ORIGINAL ARTICLE

Test of insulin resistance in nondiabetic and streptozotocin-induced diabetic rats using glycosylated hemoglobin test and other interventions

Lina AlTamimi, Zainab Z. Zakaraya[1], Mohammad Hailat[2], Mousa N. Ahmad[3], Nidal A. Qinna[4,5], Mohammed F. Hamad[6], Wael Abu Dayyih[7]

Department of Clinical Pharmacy, Faculty of Pharmacy, Zarqa University, Zarqa, Jordan, [1]Department of Biopharmaceutics and Clinical Pharmacy, Faculty of Pharmacy, Al-Ahliyya Amman University, [2]Department of Pharmacy, Faculty of Pharmacy, Al-Zaytoonah University of Jordan, [3]Department of Nutrition and Food Technology, Human Nutrition and Dietetics, The University of Jordan, [4]Department of Pharmacology and Biomedical Sciences, Faculty of Pharmacy and Medical Sciences, University of Petra, [5]University of Petra Pharmaceutical Centre (UPPC), University of Petra, Amman, [6]Department of Basic Medical Sciences, Faculty of Medicine, Al-Balqa Applied University, Al-Salt, [7]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mutah University, Al-Karak, Jordan

J. Adv. Pharm. Technol. Res.

ABSTRACT

Type 2 diabetes is common globally. Pioglitazone (PGZ) is an oral TZD antidiabetic, whereas chromium-picolinate (Cr-PL) and Cr-glucose tolerance factor (Cr-GTF) are useful type 2 diabetes mellitus (T2DM) supplements. Cr-PL/GTF antioxidants cure T2DM. They may fail in diabetes with or without insulin-sensitizing medications. It examined how Cr-PL, Cr-GTF, PGZ, and their combination affected glucose, glycosylated hemoglobin, insulin, and HOMA-IR. Sixty-three adult Sprague-Dawley rats (220–300 g) were selected, and nine rats were randomly assigned to a normal nondiabetic group. In contrast, 54 rats were randomly split into 9 rats per each of the 6 major groups and injected intraperitoneally with 40 mg/kg STZ to induce T2DM. Rats were administered PGZ = 0.65 mg/kg (rat weight)/day, Cr-PL = 1 mg/kg, Cr-GTF = 1 mg/kg, and their combinations (PGZ + Cr-PL and Cr-GTF) daily for 6 weeks per intervention. The PGZ + Cr-PL and PGZ + Cr-GTF groups had substantially lower insulin levels than the PGZ group (13.38 ± 0.06, 12.98 ± 0.19 vs. 14.11 ± 0.02, respectively), with the PGZ + Cr-GTF group having the lowest insulin levels (12.98 ± 0.19 vs. 14.11 ± 0.02, 13.38±0.06, respectively). Intervention substantially reduced HOMA-IR in the PZ + Cr-PL and PZ + Cr-GTF groups compared to PGZ (7.49 ± 0.04, 6.69 ± 0.11 vs. 8.37 ± 0.04, respectively). This research found that combining PGZ with Cr-GTF resulted in considerably lower HOMA-IR levels than the PGZ and Cr-PL groups (6.69 ± 0.11 vs. 8.37 ± 0.04, 7.49 ± 0.04, respectively). Both Cr-PL and Cr-GTF may control T2DM. Both Cr complexes improved T2DM biomarkers more than the control diabetic group without medication. PGZ alone and PGZ + Cr-PL had less pharmacological synergy than Cr-GTF and PGZ in altering insulin and HOMA-IR blood levels. These encouraging discoveries need more study.

Key words: Chromium-glucose tolerance factor, chromium-picolinate, glycated hemoglobin, pioglitazone, type 2 diabetes

Address for correspondence:
Prof. Wael Abu Dayyih,
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mutah University, Al-Karak 61710, Jordan.
E-mail: wabudayyih@mutah.edu.jo

Submitted: 03-Jul-2023 **Revised:** 26-Oct-2023
Accepted: 16-Nov-2023 **Published:** 15-Jan-2024

Access this article online	
Quick Response Code:	Website: www.japtr.org
	DOI: 10.4103/JAPTR.JAPTR_343_23

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a global health crisis with high morbidity and mortality rates due

How to cite this article: AlTamimi L, Zakaraya ZZ, Hailat M, Ahmad MN, Qinna NA, Hamad MF, *et al*. Test of insulin resistance in nondiabetic and streptozotocin-induced diabetic rats using glycosylated hemoglobin test and other interventions. J Adv Pharm Technol Res 2024;15:1-7.

