

EFFECT OF CHROMIUM PICOLINATE VS BIOTIN ON INSULIN RESISTANCE, METABOLIC INDICES, SERUM TYPE-2 DIABETES MELLITUS, AND CHOLESTEROL LEVELS AMONG STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Background: Type 2 diabetes mellitus is characterized by persistent hyperglycaemia, insulin resistance, dyslipidaemia, inflammation, and oxidative stress. Chromium picolinate and biotin may influence complementary pathways involved in glucose and lipid metabolism.

Objective: To evaluate the individual and combined effects of chromium picolinate and biotin on glycaemic control, insulin resistance, lipid profile, and related biochemical indices in streptozotocin-induced diabetic rats.

Methods: Sixty-six male rats were randomized into 11 groups of six animals. One group served as a non-diabetic control, while diabetes was induced in the remaining groups using streptozotocin at 65 mg/kg. Diabetic rats received chromium picolinate, biotin, or combined supplementation at graded doses for seven weeks. Body weight, feed and water intake, fasting glucose, HOMA-IR, HbA1c, lipid profile, haematological indices, hepatorenal markers, C-reactive protein, TBARS, superoxide dismutase, and catalase were assessed.

Results: Untreated diabetic rats showed marked metabolic deterioration, including post-treatment glucose of 430.6 ± 3.1 mg/dL, HOMA-IR of 44.90 ± 1.00 , and HbA1c of $10.92 \pm 0.50\%$. The highest combined regimen, chromium picolinate 200 µg/kg plus biotin 200 µg/kg, reduced glucose to 138.1 ± 1.7 mg/dL, HOMA-IR to 3.36 ± 0.40 , and HbA1c to $4.98 \pm 0.20\%$. Feed intake increased from 25.33 ± 1.20 to 35.50 ± 1.00 g/day and declined to 27.30 ± 2.20 g/day with combined treatment. It also produced near-control lipid, inflammatory, renal, and antioxidant values.

Conclusion: Combined chromium picolinate and biotin supplementation produced the greatest overall metabolic improvement and warrants further mechanistic and translational investigation.

KEYWORDS: Biotin; chromium picolinate; diabetes mellitus; HbA1c; insulin resistance; oxidative stress; streptozotocin.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by persistent hyperglycaemia arising from impaired insulin action, progressive pancreatic β -cell dysfunction, or both. Prolonged disturbances in glucose homeostasis are accompanied by abnormalities in carbohydrate, lipid, and protein metabolism and contribute to the development of cardiovascular, renal, hepatic, neurological, and microvascular complications [1]. Oxidative stress and chronic low-grade inflammation further aggravate insulin resistance by disrupting intracellular signalling and promoting cellular injury [2]. Consequently, therapeutic approaches capable of improving insulin sensitivity while simultaneously correcting dyslipidaemia and metabolic oxidative stress remain of considerable interest [3].

Chromium is an essential trace element involved in the regulation of carbohydrate, lipid, and protein metabolism. Chromium picolinate (CrPic) is among the most extensively investigated supplemental forms because of its relatively favourable bioavailability and proposed insulin-sensitizing activity [4]. Chromium has been reported to enhance insulin-receptor signalling, facilitate intracellular glucose uptake, and influence metabolic pathways involved in glucose and lipid utilization [5]. Experimental studies have additionally suggested that chromium supplementation

may attenuate oxidative disturbances associated with insulin resistance, although the magnitude of its metabolic effect varies according to dose, formulation, duration of supplementation, and severity of metabolic dysfunction [6].

Biotin, or vitamin B7, is a water-soluble micronutrient that functions as a cofactor for several carboxylases involved in gluconeogenesis, fatty acid synthesis, amino acid metabolism, and cellular energy production [7]. In addition to its coenzyme role, biotin can affect glucose metabolism and lipid homeostasis pathways and their expression [8]. Experimental studies showed that biotin supplementation has beneficial effects on glucose metabolism and insulin-related responses, and the simultaneous intake of chromium and biotin has shown beneficial effects on insulin sensitivity and related metabolic indicators in experimental models [9].

Among the various metabolic abnormalities associated with diabetes, dyslipidaemia is an important one and is generally associated with low HDL cholesterol and high total cholesterol, LDL cholesterol and triglycerides. Experimental studies have shown that chromium supplementation can influence selected glucose and lipid parameters [10], and human studies revealed an association between chromium status and glycaemic regulation and cardiovascular risk factors [11]. Moreover, metabolic improvement can be seen simultaneously with oxidative injury reduction, indicating that micronutrient interventions may impact a number of interdependent aspects of metabolic stress associated with diabetes [12].

The potential benefit of combining chromium picolinate with biotin is based on their complementary biological actions. Biotin is involved in enzymatic reactions that regulate glucose production, lipid synthesis and intermediary metabolism, while chromium primarily affects insulin signalling and glucose transport [13]. The effect of chromium on insulin-associated inflammatory signalling pathways has also been studied, and clinical trials have shown measurable effects of chromium supplementation on glycaemic and cardiometabolic indices in insulin-resistant individuals [14,15]. However, currently, there is little evidence to make direct comparisons between the dose-response of CrP and biotin alone, and in combination [16].

Thus, the present study was designed to investigate the effect of combined and individual supplementation of chromium picolinate and biotin on insulin resistance, fasting blood glucose, glycated hemicelluloses, body weight, feed intake, water intake, serum lipid profile and related metabolic indices in streptozotocin-induced diabetic rats. To assess the systemic inflammatory response to supplementation, as well as hepatic and renal biochemical markers and oxidative-stress indices, these were also evaluated to characterize the larger picture of metabolic response to supplementation [17,18].

MATERIALS AND METHODS

Study Design and Setting

The study was carried out in the Animal House, Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore, Pakistan, in a controlled experimental animal study. The study aimed to evaluate the effect of chromium picolinate and biotin alone and in combination on glycaemic regulation, insulin resistance, lipid metabolism, haematological indices, renal biochemical parameters, hepatic biochemical parameters, inflammatory status and oxidative stress in streptozotocin-induced diabetic rats.

