg/kg/day.)



P-values for multiple comparisons

	G1	G2	G3	G4	G5	G6
G0	<0.001	0.067	1.000	1.000	<0.001	0.001
G1		0.037	0.001	<0.001	1.000	0.779
G2			1.000	0.494	0.779	1.000
G3				1.000	0.037	0.779
G4					<0.001	0.020
G5						1.000

Figure 3: Significant differences in total cholesterol (TC) levels (mg/dl) among non-diabetic and streptozotocin-induced diabetic rats with various interventions for 6 weeks. (Oral intervention groups: G(1): no intervention; G(2): PGZ=0.65 mg/kg/day; G(3): PGZ=0.65 mg/kg/day+ Cr-PL =1 mg/kg/day; G(4): PGZ=0.65 mg/kg/day+ Cr-GTF=1 mg/kg/day; G(5): Cr-PL=1 mg/kg/day; G(6): Cr-GTF=1 mg/kg/day.)



P-values for multiple comparisons

	G1	G2	G3	G4	G5	G6
G0	<0.001	0.054	1.000	1.000	<0.001	0.002
G1		0.048	<0.001	<0.001	1.000	0.646
G2			1.000	0.615	0.942	1.000
G3				1.000	0.021	0.625
G4					0.001	0.045
G5						1.000

Figure 4: Significant differences in low-density lipoprotein-cholesterol (LDL-C) levels (mg/dl) among non-diabetic and streptozotocin-induced diabetic rats with various interventions for 6 weeks. (Oral intervention groups: G(1): no intervention; G(2): PGZ=0.65 mg/kg/day; G(3): PGZ=0.65 mg/kg/day+ Cr-PL =1 mg/kg/day; G(4): PGZ=0.65 mg/kg/day+ Cr-GTF=1 mg/kg/day; G(5): Cr-PL=1 mg/kg/day; G(6): Cr-GTF=1 mg/kg/day.)

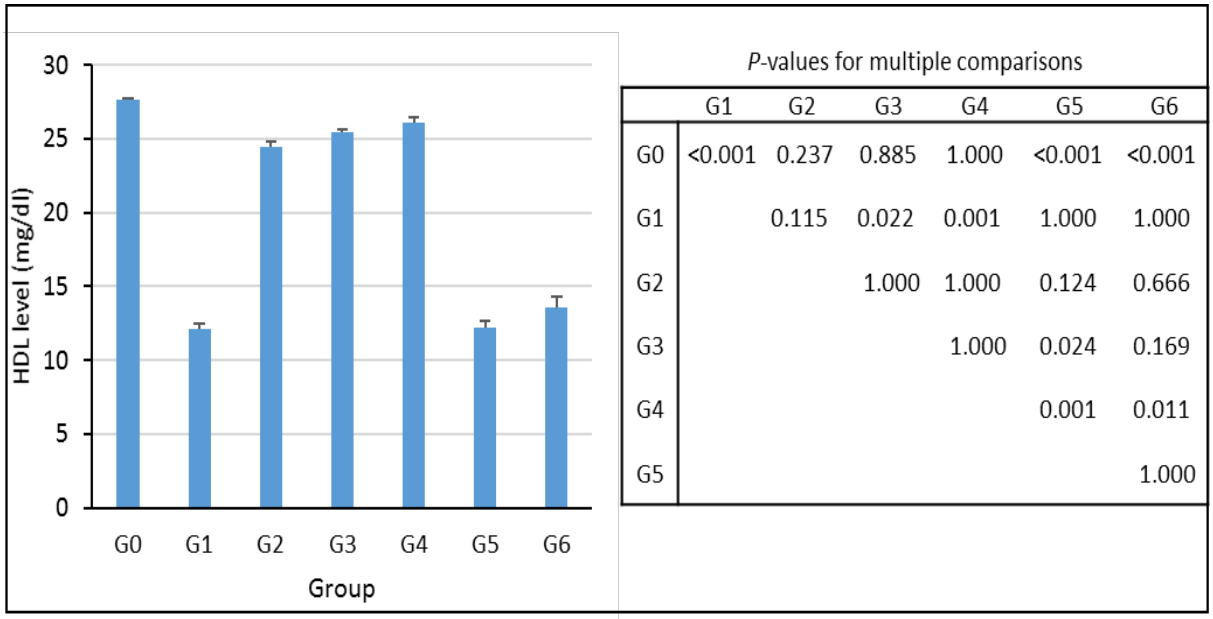
HDL-C (mg/dl) has significantly lower mean values ($P<$ 0.05) of G (1), G (5), and G (6) than G (0) with mean and SEM values of (12.13±00.30, 12.21±00.42, 13.59±00.69 vs. 27.62±00.13, respectively). Also, values for G(1) are significantly lower ($P<$ 0.05) than G (3) and G (4) (12.13±00.30 vs. 24.72±00.51, 26.11±00.33 mg/dl, respectively) (table 1, figure 5).