to its increased prevalence.[1,2] Its link with oxidative stress may reflect excessive oxidative deterioration in hyperglycemia and dyslipidemia.[3,4] T2DM is the most common form of the disease, and it is caused by a combination of hereditary and lifestyle factors, particularly obesity.[5,6]

The World Health Organization states that chronic hyperglycemia, characterized by fasting blood glucose (FBG) levels of 126 mg/dL or higher, is a significant global health issue due to decreased insulin production and beta-cell dysfunction, a condition influenced by genetic and environmental factors.[7]

Pioglitazone (PGZ), an effective insulin sensitizer, reduces insulin sensitivity in DM patients by maintaining β-cell function, decreasing glycosylated hemoglobin (HbA1c), and improving metabolic syndrome symptoms. It also enhances insulin sensitivity in fat and muscle, reducing hypertension and dyslipidemia.[8]

Sharma and Verma's study on PGZ's impact on glucose homeostasis revealed that it enhances glycogen synthesis and transport, potentially increasing glucose uptake or oxidation, leading to skeletal muscle glucose disposal through nuclear peroxisome proliferator-activated receptor-gamma (PPAR-γ). This study also explored the relationship between blood chromium, leptin, PPAR-γ, glucose, insulin, and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR).[9]

The study examined the effects of PZ, chromium-picolinate (Cr-PL), and Cr-glucose tolerance factor (Cr-GTF) on glucose, HbA1c, insulin, and HOMA-IR in rats with type 2 diabetes. Groups of rats received different interventions for 6 weeks. PGZ/Cr-GTF had the lowest insulin and HOMA-IR levels. Cr-PL and Cr-GTF could curb type 2 diabetes and improve biomarkers. Exploring these findings requires more research.

MATERIALS AND METHODS

Materials

The HCl-conjugated derivative of PGZ was employed. Dar Al Dawa provided an oral crystalline powder sample of PGZ HCl (Amman, Jordan, Batch number: BWP200007-Cipla Ltd.). Cr-PL and Cr-GTF came from Now® Foods (USA, Batches 20129861 and 3172406). Freshly dissolved PGZ HCl and Cr powder samples in CMC 10% were used to compute rat dosage quantities. Using the highest single dosage of 45 mg/70 kg adult human/day, the PGZ HCl dose was estimated as 0.65 mg/kg (rat weight)/day. Rats received daily doses of PGZ, Cr-PL, and Cr-GTF and their combinations through gastric gavage between 8:00 and 10:00 am to guarantee precise digestion.[10]

Design and animal experiments

The Institutional Animal Ethics Committee authorized a randomized controlled design experiment using male Sprague-Dawley rats as animal models for T2DM. The rats (n = 63) were acquired from the University of Petra (Amman, Jordan) and maintained in a well-ventilated, controlled environment. The rats' natural diet included powder yellow corn, soybean food, DL-methionine, choline chloride, mineral mix, vitamin mix, and corn oil. The animal ethical clearance number and the institutional clearance certificate number were UOPEC-2021/321.[11-13]

Type 2 diabetes induction

After acclimation, nine rats were randomly assigned to a healthy nondiabetic group (G0), while the other 54 rats were selected for T2DM induction by intraperitoneal injection with 40 mg/kg STZ.[14] Streptozotocin doses were calculated according to dose and rats' weight. Cayman–USA (188883-66-4) supplied 95% streptozotocin kept at 4°C. STZ was produced shortly before injection using 10% buffer citrate at pH 4.5. Dose and rat weight determine streptozotocin dosages. Rats were fasted for 4 h before injection and received 10% sucrose drinking water for 24 h.

Criteria for inclusion were blood glucose levels after T2DM induction: FBG (12 h) ≥170 mg/dl and non-FBG ≥250 mg/dl, not surpassing 300 mg/dl.[14] The Glucocard-S (Arkray, Japan) blood glucose meter was utilized.

After 1 week of STZ injections, rats' blood glucose levels were constant, indicating their inclusion in the experimental intervention stage. After induction and inclusive selection, 54 rats were randomly divided into six groups (1–6) of 9 rats, each labeled according to the intervention type [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 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.