Experimental Animals

A total of 66 healthy male rats, approximately seven weeks of age and weighing 180–250 g, were included in the study. Animals that were previously used in another experimental study were excluded. The rats were kept in properly ventilated, clean cages and provided with the proper light-dark schedule. Throughout the experimental period, standard rodent feed and drinking water were freely available. All the animals were acclimatized for 7 days before the start of the intervention.

Ethical Considerations

All the experimental procedures were conducted according to the rules and regulations laid down by the Research Ethical Committee (REC), The University of Lahore. Throughout the study, appropriate housing, feeding, environmental and animal-handling procedures were maintained. Special efforts were made to reduce all unnecessary stress, discomfort, and injury during induction of diabetes, treatment of diabetes, diabetes monitoring, collection of blood, and during all terminal procedures.

Materials and Reagents Procurement

All experimental materials, chemicals, reagents, and diagnostic kits were purchased from certified and authorized suppliers. Chromium picolinate and biotin were purchased as analytical grade. Streptozotocin was purchased from a reputable supplier and kept until use as per the manufacturer's guidelines. Commercial diagnostic kits were procured for the assessment of fasting blood glucose, insulin, lipid profile, liver and kidney biochemical indices, inflammatory markers, and oxidative-stress indices. Standard rodent feed was purchased from a commercial feed manufacturer known to produce good quality feed.

Experimental Diet

The basal diet was the same for all experimental groups throughout the trial. The basal diet was comprised of corn oil, corn starch, protein, cellulose, mineral mixture, and vitamin mixture. The drinking water and feed were given freely during the intervention period.

Induction of Diabetes

After acclimatization, the animals were weighed and then starved overnight. Experimental diabetes was achieved by injecting STZ in a single dose of 65 mg/kg bw, in citrate buffer (pH 4.5) i.p. Citrate buffer alone was administered to rats of the negative-control group.

Hyperglycaemia was verified by measuring the blood glucose concentration on days 3 and 7 after STZ administration. Blood was collected from the tail, and glucose was measured using an Advantage Accu-Chek glucometer. Rats were considered diabetic when their blood glucose concentrations exceeded 300 mg/dL and were assigned to the diabetic-control and intervention groups.

Experimental Grouping and Treatment Protocol

Rats were divided into 11 groups of 6 each, randomly distributed. Group 1 was the normal negative control, and Group 2 was the untreated diabetic control. Groups 3-5 were fed chromium picolinate at progressively increasing levels, groups 6-8 were provided with biotin at progressively increasing levels, and groups 9-11 were fed chromium picolinate and biotin together at increasing levels. Seven weeks were used for all interventions. The entire experimental design is given in Table 1.

Table 1. Experimental Grouping and Treatment Allocation

Group	Diabetes Induction	Treatment
G1	Citrate buffer, pH 4.5	Negative control; no supplementation
G2	STZ 65 mg/kg	Diabetic control; no supplementation
G3	STZ 65 mg/kg	Chromium picolinate 200 µg/kg body weight
G4	STZ 65 mg/kg	Chromium picolinate 300 µg/kg body weight
G5	STZ 65 mg/kg	Chromium picolinate 400 µg/kg body weight
G6	STZ 65 mg/kg	Biotin 200 µg/kg body weight
G7	STZ 65 mg/kg	Biotin 300 µg/kg body weight
G8	STZ 65 mg/kg	Biotin 400 µg/kg body weight
G9	STZ 65 mg/kg	Chromium picolinate 100 µg/kg + biotin 100 µg/kg body weight
G10	STZ 65 mg/kg	Chromium picolinate 150 µg/kg + biotin 150 µg/kg body weight
G11	STZ 65 mg/kg	Chromium picolinate 200 µg/kg + biotin 200 µg/kg body weight

Assessment of Body Weight, Feed Intake, and Water Intake

Body weight, feed intake, and water intake were monitored throughout the experimental period. Body weight was measured using a calibrated weighing balance and recorded in grams. Feed consumption was determined from the quantity of feed provided and the amount remaining, while water consumption was monitored from the volume supplied and the residual volume. These measurements were used to evaluate changes in nutritional and metabolic status associated with diabetes induction and supplementation.

Fasting Blood Glucose Measurement

Fasting blood glucose was measured after an overnight fast using blood obtained from the tail. Glucose concentration was determined using the glucose oxidase method with an Accu-Chek Advantage glucometer and was expressed in mg/dL.

Fasting Insulin and HOMA-IR

Fasting serum insulin concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Serum samples and standards were added to the ELISA plate, and absorbance was measured at 450 nm using a microplate reader. Insulin concentrations were calculated from the corresponding standard calibration curve.

Insulin resistance was estimated using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). HOMA-IR was calculated from fasting insulin and fasting glucose concentrations according to the following equation: $HOMA-IR = \frac{405 \times \text{Fasting insulin} \times \text{Fasting glucose (mg/dL)}}{100}$

The calculated HOMA-IR values were used to compare insulin resistance among the experimental groups.

Glycated Haemoglobin Measurement

Glycated haemoglobin (HbA1c) was determined using a commercially available assay according to the manufacturer's instructions and expressed as a percentage of total haemoglobin. Glyco-Tek affinity column kits were additionally used for assessment of HbA1c as an indicator of longer-term glycaemic status.

Serum Lipid Profile

The serum lipid profile comprised total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). These parameters were evaluated to determine the extent of diabetes-associated dyslipidaemia and the response to chromium picolinate and biotin supplementation.

HDL-C and LDL-C were determined using commercial enzymatic colorimetric procedures based on the cholesterol oxidase–peroxidase (CHOD-POD) method. Absorbance was measured at 505 nm using a spectrophotometer, and concentrations were expressed as mg/dL. Total cholesterol was similarly determined using an enzymatic CHOD-POD assay. Serum triglycerides were measured using the glycerol-3-phosphate oxidase–peroxidase (GPO-POD) method, with absorbance measured at 505 nm and results expressed as mg/dL.