HDL-C mean values are significantly higher ($P<$ 0.05) in G (3) than G (5) with mean and SEM values of (25.47±00.14 vs. 12.21±00.42 mg/dl). Group (4) has significantly lower mean values ($P<$ 0.05) than G (5) and G (6) (26.11±00.33 vs. 12.21±00.42, 13.59±00.69, respectively). Different but non-significant levels were found between other groups. Ratios of LDL-C/HD-CL found to be significant ($P<$0.05) higher in G(1) (5.56±0.15), G(2) (1.50±0.05), G(5) (4.61±0.15), and G(6) (3.11±0.19) compared to that of G(0) (0.76± 0.02). Significantly higher ratios were found in G(5) and G(6) compared to G(4).).

As significantly (p< 0.05) higher values of LDL/HDL-C are found in G(5) but not in G(3), LDL/HDL-C values of G(4) and G(6) are not significantly different from that of

G(3). Also, significantly lower values of LDL/HDL-C are found in G(2), G(3), and G(4) compared to G(1) (table 1 and Figure 6). Non-significant values were detected among different groups.TC/TG for G (1), G (2), G (5), and G (6) then G (0) is significantly lower mean values ($P<0.05$) with mean and SEM values of (0.79±0.01, 0.82±0.01, 0.81±0.01, 0.82±0.00 vs. 1.40±0.04, respectively). TC/TG mean values are significantly lower ($P<0.05$) in G(1) than in G (3) and G (4) (0.79±0.01 vs. 1.05±0.01, 1.11±0.01, respectively). In addition, G (4) has significantly lower mean values ($P<0.05$) than G (5) and G (6) (1.11±0.01 vs. 0.81±0.01, 0.82±0.00, respectively) (table 1, figure 7). Ratios of AIP showed significant ($P<0.05$) higher levels of TG (mg/dl) in G(1) (1.05±0.01), G(2) (0.55±0.01), G(5) (0.96±0.01), and G(6) (0.87±0.02) than that of G(0) (0.15±0.01).).

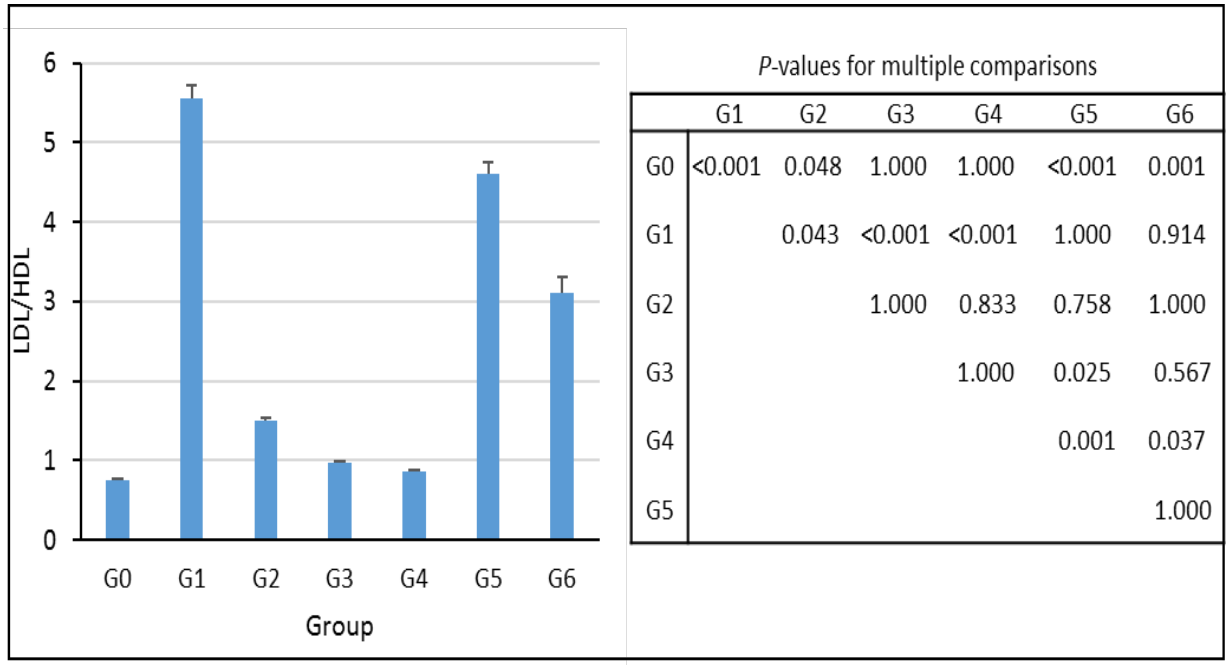
Significantly higher values were found in G(5) and G(6) compared to G(4). As significantly lower values of AIP are found in G(2), G(3), and G(4) compared to G(1), non-significant ratios are found among other groups. (table 1 and figure 8).