Dose calculation

Cr-PL and Cr-GTF were purchased from Now Foods, USA (Batch 20129861 and 3172406). Freshly dissolved PGZ HCl and Cr powder samples in CMC 10% were used to compute rat dosage quantities. The maximal single dosage of 45 mg/70 kg adult human/day was used to compute the PGZ HCl dose: 0.65 mg/kg (rat weight)/day.[15] A prior animal study showed that the Cr maximum safe dosage of 1 mg/kg (rat weight)/day was utilized safely and effectively.[10] To achieve correct dosing, rats were gastric-gavaged daily with PGZ, Cr-PL, Cr-GTF, and combinations between 8:00 and 10:00 a.m. The five experimental groups of rats were

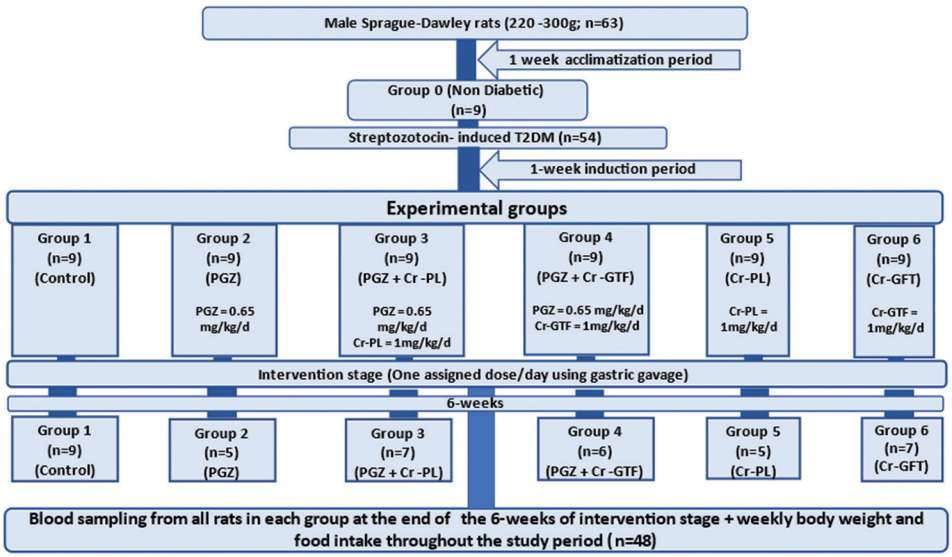

Figure 1: Streptozotocin-induced diabetic and control rats underwent a series of therapies over 6 weeks. PGZ: Pioglitazone, Cr-PL: Chromium-picolinate, Cr-GTF: Chromium-glucose tolerance factor

sorted and given Cr-PL, Cr-GTF, and PGZ in the following combinations:

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

After the 6-week intervention, 48 rats were fasted for 8 h and anesthetized with isoflurane. Blood was slowly drawn from the left ventricle using a 19–21-G needle per rat. After aliquoting, samples were placed in labeled 3 mL EDTA Eppendorf tubes. To analyze HbA1c and FBG, collect 0.3 ml of blood, and keep it at 4°C until analysis (≤24 h.). Leave the remaining blood at room temperature to coagulate. Following 15 min of centrifugation at 300 rpm, serum was stored at −80°C for analysis. Blood samples were obtained while participants were fasting following week 6 of the study using standard experimental techniques.

Body weight, water, and food intake

Body weight, water intake, and food consumption measurements were taken weekly throughout the trial and again after week 6. The researcher performed all biochemical experimental procedures and analyses at Aurum Biotech Laboratories, Amman, Jordan.

Biochemical analyses

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

Fasting blood glucose and glycated hemoglobin A1c analysis

Genrui PA120 Analyzer quantified fresh blood samples for glycated hemoglobin A1c analysis, whereas Snibe Biossays 240 Plus Analyzer serum samples were used for FBG and lipid profile analysis. Within 30 min of thawing, frozen serum was centrifuged at 3000 rpm for 20 min before analysis. The supernatants were immediately collected and analyzed without freeze/thaw cycles. 10 µL of serum and 1 ml of reagent were mixed after 10 min of incubation at 37°C. Absorbance was then read against blank at 450 nm. All reagents were at room temperature (20°C–25°C) before analysis.

Insulin analysis and homeostasis model assessment of insulin resistance

All samples and analysis reagents were placed at room temperature (18°C–25°C) before analysis. An appropriate ELISA kit from MyBioSource (USA) manufacturer was used to analyze and quantify insulin concentrations. According to Matthews *et al*., IR was evaluated using the HOMA-IR.[16] The following formula determined each rat's HOMA-IR:

$$\text{HOMA - IR} = \frac{\text{fasting insulin (mIU / mL)} \times \text{fasting glucose (mg / dL)}}{405}$$

Statistical analysis

The study utilized SAS 9 for statistical analysis (Statistical Analysis Software, SAS Institute Inc., Cary, North Carolina, US), utilizing tests such as Kolmogorov–Smirnov, Shapiro–Wilk, Levene's, Kruskal–Wallis multiple comparison test, Bonferroni correction, and Pearson's correlation test to determine statistical significance ($P < 0.05$).