Haematological Assessment

Haematological evaluation included red blood cell count (RBC), haemoglobin concentration (Hb), and haematocrit (Hct). Blood samples intended for haematological assessment were collected in EDTA-containing tubes and processed using standard haematological procedures. These indices were evaluated to assess alterations in erythrocyte-related parameters associated with experimental diabetes and subsequent supplementation.

Hepatic Biochemical Assessment

Hepatic biochemical status was assessed by measuring alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), serum albumin, and total bilirubin. These parameters were evaluated as secondary biochemical outcomes reflecting hepatic alterations associated with experimental diabetes.

AST was determined using a commercially available DiaSys Diagnostic Systems assay kit, with absorbance measured at 340 nm. ALT was measured using the corresponding commercial assay according to the manufacturer's protocol. ALP activity was determined using a commercial assay kit, with absorbance recorded at 405 nm. Serum albumin was measured using the bromocresol green colorimetric method, with absorbance measured at 630 nm, and the results were expressed as g/dL. Total bilirubin was determined using a commercial diazo-reaction colorimetric assay, with absorbance measured at 540 nm and results expressed as mg/dL.

Renal Biochemical Assessment

Renal biochemical status was evaluated by measuring blood urea and serum creatinine. Blood urea was determined using a commercial enzymatic colorimetric assay based on the urease–glutamate dehydrogenase method, with absorbance measured at 340 nm and results expressed as mg/dL. Serum creatinine was measured using a commercial colorimetric assay based on the Jaffe kinetic principle, with absorbance measured at 510 nm and concentrations expressed as mg/dL.

Assessment of C-Reactive Protein

Serum C-reactive protein (CRP) was measured as a marker of systemic inflammation using a commercially available ELISA kit in accordance with the manufacturer's instructions. Serum samples and standards were added to the assay plate, and absorbance was measured at 450 nm using a microplate reader. CRP concentrations were calculated from the standard calibration curve and expressed as mg/L.

Assessment of Lipid Peroxidation

Lipid peroxidation was evaluated through measurement of thiobarbituric acid-reactive substances (TBARS). Samples were reacted with thiobarbituric acid under acidic conditions and heated to produce a coloured chromogen. Following cooling, absorbance was measured spectrophotometrically at 532 nm. TBARS values were used as an indicator of lipid peroxidation and oxidative membrane damage.

Assessment of Antioxidant Status

Antioxidant status was evaluated by measuring the activities of superoxide dismutase (SOD) and catalase (CAT). SOD activity was determined using a commercially available assay based on its ability to inhibit superoxide-radical production, and activity was expressed as U/mg protein. Catalase activity was measured using a commercial assay based on the decomposition of hydrogen peroxide. Absorbance was recorded spectrophotometrically at 240 nm, and catalase activity was expressed as U/mg protein.

Terminal Blood Collection and Sample Processing

At the end of the seven-week experimental period, the animals were fasted before terminal sample collection. Rats were euthanized by exposure to halothane (2-bromo-2-chloro-1,1,1-trifluoroethane). Blood was subsequently collected by cardiac puncture using a 19½-gauge needle and processed according to the requirements of the respective haematological and biochemical assays.

Statistical Analysis

Data were entered and analyzed using Statistical Package for the Social Sciences (SPSS), version 25. Quantitative variables were expressed as mean $\pm$ standard deviation. Differences among the 11 experimental groups were assessed using one-way analysis of variance (ANOVA). When a statistically significant overall difference was identified, Tukey's Honestly Significant Difference (HSD) post-hoc test was used for multiple comparisons among groups. A p -value ≤ 0.05 was considered statistically significant.

RESULTS

All 66 experimental animals completed the study and were included in the final analysis. Data are presented as mean $\pm$ standard deviation. The effects of chromium picolinate, biotin, and their combined administration on anthropometric, glycaemic, haematological, biochemical, lipid, inflammatory, and oxidative-stress parameters are presented in Tables 1–5 and Figure 1.

Body Weight, Feed Intake, and Water Intake

Streptozotocin-induced diabetes produced a marked reduction in body weight, accompanied by increased feed and water consumption. Mean body weight decreased from 247.0 ± 2.6 g in the negative-control group (G1) to 189.3 ± 2.7 g in the diabetic-control group (G2). Chromium picolinate and biotin supplementation improved body weight to varying degrees, with the greatest increases observed in the combination groups. Mean body weight reached 262.0 ± 1.4 g in G9, 288.0 ± 1.0 g in G10, and 302.5 ± 1.3 g in G11 (Table 1).

Daily feed intake increased from 25.33 ± 1.20 g/day in G1 to 35.50 ± 1.00 g/day in G2, demonstrating the polyphagic response associated with uncontrolled diabetes. Water intake similarly increased from 31.4 ± 2.1 mL/day to 85.7 ± 4.2 mL/day. Both parameters progressively approached control values following supplementation. In G11, feed intake was reduced to 27.30 ± 2.20 g/day and water intake to 34.1 ± 1.2 mL/day (Table 1).