P-values for multiple comparisons

	G1	G2	G3	G4	G5	G6
G0	<0.001	0.237	0.885	1.000	<0.001	<0.001
G1		0.115	0.022	0.001	1.000	1.000
G2			1.000	1.000	0.124	0.666
G3				1.000	0.024	0.169
G4					0.001	0.011
G5						1.000

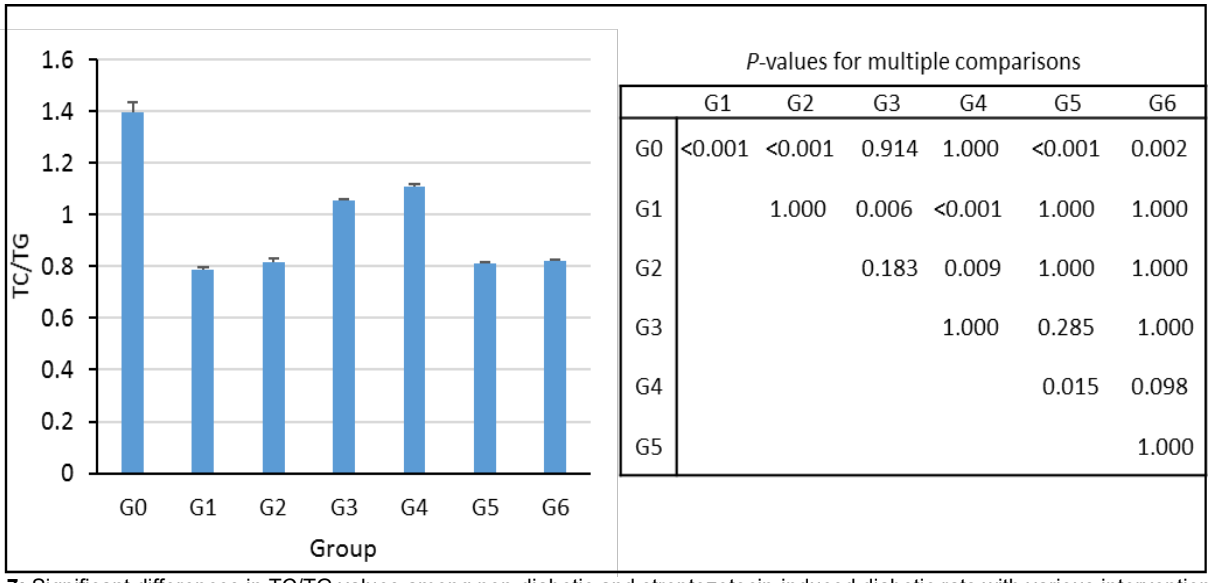
Figure 5: Significant differences in high-density lipoprotein -cholesterol (HDL-C) levels (mg/dl) among non-diabetic and streptozotocin-induced diabetic rats with various interventions for 6 weeks. (Oral intervention groups: G(1): no intervention; G(2): PGZ=0.65 mg/kg/day; G(3): PGZ=0.65 mg/kg/day+ Cr-PL =1 mg/kg/day; G(4): PGZ=0.65 mg/kg/day+ Cr-GTF=1 mg/kg/day; G(5): Cr-PL=1 mg/kg/day; G(6): Cr-GTF=1 mg/kg/day.)



P-values for multiple comparisons

	G1	G2	G3	G4	G5	G6
G0	<0.001	0.048	1.000	1.000	<0.001	0.001
G1		0.043	<0.001	<0.001	1.000	0.914
G2			1.000	0.833	0.758	1.000
G3				1.000	0.025	0.567
G4					0.001	0.037
G5						1.000