RESULTS

Six-week therapies evaluated IR values in FBG, HbA1c, insulin, and homeostatic model. G(0) showed significantly lower values, with mean and standard error of mean (SEM) values. HbA1c and insulin mean values were also lower, with mean and SEM values indicating a significant difference.

The mean values of FBG, HbA1c, insulin, and HOMA-IR were significantly higher in G(1) compared to G(2), G(3), and G(4). However, G(3) has lesser significance in insulin and HOMA-IR, with mean and SEM values of 13.38 ± 0.06 and 7.49 ± 0.04, respectively.

The mean and SEM values for FBG, HbA1c, insulin, and HOMA-IR showed significant differences between groups, with G(4) having lower values than others. However, G(0) values were nonsignificant [Table 1].

Figure 2 shows that G(1), G(2), G(5), and G(6) have significantly ($P < 0.05$) greater HOMA-IR values compared to G(0). Furthermore, HOMA-IR readings were considerably ($P < 0.05$) lower in G(2), G(3), and G(4) compared to G(1). Compared to Group 3, Group 5 has substantially higher HOMA-IR values ($P < 0.05$). Furthermore, the HOMA-IR levels in G(5) and G(6) were considerably ($P < 0.05$) greater than in G(4). Group (3) and G(4) show significantly lower values of HOMA-IR than G(2), while G(4) shows significantly lower HOMA-IR values than G(3). On the contrary, HOMA-IR values of G(3) and G(4) were equivalent to G(0) in a statistical sense. It is worth noting that G(5) and G(6) were not all that different from G(1) and G(2), either. HOMA-IR values of G(6) were not significantly different from that of G(3). HOMA-IR values of G(6) were not significantly different from that of G(5).

Figure 3 shows substantial changes ($P < 0.05$) in FBG levels between nondiabetic and streptozotocin-induced diabetic rats after 6 weeks of intervention, including healthy and treated groups. FBG was much greater in G(1), G(2), G(5), and G(6) than G(0). FBG levels were much lower in G(2), G(3), and G(4) than G(1). G(5) also has a much greater FBG than G(3). FBG levels were much greater in G(5) and G(6) than in G(4). FBG levels were not statistically different between G(6) and G(5) or G(3) and G(4) with G(0). Differences between G(5) and G(6) with G(1), G(3), G(4), G(5), and G(6) with G(2) were not significant.

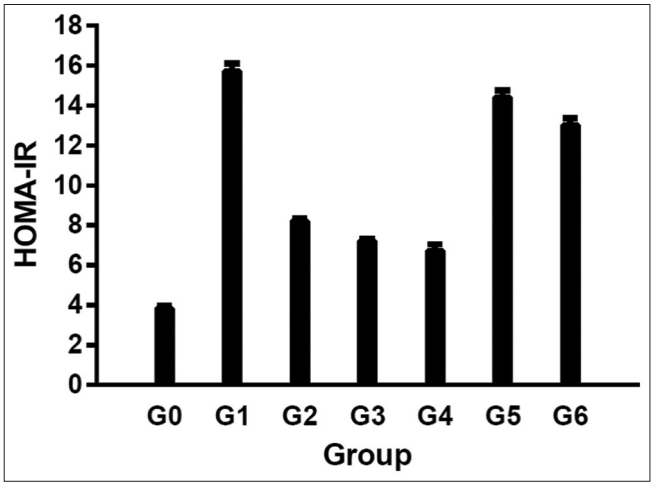

Figure 2: Significant differences in homeostatic model assessment of insulin resistance values among nondiabetic and streptozotocin-induced diabetic rats with various 6-week interventions. G(1): No intervention, G(2): PGZ=0.65mg/kg/day, G(3): PGZ=0.65mg/kg/day+Cr-PL=1mg/kg/day, G(4): PGZ = 0.65 mg/kg/day + Cr-GTF = 1 mg/kg/day, G(5): Cr-PL = 1 mg/kg/day, G(6): Cr-GTF = 1 mg/kg/day. PGZ: Pioglitazone, Cr-PL: Chromium-picolinate, Cr-GTF: Chromium-glucose tolerance factor, HOMA-IR: Homeostatic model assessment of insulin resistance