Table 1. Effect of Chromium Picolinate and Biotin Supplementation on Body Weight, Feed Intake, and Water Intake

Group	Treatment	Body Weight (g)	Feed Intake (g/day)	Water Intake (mL/day)
G1	Negative control	247.0 ± 2.6	25.33 ± 1.20	31.4 ± 2.1
G2	Diabetic control	$189.3 \pm 2.7^{***}$	$35.50 \pm 1.00^{***}$	$85.7 \pm 4.2^{***}$
G3	CrPic 200 μ g/kg	$192.3 \pm 1.1^*$	$35.83 \pm 1.40^*$	$60.3 \pm 3.5^*$
G4	CrPic 300 μ g/kg	$230.3 \pm 1.2^*$	$34.30 \pm 1.10^*$	$52.9 \pm 1.1^*$
G5	CrPic 400 μ g/kg	$232.5 \pm 1.5^{**}$	$28.00 \pm 1.20^*$	$46.2 \pm 2.6^*$
G6	Biotin 200 μ g/kg	$198.8 \pm 2.2^*$	$33.60 \pm 1.20^*$	$55.2 \pm 3.8^*$
G7	Biotin 300 μ g/kg	$235.6 \pm 1.4^*$	$31.00 \pm 1.90^*$	$42.3 \pm 2.4^*$
G8	Biotin 400 μ g/kg	$250.0 \pm 1.6^{**}$	$29.17 \pm 1.70^*$	$39.1 \pm 1.7^*$
G9	CrPic 100 μ g/kg + Biotin 100 μ g/kg	$262.0 \pm 1.4^{**}$	$32.80 \pm 0.02^*$	$52.5 \pm 2.9^*$
G10	CrPic 150 μ g/kg + Biotin 150 μ g/kg	$288.0 \pm 1.0^{**}$	$30.50 \pm 2.30^*$	$38.3 \pm 1.7^*$
G11	CrPic 200 μ g/kg + Biotin 200 μ g/kg	$302.5 \pm 1.3^{***}$	$27.30 \pm 2.20^*$	$34.1 \pm 1.2^*$

Values are expressed as mean $\pm$ SD. CrPic: chromium picolinate. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Glycaemic Status, Fasting Insulin, HOMA-IR, and HbA1c

Fasting blood glucose was markedly elevated following STZ administration. At week 7, fasting blood glucose increased from 102.3 ± 1.3 mg/dL in G1 to 300.6 ± 1.6 mg/dL in G2. Chromium picolinate and biotin supplementation reduced fasting glucose, with the greatest improvement observed in the combined-treatment groups. Fasting glucose values were 195.6 ± 1.5 mg/dL in G9, 165.1 ± 2.8 mg/dL in G10, and 101.6 ± 2.6 mg/dL in G11 (Table 2).

Post-treatment blood glucose increased to 430.6 ± 3.1 mg/dL in the diabetic-control group compared with 110.2 ± 3.0 mg/dL in the negative control. Chromium picolinate at 200, 300, and 400 μ g/kg reduced glucose to 400.0 ± 1.0 , 365.5 ± 3.2 , and 360.1 ± 2.6 mg/dL, respectively. Corresponding values in the biotin-treated groups were 410.1 ± 3.1 , 315.8 ± 2.0 , and 260.6 ± 3.5 mg/dL. The combined treatments produced greater reductions, with glucose concentrations of 270.6 ± 2.4 , 185.7 ± 1.2 , and 138.1 ± 1.7 mg/dL in G9, G10, and G11, respectively (Table 2).

Insulin resistance was substantially increased following diabetes induction. HOMA-IR increased from 2.37 ± 0.40 in G1 to 44.90 ± 1.00 in G2. Chromium picolinate reduced HOMA-IR to 28.84 ± 0.60 , 16.69 ± 0.40 , and 16.77 ± 0.10 in G3–G5, while the corresponding values in G6–G8 were 29.06 ± 0.70 , 20.44 ± 1.80 , and 14.30 ± 1.50 . The combined-treatment groups showed further reductions to 13.86 ± 1.10 in G9, 6.23 ± 0.60 in G10, and 3.36 ± 0.40 in G11. The distribution of HOMA-IR across the experimental groups is illustrated in Figure 1.

HbA1c increased from $4.86 \pm 0.28\%$ in G1 to $10.92 \pm 0.50\%$ in G2. Treatment progressively reduced HbA1c, with values of $5.82 \pm 0.20\%$, $5.24 \pm 0.20\%$, and $4.98 \pm 0.20\%$ in G9, G10, and G11, respectively. The highest combined-treatment group therefore demonstrated an HbA1c value closely approaching that of the negative control (Table 2).

Table 2. Effect of Chromium Picolinate and Biotin Supplementation on Glycaemic Parameters and Insulin Resistance

Group	Treatment	Week-7 FBG (mg/dL)	Post-Treatment Glucose (mg/dL)	Fasting Insulin	HOMA-IR	HbA1c (%)
G1	Negative control	102.3 ± 1.3	110.2 ± 3.0	1.10 ± 0.10	2.37 ± 0.40	4.86 ± 0.28
G2	Diabetic control	$300.6 \pm 1.6^{***}$	$430.6 \pm 3.1^{***}$	$1.85 \pm 0.20^{***}$	$44.90 \pm 1.00^{***}$	$10.92 \pm 0.50^{**}$
G3	CrPic 200 µg/kg	$295.5 \pm 2.0^*$	$400.0 \pm 1.0^{**}$	$1.65 \pm 0.08^{**}$	$28.84 \pm 0.60^{**}$	$8.74 \pm 0.40^*$
G4	CrPic 300 µg/kg	$245.6 \pm 1.4^*$	$365.5 \pm 3.2^{**}$	$1.15 \pm 0.20^{**}$	$16.69 \pm 0.40^*$	$7.36 \pm 0.30^{**}$
G5	CrPic 400 µg/kg	$245.7 \pm 2.1^*$	$360.1 \pm 2.6^{**}$	$1.18 \pm 0.30^{**}$	$16.77 \pm 0.10^{**}$	$7.41 \pm 0.30^*$
G6	Biotin 200 µg/kg	$285.0 \pm 0.14^*$	$410.1 \pm 3.1^{**}$	$1.72 \pm 0.30^{**}$	$29.06 \pm 0.70^{**}$	$8.98 \pm 0.40^*$
G7	Biotin 300 µg/kg	$230.3 \pm 2.2^*$	$315.8 \pm 2.0^{**}$	$1.50 \pm 0.09^{**}$	$20.44 \pm 1.80^{**}$	$7.64 \pm 0.30^{**}$
G8	Biotin 400 µg/kg	$175.6 \pm 1.0^*$	$260.6 \pm 3.5^*$	$1.36 \pm 0.50^*$	14.30 ± 1.50	$7.80 \pm 0.30^*$
G9	CrPic 100 µg/kg + Biotin 100 µg/kg	$195.6 \pm 1.5^*$	$270.6 \pm 2.4^{***}$	$1.20 \pm 0.40^{***}$	$13.86 \pm 1.10^*$	$5.82 \pm 0.20^*$
G10	CrPic 150 µg/kg + Biotin 150 µg/kg	$165.1 \pm 2.8^*$	$185.7 \pm 1.2^{***}$	$0.78 \pm 0.20^{***}$	$6.23 \pm 0.60^{**}$	$5.24 \pm 0.20^{**}$
G11	CrPic 200 µg/kg + Biotin 200 µg/kg	$101.6 \pm 2.6^*$	$138.1 \pm 1.7^{***}$	$0.58 \pm 0.10^{***}$	$3.36 \pm 0.40^{***}$	$4.98 \pm 0.20^{**}$