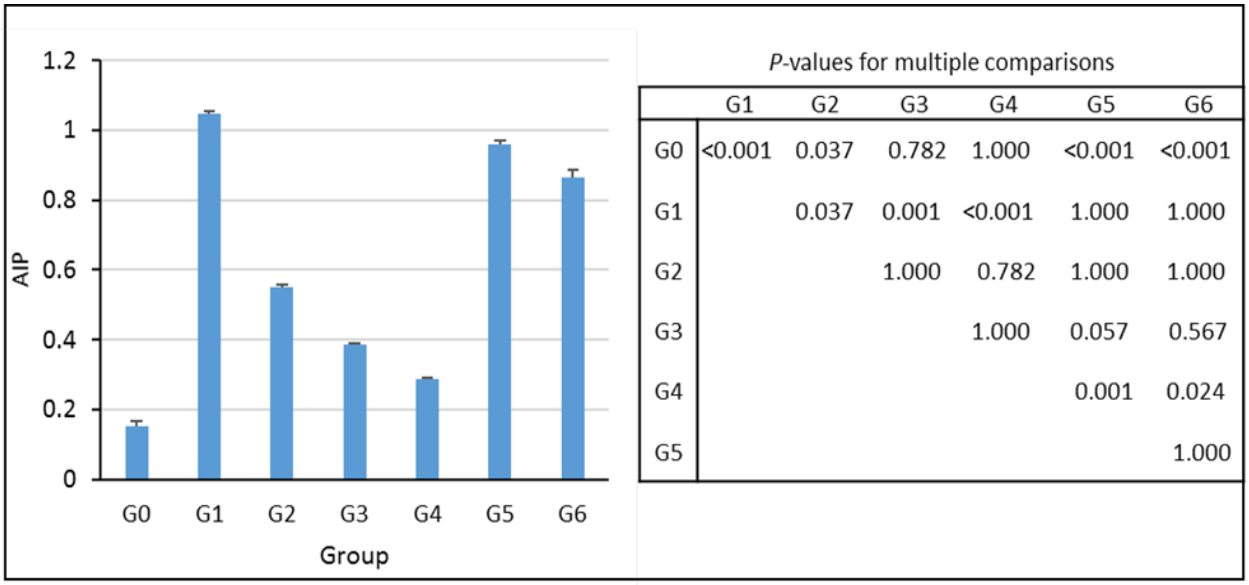
Figure 6: Significant differences in LDL/HDL-C values among non-diabetic and streptozotocin-induced diabetic rats with various interventions for 6 weeks. (Oral intervention groups: G(1): no intervention; G(2): PGZ=0.65 mg/kg/day; G(3): PGZ=0.65 mg/kg/day+ Cr-PL =1 mg/kg/day; G(4): PGZ=0.65 mg/kg/day+ Cr-GTF=1 mg/kg/day; G(5): Cr-PL=1 mg/kg/day; G(6): Cr-GTF=1 mg/kg/day.)



P-values for multiple comparisons

	G1	G2	G3	G4	G5	G6
G0	<0.001	<0.001	0.914	1.000	<0.001	0.002
G1		1.000	0.006	<0.001	1.000	1.000
G2			0.183	0.009	1.000	1.000
G3				1.000	0.285	1.000
G4					0.015	0.098
G5						1.000

Figure 7: Significant differences in TC/TG values among non-diabetic and streptozotocin-induced diabetic rats with various interventions for 6 weeks. (Oral intervention groups: G(1): no intervention; G(2): PGZ=0.65 mg/kg/day; G(3): PGZ=0.65 mg/kg/day+ Cr-PL =1 mg/kg/day; G(4): PGZ=0.65 mg/kg/day+ Cr-GTF=1 mg/kg/day; G(5): Cr-PL=1 mg/kg/day; G(6): Cr-GTF=1 mg/kg/day.)



P-values for multiple comparisons

	G1	G2	G3	G4	G5	G6
G0	<0.001	0.037	0.782	1.000	<0.001	<0.001
G1		0.037	0.001	<0.001	1.000	1.000
G2			1.000	0.782	1.000	1.000
G3				1.000	0.057	0.567
G4					0.001	0.024
G5						1.000

Figure 8: Significant differences in atherogenic index of plasma (AIP) values among non-diabetic and streptozotocin-induced diabetic rats with various interventions. (Oral intervention groups : G(1): no intervention; G(2): PGZ=0.65 mg/kg/day; G(3): PGZ=0.65 mg/kg/day+ Cr-PL =1 mg/kg/day; G(4): PGZ=0.65 mg/kg/day+ Cr-GTF=1 mg/kg/day; G(5): Cr-PL=1 mg/kg/day; G(6): Cr-GTF=1 mg/kg/day.)

Significantly (*P*< 0.05) higher correlations detected for the groups (G(1), G(5), and G(6) compared to G(0)) for AC values (7.79±0.19, 6.39±0.23, 5.09±0.33vs. 0.99±0.02, respectively). Group (1) has significantly higher mean values (*P*< 0.05) than G(2), G(3), and G(4) (7.79±0.19, vs. 1.90±0.06, 1.57±, 1.14±0.03, respectively) (table 1, figures 9). In addition, G(4) has significantly lower mean values (*P*< 0.05) than G(5) and G(6) (1.14±0.03 vs. 6.39±0.23, 5.09±0.33, respectively) for AC.