Table 1: Evaluation of insulin resistance in 6-week-old nondiabetic and streptozotocin-induced diabetic rats using fasting blood glucose, hemoglobin A1c, insulin, and a homeostatic model

Variables	Nondiabetic group G (0) (*n*=9)	Streptozotocin-induced diabetic groups					
		Control G (1) (*n*=9)	PGZ G(2) (*n*=5)	PGZ + Cr-PL G (3) (*n*=7)	PGZ + Cr-GTF G (4) (*n*=6)	Cr-PL G (5) (*n*=5)	Cr-GTF G (6) (*n*=7)
FBG (mg/dL)	116.0±1.3	347.2±0.9[a]	240.2±01.2[a,b]	226.8±0.6[b]	208.6±0.7[b]	334.4±0.6[a,c,d]	325.7±1.4[a,d]
HbA1c	3.39±0.05	4.71±0.05[a]	3.76±0.02[b]	3.61±0.02[b]	3.41±0.02[b]	4.32±0.02[a,c,d]	3.96±0.03[a,d]
Insulin (mIU/L)	12.84±0.33	18.25±0.18[a]	14.11±0.02[b,c,d]	13.38±0.06[b,d]	12.98±0.19[b]	17.55±0.08[a,c,d]	16.15±0.15[a,d]
HOMA-IR	3.67±0.07	15.65±0.18[a]	8.37±0.04[a,b,c,d]	7.49±0.04[b,d]	6.69±0.11[b]	14.49±0.07[a,c,d]	12.98±0.10[a,d]

[a]Significantly different from G(0), [b]Significantly different from G(1), [c]Significantly different from G(3), [d]Significantly different from G(4). Means are expressed as a value±SD (SEM). Oral intervention doses: 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. Significant differences ($P<0.05$) exist between values in rows with different superscripts. PGZ: Pioglitazone, Cr-PL: Chromium-picolinate, Cr-GTF: Chromium-glucose tolerance factor, FBG: Fasting blood glucose, HbA1c: Glycosylated hemoglobin, HOMA-IR: Homeostatic model assessment of insulin resistance, SEM: Standard error of mean, SD: Standard deviation

Figure 4 shows substantial ($P < 0.05$) and nonsignificant changes in glycated hemoglobin levels between nondiabetic and treated groups after 6 weeks of treatments. G(1), G(5), and G(6) have much greater HbA1c than G(0), however G(2), G(3), and G(4) did not. There was no substantial variation in G(5) and G(6) levels from G(1), however G(2), G(3), and G(4) were significantly lower. G(3), G(4), G(5), and G(6) HbA1c readings were also similar. G(5) has much greater levels than G(3); however, G(4) and G(6) did not. Finally, G(5) and G(6) have considerably higher HbA1c values than G(4), while G(6) was not significant.

Figure 5 reveals that after comparing insulin levels between control and treated groups, G(1), G(5), and G(6) showed significant ($P < 0.05$) increases compared to G(0). Insulin levels were substantially ($P < 0.05$) higher in G(2), G(3), and G(4) than G(1). Compared to G(3), G(5) has significantly higher concentrations ($P < 0.05$). Insulin levels in G(5) and G(6) were significantly higher ($P < 0.05$) than in G(4). G(3) and G(4) were substantially lower than G(2), and G(4) was much lower. Other group differences were not statistically significant.

DISCUSSION

Our study contradicts previous research suggesting PGZ activates the P38 pathway for glucose absorption in insulin-resistant adipocytes, suggesting further research is needed to confirm its usefulness.[17] Our study contradicts previous research suggesting PGZ activates the P38 pathway for glucose absorption in insulin-resistant adipocytes, suggesting further research is needed to confirm its usefulness.[18,19]

The study found no significant differences in FBG and HbA1c levels between the PGZ, PGZ + Cr-PL, and PGZ + Cr-GTF groups, resembling the T2DM group level, unlike previous research.[20] The study found no significant bioactive differences between Cr-PL or Cr-GTF single administration and Cr-PL + PGZ or Cr-GTF + PGZ co-administration, and Cr-PL + PGZ and Cr-GTF + PGZ did not differ significantly.

PGZ lowers insulin and HOMA-IR values compared to diabetic and nondiabetic groups in our research. No prior research has shown that all therapies significantly reduced insulin and HOMA-IR levels in T2DM models when given individually but substantially less when administered with PGZ + Cr-GTF compared to PL and PGZ.[21] PGZ with both Cr compounds improved FBG and HbA1c levels but did not improve pharmacological responsiveness.