FBG: fasting blood glucose; HOMA-IR: homeostatic model assessment of insulin resistance; CrPic: chromium picolinate.

Figure 1 shows HOMA-IR across experimental groups. HOMA-IR was highest in the untreated diabetic-control group and progressively decreased following chromium picolinate, biotin, and combined supplementation, with the lowest value among diabetic treatment groups observed in G11.

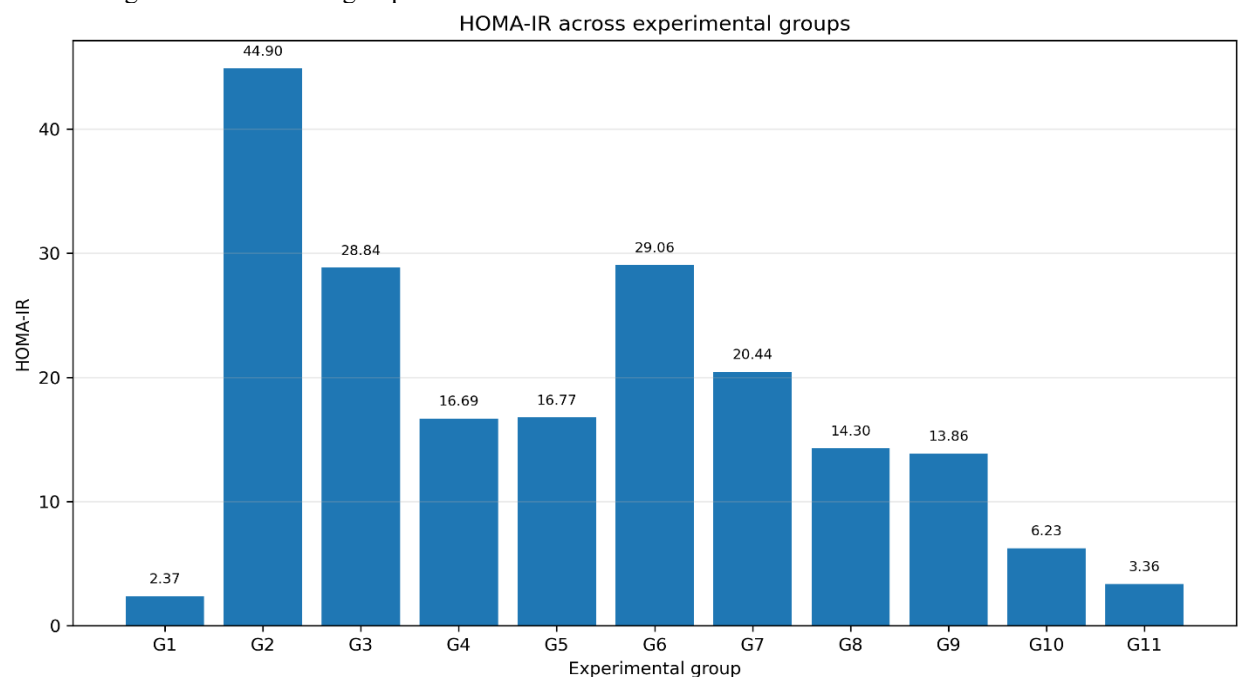


Figure 1 – HOMA-IR across experimental groups

Haematological Parameters

The diabetic-control group demonstrated reductions in red blood cell count, haemoglobin concentration, and haematocrit. RBC count decreased from 7.85 ± 0.42 million/mm³ in G1 to 5.40 ± 0.38 million/mm³ in G2. Haemoglobin similarly declined from 14.6 ± 0.6 to 10.2 ± 0.73 g/dL, whereas haematocrit decreased from $43.8 \pm 1.8\%$ to $32.0 \pm 2.2\%$. Treatment with chromium picolinate and biotin improved these haematological indices, with the combined-treatment groups generally showing values closer to those of the negative control. G11 demonstrated an RBC count of 7.72 ± 0.25 million/mm³, haemoglobin concentration of 14.4 ± 0.4 g/dL, and haematocrit of $43.2 \pm 1.2\%$ (Table 3).

One-way analysis of variance demonstrated significant differences among groups for RBC count ($F_{10,55} = 4.08$, $p = 0.0003$), haemoglobin concentration ($F_{10,55} = 4.47$, $p = 0.0001$), and haematocrit percentage ($F_{10,55} = 2.50$, $p = 0.015$).

Hepatic Biochemical Parameters

STZ-induced diabetes substantially altered hepatic biochemical markers. Serum albumin decreased from 3.58 ± 0.16 g/dL in G1 to 2.57 ± 0.19 g/dL in G2, whereas total bilirubin increased from 0.20 ± 0.03 to 0.48 ± 0.05 mg/dL. Supplementation improved both parameters, and G11 demonstrated an albumin concentration of 3.79 ± 0.10 g/dL and total bilirubin of 0.25 ± 0.01 mg/dL (Table 3).