Where among other groups some non-significant relations were observed. Again, for CRR ratios, significant (*P*< 0.05) higher correlations detected for the groups (G(1), G(5), and G(6) compared to G(0) (8.79±0.19, 7.39±0.23, 6.09± 0.33 vs. 1.99±0.02, respectively). Group (1) has significantly higher mean values (*P*< 0.05) than G(2), G(3), and G(4) (8.79±0.19 vs. 2.90±0.06, 2.57±0.02, 2.14±0.03, respectively) (table 1, figures 10). In addition, G(4) has significantly lower mean values (*P*< 0.05) than G(5) and G(6) (2.14±0.03 vs. 7.39±0.23, 6.09±0.33, respectively) for CRR. Non-significant correlations were found among groups.

Table 1 displays that G(1), G(5), and G(6) have significantly lower mean values (P<0.05) than G(0) concerning PPAR-γ (pg/ml) with mean and SEM values of (125.4±001.1, 210.5±000.6, 222.6±000.6 vs. 305.9±004.6, respectively). Group (1) has significantly lower mean values (P< 0.05) than G (2), G (3), and G (4) concerning PPARγ with mean and SEM values of (125.4± 001.1 vs. 231.8±000.9, 244.5±000.6, 298.4± 006.8 pg/ml, respectively). Group (3) has significantly higher values

(P<0.05) than G(5) (244.5±000.6 vs. 210.5±000.6). For G (4), it shows significantly higher mean values (P< 0.05) than G (5) and G (6) (298.4±006.8 vs. 210.5±000.6, 222.6±000.6, respectively). G (6) shows significantly (p< 0.05) lower mean values than G (4) in PPAR-γ levels. Non-significant relations were detected among groups. For all studied parameters, non-significant differences between values were observed among non-diabetic and streptozotocin-induced diabetic rats of healthy non-diabetic group G (0), control diabetic group G (1), PGZ treated group G (2), PGZ +Cr-PL treated group G (3), PGZ +Cr-GTF treated group G (4), Cr-PL treated group G (5), and Cr-GTF treated group G (6) with various interventions for 6 weeks.

Table 1: Serum lipids and lipoproteins and lipid cardiovascular indices of non-diabetic and streptozotocin-induced diabetic rats with various interventions for 6 weeks

Variables	Non-Diabetic Group	Streptozotocin-Induced Diabetic Groups					
		Control	PGZ	PGZ + Cr-PL	PGZ + Cr-GTF	Cr-PL	Cr-GTF
	G (0) (n=9)	G (1) (n=9)	G (2) (n=5)	G (3) (n=7)	G (4) (n=6)	G (5) (n=5)	G (6) (n=7)
TC (mg/dl)	54.89± 0.26	106.2± 01.0 [a]	70.83± 0.87 [b]	65.44± 0.18 [b]	55.89± 0.25 [b]	89.44± 0.19 [a, d, e]	80.94± 0.41 [a, e]
LDL-C (mg/dl)	20.88± 0.49	67.12± 0.79 [a]	36.55± 1.05 [b]	24.72± 0.51 [b]	22.62± 0.31 [b]	55.73± 0.26 [a, d, e]	41.20± 0.44 [a, e]
HDL-C (mg/dl)	27.62± 0.13	12.13± 0.30 [a]	24.44± 0.41	25.47± 0.14 [b]	26.11± 0.33 [b]	12.21± 0.42 [a, d, e]	13.59± 0.69 [a, e]
TG (mg/dl)	39.50± 1.09	134.8± 01.1 [a]	86.78± 0.61 [a, b]	62.17± 0.52 [b]	50.50± 0.34 [b]	110.4± 0.63 [a, d, e]	98.61± 0.53 [a, e]
LDL-C/ HDL-C	0.76± 0.02	5.56± 0.15 [a]	1.50± 0.05 [a, b]	0.97± 0.02 [b]	0.87± 0.02 [b]	4.61± 0.15 [a, d, e]	3.11± 0.19 [a, e]
TC/TG	1.40± 0.04	0.79± 0.01 [a]	0.82± 0.01 [a]	1.05± 0.01 [b]	1.11± 0.01 [b, c]	0.81± 0.01 [a, e]	0.82± 0.00 [a]
AIP	0.15± 0.01	1.05± 0.01 [a]	0.55± 0.01 [a, b]	0.39± 0.00 [b]	0.29± 0.00 [b]	0.96± 0.01 [a, e]	0.87± 0.02 [a, e]
CRR	1.99± 0.02	8.79± 0.19 [a]	2.90± 0.06 [b]	2.57± 0.02 [b]	2.14± 0.03 [b]	7.39± 0.23 [a, e]	6.09± 0.33 [a, e]
AC	0.99± 0.02	7.79± 0.19 [a]	1.90± 0.06 [b]	1.57± 0.02 [b]	1.14± 0.03 [b]	6.39± 0.23 [a, e]	5.09± 0.33 [a, e]
PPAR-γ (pg/ml)	305.9± 4.6	125.4± 1.1 [a]	231.8± 0.9 [b]	244.5± 0.6 [b]	298.4± 6.8 [b]	210.5± 0.6 [a, c, d]	222.6± 0.6 [a, d]