Antioxidant Cr compounds may enhance insulin sensitivity in T2DM by promoting GLU4 opening and glucose cell entry, potentially enhancing glucose tolerance. Previous research has not addressed this effect's mechanism.[22,23]

According to Refaie *et al*.,[24] Cr-PL significantly lowered blood glucose in diabetic rats but not normal rats. Cr-PL affected glucose metabolism mechanistically. Cr-PL lowered blood glucose and improved carbohydrate metabolism in T2DM rats, according to Sundaram *et al*.[25] Chen *et al*.[26] found that Cr-PL has unique insulin-sensitizing and antihyperglycemic effects. The main approaches are to increase glucose transporter GLUT4 expression and regulate carbohydrate and lipid abnormal metabolism.[24-26]

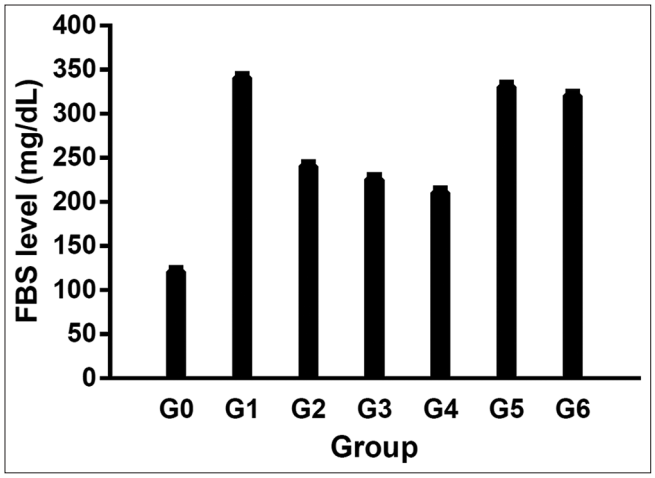

Figure 3: Fasting blood glucose levels (mg/dl) significantly differed between nondiabetic and streptozotocin-induced diabetic rats after 6 weeks of treatment. G(1): No intervention, G(2): PGZ=0.65mg/kg/day,G(3):PGZ=0.65mg/kg/day+Cr-PL=1mg/kg/day, G(4): PGZ = 0.65 mg/kg/day + Cr-GTF = 1 mg/kg/day, G(5): Cr-PL = 1 mg/kg/day, G(6): Cr-GTF = 1 mg/kg/day. FBS: Fasting blood glucose, PGZ: Pioglitazone, Cr-PL: Chromium-picolinate, Cr-GTF: Chromium-glucose tolerance factor

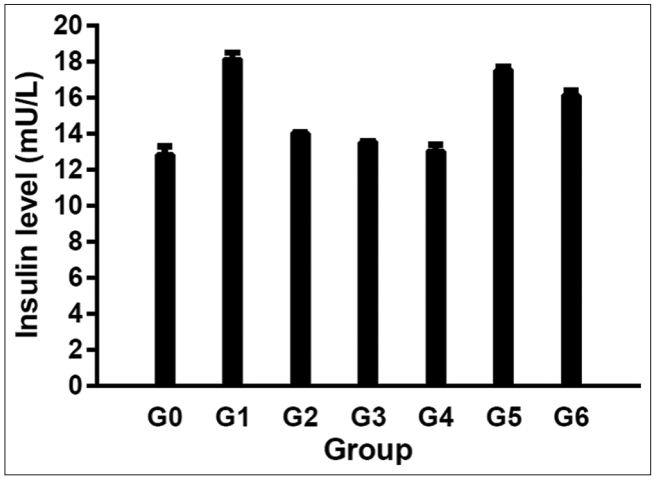

Figure 4: Significant differences in insulin levels (mIU/L) among nondiabetic and streptozotocin-induced diabetic rats with 6-week interventions. G(1): No intervention, G(2): PGZ=0.65mg/kg/day,G(3):PGZ=0.65mg/kg/day+Cr-PL=1mg/kg/day, G(4): PGZ = 0.65 mg/kg/day + Cr-GTF = 1 mg/kg/day, G(5): Cr-PL = 1 mg/kg/day, G(6): Cr-GTF = 1 mg/kg/day. PGZ: Pioglitazone, Cr-PL: Chromium-picolinate, Cr-GTF: Chromium-glucose tolerance factor