Hepatic enzyme activities were also markedly elevated in the untreated diabetic group. ALT increased from 45.0 ± 1.5 U/L in G1 to 168.0 ± 1.4 U/L in G2, ALP increased from 145.4 ± 1.4 to 420.0 ± 4.1 U/L, and AST increased from 112.0 ± 0.8 to 385.0 ± 2.1 U/L. These abnormalities were progressively attenuated following treatment. G11 showed ALT, ALP, and AST activities of 48.0 ± 0.5 , 150.4 ± 1.7 , and 118.15 ± 0.9 U/L, respectively (Table 3).

Renal Biochemical Parameters

Renal biochemical markers were significantly altered following diabetes induction. Serum creatinine increased from 0.48 ± 0.05 mg/dL in G1 to 1.35 ± 0.12 mg/dL in G2, while blood urea increased from 18.5 ± 1.6 to 54.8 ± 2.1 mg/dL. Chromium picolinate and biotin supplementation reduced both markers, with the greatest normalization observed in the combination groups. Creatinine decreased to 0.58 ± 0.03 mg/dL in G10 and 0.51 ± 0.03 mg/dL in G11, while blood urea decreased to 23.6 ± 0.8 and 19.9 ± 0.8 mg/dL, respectively (Table 3).

Table 3. Effect of Chromium Picolinate and Biotin Supplementation on Haematological, Hepatic, and Renal Biochemical Parameters

Gro up	RBC (million/ mm ³)	Hb (g/dL)	Hct (%)	Albumi n (g/dL)	Bilirubi n (mg/dL)	ALT (U/L)	ALP (U/L)	AST (U/L)	Creatini ne (mg/dL)	Urea (mg/dL)
G1	7.85±0.42	14.6±0.6	43.8±1.8	3.58±0.16	0.20±0.03	45±1.5	145.4±1.4	112±0.8	0.48±0.05	18.5±1.6
G2	5.40±0.38**	10.2±0.73**	32.0±2.2**	2.57±0.19***	0.48±0.05***	168±1.4***	420±4.1***	385±2.1**	1.35±0.12***	54.8±2.1***
G3	5.85±0.31*	10.7±0.5*	33.5±1.6*	2.97±0.13**	0.40±0.04**	150±0.8*	390.2±2.5*	342.3±2.6**	1.22±0.10*	48.2±1.0*
G4	6.40±0.26*	11.9±0.5*	36.8±1.4*	3.03±0.14**	0.37±0.03**	122.8±0.6**	315.7±2.4**	278.2±1.0**	0.88±0.08**	35.9±0.9**
G5	6.80±0.33*	11.8±0.7*	36.5±0.3*	3.09±0.15**	0.33±0.02**	126±1.3**	328.5±2.3**	285.3±0.7**	0.94±0.08**	37.7±0.9**
G6	5.60±0.80*	11.1±0.4*	34.8±1.3	3.14±0.13**	0.31±0.02**	142±1.1**	370.5±3.3**	330.1±8.719**	1.18±0.09**	46.5±1.5**
G7	6.32±0.29*	12.1±0.4*	37.2±1.7*	3.21±0.11**	0.30±0.01**	110±1.9**	298.3±3.0**	265.7±1.781**	0.92±0.04**	38.8±1.1**
G8	6.28±0.35*	12.8±0.6*	39.1±1.9*	3.44±0.12**	0.29±0.01**	88±1.7*	242.6±2.6**	195.8±0.59**	0.76±0.06*	31.4±1.4*
G9	5.68±0.40*	10.7±0.8*	33.8±2.0*	3.50±0.13**	0.28±0.01**	82.01±1.6**	220.8±2.2**	188.8±1.4**	0.82±0.08**	29.3±0.9**
G10	7.38±0.32*	13.9±0.5*	41.9±1.3*	3.67±0.12**	0.27±0.01**	58.4±1.4**	172.6±1.9**	135.8±1.1**	0.58±0.03**	23.6±0.8**
G11	7.72±0.25*	14.4±0.4*	43.2±1.2*	3.79±0.10**	0.25±0.01**	48±0.5*	150.4±1.7**	118.15±0.9*	0.51±0.03**	19.9±0.8**

Serum Lipid Profile

STZ-induced diabetes resulted in pronounced dyslipidaemia. LDL-C increased from 22.6 ± 2.4 mg/dL in G1 to 98.2 ± 2.6 mg/dL in G2, while HDL-C decreased from 39.0 ± 0.4 to 18.4 ± 0.5 mg/dL. Total cholesterol increased from 78.3 ± 1.0 to 192.2 ± 0.4 mg/dL, and triglycerides increased from 85.5 ± 1.1 to 245.5 ± 1.8 mg/dL (Table 4).

Treatment with both supplements improved the lipid profile. The greatest overall improvement was observed with combined treatment. In G9, LDL-C, HDL-C, total cholesterol, and triglycerides were 49.4 ± 1.4 , 32.1 ± 0.2 , 112.2 ± 0.9 , and 130.3 ± 1.3 mg/dL, respectively. Further improvement occurred in G10, while G11 showed LDL-C of 24.3 ± 0.1 mg/dL, HDL-C of 38.3 ± 0.08 mg/dL, total cholesterol of 81.7 ± 2.7 mg/dL, and triglycerides of 88.3 ± 1.6 mg/dL, closely approaching the corresponding negative-control values (Table 4).