- Values are means ± standard error of mean (SEM).
- Abbreviations: PGZ: pioglitazone; Cr-PL: chromium-picolinate; Cr-GTF: chromium-glucose tolerance factor; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol;TG: triglyceride; AIP: atherogenic index of plasma [log (Triglyceride/HDL-C)]; AC: atherogenic coefficient [TC-HDL-C/HDL-C]; CRR: cardiac risk ratio (TC/HDL-C), PPAR -γ: Peroxisome proliferator-activated receptor-gamma..
- Oral intervention doses: (1): no intervention; (2): PGZ=0.65 mg/kg/day; (3): PGZ=0.65 mg/kg/day+ Cr-PL =1 mg/kg/day; (4): PGZ=0.65 mg/kg/day+ Cr-GTF=1 mg/kg/day; (5): Cr-PL=1 mg/kg/day; (6): Cr-GTF=1 mg/kg/day.
- Values in rows with different superscripts are significantly different (p< 0.05).
[a] significantly different from G (0), [b] significantly different from G (1), [c] significantly different from G2, [d] significantly different from G3, [e] significantly different from G4.

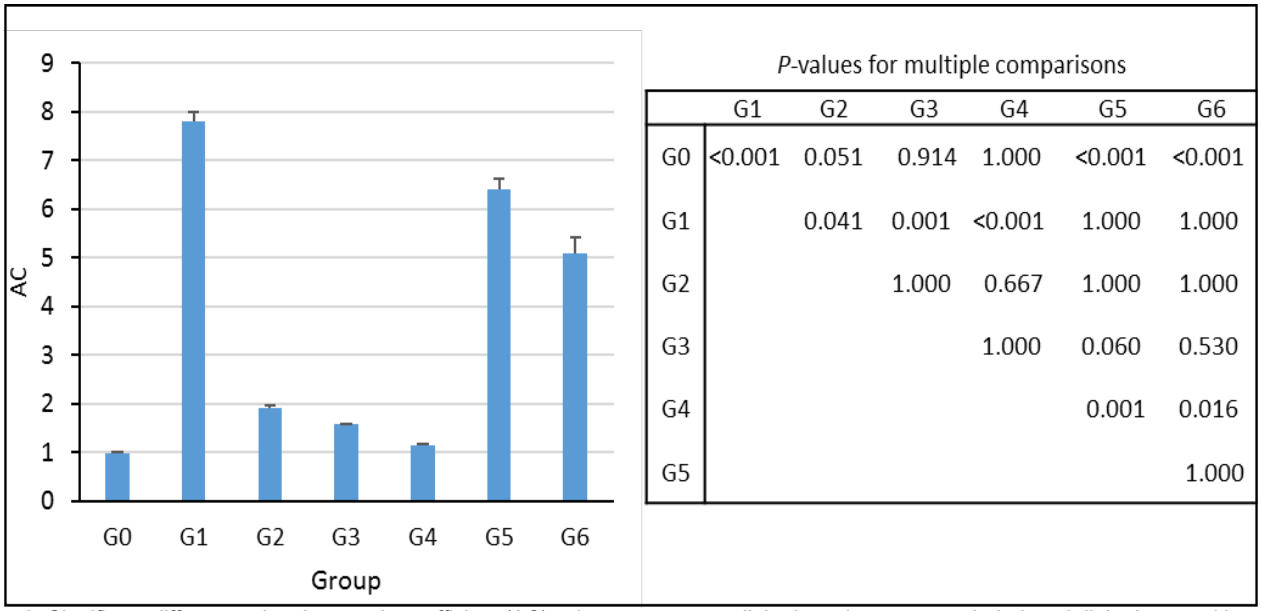
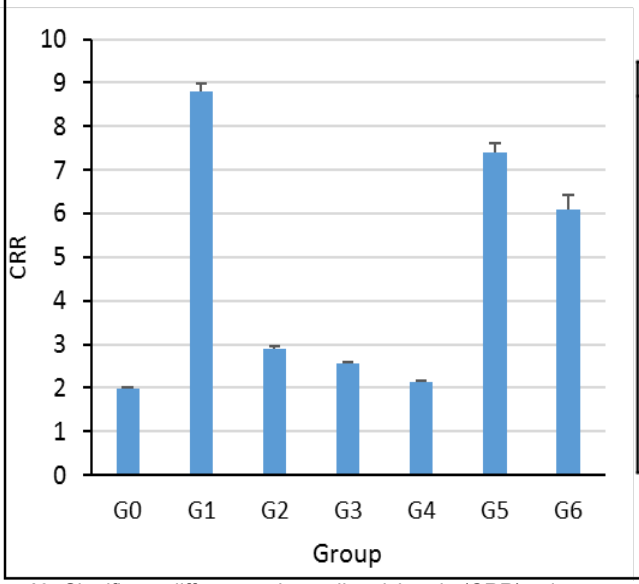