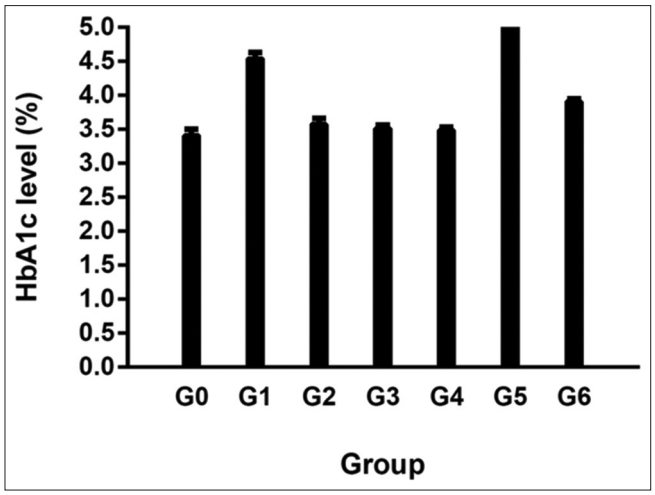

Figure 5: Significant differences in glycosylated hemoglobin levels (mmol/mol) among nondiabetic and streptozotocin-induced diabetic rats with 6-week interventions. G(1): No intervention, G(2): PGZ=0.65mg/kg/day,G(3):PGZ=0.65mg/kg/day+Cr-PL=1mg/kg/day, G(4): PGZ = 0.65 mg/kg/day + Cr-GTF = 1 mg/kg/day, G(5): Cr-PL = 1 mg/kg/day, G(6): Cr-GTF = 1 mg/kg/day. PGZ: Pioglitazone, Cr-PL: Chromium-picolinate, Cr-GTF: Chromium-glucose tolerance factor, HbA1c: Glycosylated hemoglobin

This study examines diabetes management using a controlled experimental design and a Sprague-Dawley rat model. It evaluates biomarkers, single interventions, and combination therapies, revealing potential synergistic effects. However, it has limitations such as small sample size, brief intervention duration, lack of human data, and lack of long-term effects on diabetes-related biomarkers.

CONCLUSIONS

Insulin resistance is a global health concern, with T2DM patients at high risk for complications. PGZ, an insulin-sensitizing drug, was used to treat T2DM orally. Cr, an essential mineral, has been shown to reduce resistance. Combinations of PGZ with Cr-GTF and Cr-PL showed lower insulin and HOMA-IR biomarker levels, reducing side effects. Further metabolic examinations are needed.

Acknowledgment

Dr. Bayan Ghanim's assistance and the University of Petra Pharmaceutical Centre's helpers in the experimental unit provided the necessary animals and lodging for the study.