Table 4. Effect of Chromium Picolinate and Biotin Supplementation on Serum Lipid Profile

Group	Treatment	LDL-C (mg/dL)	HDL-C (mg/dL)	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)
G1	Negative control	22.6 ± 2.4	39.0 ± 0.4	78.3 ± 1.0	85.5 ± 1.1
G2	Diabetic control	$98.2 \pm 2.6^{***}$	$18.4 \pm 0.5^*$	$192.2 \pm 0.4^{***}$	$245.5 \pm 1.8^{***}$
G3	CrPic 200 µg/kg	$88.9 \pm 1.2^{***}$	$20.3 \pm 0.1^*$	$175.9 \pm 1.6^{***}$	$222.0 \pm 1.8^{***}$
G4	CrPic 300 µg/kg	$61.8 \pm 0.8^{***}$	$28.7 \pm 0.1^*$	$138.7 \pm 0.1^{**}$	$160.1 \pm 2.3^{***}$
G5	CrPic 400 µg/kg	$63.5 \pm 0.9^*$	$27.08 \pm 0.2^*$	$140.3 \pm 1.4^*$	$164.3 \pm 1.8^*$
G6	Biotin 200 µg/kg	$84.02 \pm 0.7^{***}$	$23.02 \pm 0.1^*$	$170.5 \pm 0.9^{***}$	$210.4 \pm 1.5^{***}$
G7	Biotin 300 µg/kg	$68.2 \pm 0.4^{***}$	$26.5 \pm 0.08^*$	$145.2 \pm 1.2^{**}$	$172.1 \pm 1.1^{***}$
G8	Biotin 400 µg/kg	$52.4 \pm 1.2^{***}$	$30.8 \pm 0.2^*$	$124.9 \pm 1.9^{***}$	$138.3 \pm 2.3^{***}$
G9	CrPic 100 µg/kg + Biotin 100 µg/kg	$49.4 \pm 1.4^{***}$	$32.1 \pm 0.2^{**}$	$112.2 \pm 0.9^{***}$	$130.3 \pm 1.3^{***}$
G10	CrPic 150 µg/kg + Biotin 150 µg/kg	$31.3 \pm 1.3^{***}$	$36.8 \pm 0.1^{**}$	$90.5 \pm 1.0^{***}$	$102.5 \pm 2.0^{***}$
G11	CrPic 200 µg/kg + Biotin 200 µg/kg	$24.3 \pm 0.1^{***}$	$38.3 \pm 0.08^{***}$	$81.7 \pm 2.7^{***}$	$88.3 \pm 1.6^{***}$

LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol.

Inflammatory and Oxidative-Stress Parameters

Serum CRP was markedly elevated following diabetes induction, increasing from 1.42 ± 0.18 mg/L in G1 to 7.84 ± 0.52 mg/L in G2. Both chromium picolinate and biotin reduced CRP concentrations, while the greatest reductions occurred in the combined-treatment groups. CRP declined to 3.46 ± 0.27 mg/L in G9, 2.64 ± 0.21 mg/L in G10, and 1.79 ± 0.16 mg/L in G11 (Table 5).

Lipid peroxidation followed a similar pattern. TBARS increased from 2.72 ± 0.22 nmol/mL in G1 to 8.65 ± 0.54 nmol/mL in G2. Supplementation progressively reduced TBARS, with concentrations of 3.94 ± 0.28 , 3.18 ± 0.24 , and 2.81 ± 0.21 nmol/mL in G9, G10, and G11, respectively (Table 5).

Antioxidant enzyme activities were substantially suppressed in untreated diabetic animals. SOD decreased from 18.84 ± 0.90 U/mg protein in G1 to 8.46 ± 0.50 U/mg protein in G2, while catalase declined from 65.48 ± 2.60 to 28.36 ± 1.80 U/mg protein. Chromium picolinate and biotin supplementation increased both antioxidant enzymes. G11 demonstrated SOD activity of 18.52 ± 0.80 U/mg protein and catalase activity of 64.86 ± 2.60 U/mg protein, values close to those of the negative control (Table 5).

Table 5. Effect of Chromium Picolinate and Biotin Supplementation on Inflammatory and Oxidative-Stress Parameters

Group	Treatment	CRP (mg/L)	TBARS (nmol/mL)	SOD (U/mg Protein)	Catalase (U/mg Protein)
G1	Negative control	1.42 ± 0.18	2.72 ± 0.22	18.84 ± 0.90	65.48 ± 2.60
G2	Diabetic control	$7.84 \pm 0.52^{***}$	$8.65 \pm 0.54^{***}$	$8.46 \pm 0.50^{**}$	$28.36 \pm 1.80^{**}$
G3	CrPic 200 µg/kg	$5.94 \pm 0.46^{**}$	$6.71 \pm 0.44^{**}$	$11.62 \pm 0.60^*$	$39.52 \pm 2.00^*$
G4	CrPic 300 µg/kg	$5.02 \pm 0.37^*$	$5.86 \pm 0.35^{**}$	$13.85 \pm 0.70^{**}$	$48.74 \pm 2.20^{**}$
G5	CrPic 400 µg/kg	$4.18 \pm 0.31^*$	$4.81 \pm 0.32^*$	$15.96 \pm 0.80^*$	$56.18 \pm 2.40^*$
G6	Biotin 200 µg/kg	$5.62 \pm 0.41^{**}$	$6.48 \pm 0.41^*$	$10.84 \pm 0.60^*$	$37.86 \pm 1.90^*$
G7	Biotin 300 µg/kg	$4.71 \pm 0.35^{**}$	$5.54 \pm 0.36^{**}$	$13.14 \pm 0.60^{**}$	$46.92 \pm 2.10^{**}$
G8	Biotin 400 µg/kg	$3.85 \pm 0.29^{**}$	$4.46 \pm 0.29^{**}$	$15.42 \pm 0.70^*$	$54.62 \pm 2.30^*$
G9	CrPic 100 µg/kg + Biotin 100 µg/kg	$3.46 \pm 0.27^{**}$	$3.94 \pm 0.28^{**}$	$16.82 \pm 0.80^*$	$59.74 \pm 2.40^*$

G10	CrPic 150 µg/kg + Biotin 150 µg/kg	2.64 ± 0.21**	3.18 ± 0.24**	17.95 ± 0.80**	63.18 ± 2.50**
G11	CrPic 200 µg/kg + Biotin 200 µg/kg	1.79 ± 0.16**	2.81 ± 0.21**	18.52 ± 0.80**	64.86 ± 2.60**

CRP: C-reactive protein; TBARS: thiobarbituric acid-reactive substances; SOD: superoxide dismutase.