Figure 9: Significant differences in atherogenic coefficient (AC) values among non-diabetic and streptozotocin-induced diabetic rats with various interventions for 6 weeks. (Oral intervention groups: G(1): no intervention; G(2): PGZ=0.65 mg/kg/day; G(3): PGZ=0.65 mg/kg/day+ Cr-PL =1 mg/kg/day; G(4): PGZ=0.65 mg/kg/day+ Cr-GTF=1 mg/kg/day; G(5): Cr-PL=1 mg/kg/day; G(6): Cr-GTF=1 mg/kg/day.)



P-values for multiple comparisons

	G1	G2	G3	G4	G5	G6
G0	<0.001	0.051	0.914	1.000	<0.001	<0.001
G1		0.041	0.001	<0.001	1.000	1.000
G2			1.000	0.667	1.000	1.000
G3				1.000	0.060	0.530
G4					0.001	0.016
G5						1.000

Figure 10: Significant differences in cardiac risk ratio (CRR) values among non-diabetic and streptozotocin-induced diabetic rats with various interventions for 6 weeks. (Oral intervention groups: G(1): no intervention; G(2): PGZ=0.65 mg/kg/day; G(3): PGZ=0.65 mg/kg/day+ Cr-PL =1 mg/kg/day; G(4): PGZ=0.65 mg/kg/day+ Cr-GTF=1 mg/kg/day; G(5): Cr-PL=1 mg/kg/day; G(6): Cr-GTF=1 mg/kg/day.)

Discussion

Previous studies have approved the fact of supplementation efficiency in ameliorating the glycemic and lipidemic status. The unavailability of studies to investigate the antidiabetic effects of the combinations of PGZ with Cr-PL, and Cr-GTF on low-grade inflammation, PPARγ activation, OS, IR, dyslipidemia, and leptin was the motivative factor to examine the efficacy of the combination of different Cr forms in T2DM models treated by PGZ.

Type 2 Diabetes mellitus is characterized by hyperglycemia together with biochemical alterations of glucose and lipid peroxidation. T2DM is considered a free radical disease that propagates complicated consequences with increased free radical formation. Relatively, lipid peroxidation of cellular structures has an important role in cardiovascular complications of T2DM. Hyperlipidemia was reported as a causative factor for increased lipid peroxidation in T2DM [36]. Pioglitazone, the T2DM remedy, can reduce blood glucose levels in patients with T2DM. Combination with pioglitazone showed a remarkable decrease in TG levels and an increase in HDL-C levels which is expected to lead to a reduction in cardiovascular risk. Pioglitazone appeared to have lesser effects of a decrease in LDL-C [37].

It is observed that Cr deficiency leads to hyperglycemia and elevated lipid profile variables except for HDL-C. However, the precise mechanism of action of Cr combination with PGZ is still unclear; however, numerous mechanisms suggested explaining and justifying fully its specific signaling process. Chromium acts as a cofactor via its insulin secondary messenger action; it improves insulin sensitivity and facilitates glucose utilization by extrahepatic tissues. Chromium also improves insulin affinity to its receptors and activates intracellular insulin receptor kinases similarly to its action in inhibiting insulin receptor phosphatases. Cr-binding protein (Chromodulin) can promote tyrosine kinase enzyme activity of insulin receptors. Our data shows that PGZ has lowered the LDL-C, TC, TG, and atherogenic indices but raised the HDL-C levels. This pharmacological positive effect is desirable and well-known about PGZ as supporting findings are found in Derosa, et al. [36]. In patients with T2DM study, Sreekumar, et al. [38] found that lipid profile level was also improved with pioglitazone treatment versus baseline and placebo as it reduced cardiovascular risk parameters. On the other hand, Cr-GTF +PGZ has better performance over these variables against Cr-PL+PGZ or single PGZ intake. Declined TC, TG, and LDL- C, and elevated HDL serum concentration is observed in Cr-GTF +PGZ compared to PGZ alone, Cr-GTF, or Cr-PL monotherapy.