Financial support and sponsorship

This study, funded by the University of Jordan and Dr. Lina Tamimi's Medical Relief Fund, involved clinicians and researchers from Jordan and Petra Universities.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Dayyih WA, Manaysa MH, Hailat MM, Zakareia Z, El Hajji F. Influence of castor oil on glycated hemoglobin (Hba1c) on induced type 2 diabetes mellitus in rats. Jordan J Pharm Sci 2021;14:341-9.
2. Ruze R, Liu T, Zou X, Song J, Chen Y, Xu R, *et al*. Obesity and type 2 diabetes mellitus: Connections in epidemiology, pathogenesis, and treatments. Front Endocrinol (Lausanne) 2023;14:1161521.
3. Al Qirim TM, Zafir A, Banu N. Remedial antioxidant action of Withania somnifera on restraint stress induced oxidative damage. FASEB J 2008;22:611-2.
4. Molehin OR, Adefegha SA, Adeyanju AA. Role of oxidative stress in the pathophysiology of type 2 diabetes and cardiovascular diseases. In: Role of Oxidative Stress in Pathophysiology of Diseases. Singapore: Springer; 2020. p. 277-97.
5. Schnurr TM, Jakupović H, Carrasquilla GD, Ängquist L, Grarup N, Sørensen TI, *et al*. Obesity, unfavourable lifestyle and genetic risk of type 2 diabetes: A case-cohort study. Diabetologia 2020;63:1324-32.
6. Zakaraya ZZ, Al Tamimi L, Hailat M, Ahmad MN, Qinna NA, Ghanim BY, *et al*. Ameliorative effect of selenium yeast in combination with pioglitazone on diabetes outcomes in streptozotocin-induced. J Popul Ther Clin Pharmacol 2022;29:e202-10.
7. Tamimi LN, Zakaraya Z, Abu Dayyih W, ALjaraedah TY, Al Omari RH, Abu Assab M, *et al*. The impressive evaluation of selenium yeast role against diabetes mellitus and its comorbidities by streptozotocin-induced diabetes in rats. J Popul Ther Clin Pharmacol 2023;30:85-92.
8. Bell DS, Jerkins T. In praise of pioglitazone: An economically efficacious therapy for type 2 diabetes and other manifestations of the metabolic syndrome. Diabetes Obes Metab 2023;25:3093-102.
9. Sharma SK, Verma SH. A study of effects of pioglitazone and rosiglitazone on various parameters in patients of type-2 diabetes mellitus with special reference to lipid profile. J Assoc Physicians India 2016;64:24-8.
10. Iskra R, Antonyak H. Chromium in health and longevity. In: Trace Elements and Minerals in Health and Longevity. Cham: Springer; 2018. p. 133-62.
11. Barbosa-da-Silva S, Sarmento IB, Bargut TC, Souza-Mello V, Aguila MB, Mandarim-de-Lacerda CA. Animal models of nutritional induction of type 2 diabetes mellitus. Int J Morphol 2014;32:279-93.
12. Ahmad MN, Khatib FA. Effects of dietary saturated, monounsaturated and polyunsaturated fats on plasma lipids and lipoproteins in diabetic rats. Ecol Food Nutr 1990;24:141-8.
13. Rowan AN. Guide for the care and use of laboratory animals. J Med Primatol 1979;8:128.
14. Wilson RD, Islam MS. Fructose-fed streptozotocin-injected rat: An alternative model for type 2 diabetes. Pharmacol Rep 2012;64:129-39.
15. Blackburn JK, Curry DW, Thomsen AN, Roth RH, Elsworth JD. Pioglitazone activates paraoxonase-2 in the brain: A novel neuroprotective mechanism. Exp Neurol 2020;327:113234.
16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
17. Esmaeili S, Safaroghli-Azar A, Pourbagheri-Sigaroodi A, Salari S, Gharehbaghian A, Hamidpour M, *et al*. Stimulation of peroxisome proliferator-activated receptor-gamma (PPARγ) using pioglitazone decreases the survival of acute promyelocytic leukemia cells through up-regulation of PTEN expression. Anticancer Agents Med Chem 2021;21:108-19.
18. Król E, Krejpcio Z, Iwanik K. Supplementary chromium (III)

propionate complex does not protect against insulin resistance in high-fat-fed rats. Biol Trace Elem Res 2014;157:147-55.

19. Wang YQ, Yao MH. Effects of chromium picolinate on glucose uptake in insulin-resistant 3T3-L1 adipocytes involve activation of p38 MAPK. J Nutr Biochem 2009;20:982-91.
20. Broadhurst CL, Domenico P. Clinical studies on chromium picolinate supplementation in diabetes mellitus – A review. Diabetes Technol Ther 2006;8:677-87.
21. Talab AT, Abdollahzad H, Nachvak SM, Pasdar Y, Eghtesadi S, Izadi A, *et al*. Effects of chromium picolinate supplementation on cardiometabolic biomarkers in patients with type 2 diabetes mellitus: A randomized clinical trial. Clin Nutr Res 2020;9:97-106.
22. Guo WL, Chen M, Pan WL, Zhang Q, Xu JX, Lin YC, *et al*. Hypoglycemic and hypolipidemic mechanism of organic chromium derived from chelation of Grifola frondosa polysaccharide-chromium (III) and its modulation of intestinal microflora in high fat-diet and STZ-induced diabetic mice. Int J Biol Macromol 2020;145:1208-18.
23. Genchi G, Lauria G, Catalano A, Carocci A, Sinicropi MS. The double face of metals: The intriguing case of chromium. Appl Sci 2021;11:638.
24. Refaie FM, Esmat AY, Mohamed AF, Aboul Nour WH. Effect of chromium supplementation on the diabetes induced-oxidative stress in liver and brain of adult rats. Biometals 2009;22:1075-87.
25. Sundaram B, Singhal K, Sandhir R. Ameliorating effect of chromium administration on hepatic glucose metabolism in streptozotocin-induced experimental diabetes. Biofactors 2012;38:59-68.
26. Chen G, Liu P, Pattar GR, Tackett L, Bhonagiri P, Strawbridge AB, *et al*. Chromium activates glucose transporter 4 trafficking and enhances insulin-stimulated glucose transport in 3T3-L1 adipocytes via a cholesterol-dependent mechanism. Mol Endocrinol 2006;20:857-70.