Cr-PL and Cr-GTF single administration did not make a remarkable difference concerning these variables except for lowering LDL-C and TG in the Cr-GTF groups only. This equates to a reduced risk for cardiac disease and T2DM complications. Following the results of Guimaraes, et al. [39] study, no effects are shown against lipid profile and atherogenic indices with or without Cr-administration. Several investigators like Sharma, et al. [40] reported a significant improvement in glucose disposal and insulin output when Cr is administered with or without antidiabetic drugs. This effect will decrease lipolysis and fatty acid oxidations which minimize the rate of free plasma lipids levels elevation.

It is found that chromium supplements protect against hypercholesterolemia and decrease the incidence of plaques in animals. Asbaghi, et al. [29] found that Cr may also inhibit the key enzyme of cholesterol synthesis, thus improving the dyslipidemia lipid profile. These findings

are shown in our study but without significant comparative bioactive effects which illustrates that an unclear mechanism of action for the Cr-PGZ combination could lay behind these conflicting findings.

Peroxisome proliferator-activated receptor-gamma (PPARγ) is a nuclear receptor, which stimulates the transcription of genes responsible for the growth and differentiation of adipocytes upon activation with various natural and synthetic ligands. Furthermore, PPARγ is the sensible active receptor for insulin-sensitizing thiazolidinediones, the drugs that are commonly used for T2DM.

Thiazolidinediones are potent PPARγ activators that improve insulin resistance, whereas PPARα activators primarily improve dyslipidemia. A recent study of the acute effects of pioglitazone, the PPARγ agonist, on human islets from subjects without and with T2D suggested that short-term pretreatment with pioglitazone primes both healthy and diabetic human islets for enhanced glucose-sensitive insulin secretion [41].

Our findings delivered a significantly lower activity level of PPAR-gamma in the diabetic group than in the healthy one, where the PGZ-treated group presented higher activity than the control diabetic group. PGZ+Cr-PL treated group showed significantly higher activity than the control group similar to PGZ+Cr-GTF treated groups. Cr-PL and Cr-GTF single-treated groups showed lower but not significant activities than the healthy and PGZ+Cr-GTF group of intervention. Consequently, the addition of Cr different compounds did not make any significant physiological improvement in PGZ pharmacological activity.

Turgut, et al. [42] found that chromium picolinate intake has improved the levels of PPARγ activity significantly, while another study by Amiri Siavashani [43] found that chromium-GTF supplementation upregulated gene expression of PPAR-γ. Rare studies are available for examining the combination effect between Cr and PGZ over PPAR-γ expression and activity level in T2DM cases. Our study is considered one of the most comprehensive and well-detailed studies about the possible synergistic effect of these combinations.

Conclusion

The experimental combinations of therapies indicated that single administration of Cr-PL and Cr-GTF complexes has significant changes in diabetic biomarkers but not to the extent of approximate normal levels in healthy groups, while a combination between Cr and PGZ in different forms showed a healthier significant amelioration in abnormal T2DM biomarkers. Lipid profile and its variables, TC, LDL-C, TG, LDL-C/HDL-C, TC/TG, AIP, and AC are significantly higher mean values (P< 0.05) and lower HDL-C in the control group than PGZ, (PGZ+Cr-PL), and (PGZ+Cr-GTF) groups (54.89±00.26, 20.88±00.49, 27.62±00.13, 39.50±01.09, 0.76±0.02, 1.40±0.04, 0.15±0.01, 1.99±0.02, 0.99±0.02, for PGZ respectively for the control group). Total cholesterol, LDL-C, TG, and LDL-C/HDL-C mean values are significantly higher (P< 0.05) and lower HDL-C in the Cr-PL group than (PGZ+Cr-PL) group (89.44±00.19, 55.73±00.26, 12.21±00.42, 110.4±00.63, 4.61±0.15, respectively for Cr-PL group). Total cholesterol, LDL-C, TG, LDL-C/HDL-C, TC/TG, AIP, and AC are significantly higher (P< 0.05) and lower HDL-C in Cr-PL and Cr-GTF groups than (PGZ+Cr-GTF) group.

Conflict of Interest and Disclosures

This work represents the joint effort of clinicians and scientists at The University of Jordan and the University of Petra. Supported by a grant from The University of Jordan and the Dr. Lina Tamimi Fund.

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