Overall, STZ-induced diabetes was associated with reduced body weight, increased feed and water intake, marked hyperglycaemia, severe insulin resistance, elevated HbA1c, dyslipidaemia, altered haematological indices, hepatic and renal biochemical abnormalities, increased systemic inflammation and lipid peroxidation, and impaired antioxidant defence. Chromium picolinate and biotin improved these abnormalities when administered individually, while the combined regimens generally produced greater overall improvements. Across the principal glycaemic, lipid, renal, inflammatory, and oxidative-stress outcomes, the most favourable values were observed in G11 receiving chromium picolinate 200 µg/kg plus biotin 200 µg/kg (Tables 1–5 and Figure 1).

DISCUSSION

The present study demonstrates that streptozotocin-induced diabetes produced a broad metabolic phenotype characterized by hyperglycaemia, insulin resistance, dyslipidaemia, weight loss, polyphagia, polydipsia, systemic inflammation, oxidative stress, and secondary biochemical abnormalities. Similar multidimensional metabolic disruption has been documented in experimental diabetic models exposed to STZ [1]. The accompanying disturbances in redox balance and biochemical homeostasis are also consistent with previous experimental observations of diabetes-associated metabolic injury [2]. Importantly, chromium picolinate and biotin improved several of these abnormalities, with the combined regimen generally producing the most favourable overall response; the improvement in renal biochemical indices is particularly consistent with previously reported effects of chromium picolinate in experimental diabetes [3].

The clearest treatment effect was observed in glycaemic regulation and insulin resistance. Post-treatment blood glucose increased from 110.2 ± 3.0 mg/dL in the negative control to 430.6 ± 3.1 mg/dL in the diabetic control, whereas HOMA-IR increased from 2.37 ± 0.40 to 44.90 ± 1.00 . Chromium supplementation has previously been shown to modify carbohydrate metabolism and insulin-associated metabolic responses in experimental models [4]. In the present study, the highest combined dose reduced post-treatment glucose to 138.1 ± 1.7 mg/dL and HOMA-IR to 3.36 ± 0.40 , supporting the concept that chromium and biotin may exert complementary effects on insulin signalling and glucose utilization [5]. Chromium-related modulation of oxidative balance may additionally contribute to improved insulin responsiveness because oxidative stress can interfere with insulin-sensitive metabolic pathways [6].

Longer-term glycaemic control showed a comparable pattern. HbA1c levels were raised from the normal control group value of $4.86 \pm 0.28\%$ to $10.92 \pm 0.50\%$ in the untreated diabetic rats and lowered to $4.98 \pm 0.20\%$ in the highest combined-treatment group. A metabolic response, which is related to a general antioxidant and metabolic response, is supported by improvement across multiple indices, as opposed to a single glucose measurement [7]. The anticipated nutritional abnormalities were also apparent: feed intake rose from 25.33 ± 1.20 to 35.50 ± 1.00 g/day and water intake from 31.4 ± 2.1 to 85.7 ± 4.2 mL/day, and body weight dropped significantly. These observations are in line with known responses in insulin-deficient and impaired glucose-utilizing experimental models [8]. The progressive normalization of food intake, water consumption, glucose regulation, and body weight with combined supplementation is also similar to the previous experimental findings that examined the use of chromium picolinate and biotin in combination [9].

Diabetes induction was also correlated with severe dyslipidaemia. LDL-C increased from 22.6 ± 2.4 to 98.2 ± 2.6 mg/dL, total cholesterol from 78.3 ± 1.0 to 192.2 ± 0.4 mg/dL, and triglycerides from 85.5 ± 1.1 to 245.5 ± 1.8 mg/dL, whereas HDL-C decreased from 39.0 ± 0.4 to 18.4 ± 0.5 mg/dL. Modulation of glucose and lipid metabolism has previously been reported in experimental animals with chromium picolinate [10]. Chromium status has also been correlated with glucose metabolism and lipid-profile variation in clinical observations, but there is no uniformity of response between populations [11]. In the present study, the maximum overall lipid improvement was observed in G11, where all lipid parameters were brought closer to control levels, and this improvement could be due to a decrease in oxidative metabolic stress in addition to improvement in insulin action [12].

The inflammatory and oxidative-stress results are additional biological evidence to support the metabolic results. CRP increased from 1.42 ± 0.18 mg/L in controls to 7.84 ± 0.52 mg/L in diabetic rats, while TBARS increased from 2.72 ± 0.22 to 8.65 ± 0.54 nmol/mL. This is in line with previous nutritional studies that have linked chromium nutritional interventions to better cardiometabolic and oxidative-stress indices [13]. On the other hand, SOD and catalase activities were significantly decreased in diabetes and gradually increased following the supplementation, and chromium picolinate has been experimentally associated with insulin-signalling pathways which intersect with inflammatory and oxidative pathways [14]. This suggests that the near-normal CRP, TBARS, SOD and catalase values in G11 were not only a glycaemic effect but also took place with a restoration of redox balance [15].

Similar directions of change were observed in the secondary biochemical outcomes. Diabetic rats without treatment had decreased values of RBC count, haemoglobin, haematocrit and albumin, and increased values of bilirubin, liver enzymes, creatinine and blood urea. But the improved glycaemic signalling alone can decrease systemic metabolic and oxidative damage, as has been shown in other experimental diabetic models directed to insulin-sensitive signalling pathways [16]. The improved inflammatory and antioxidant profile found in the present study [17] following the use of biotin-containing interventions also supports a biologic explanation for the general biochemical improvement found. However, these decreases in ALT, AST, ALP, creatinine and urea should be taken as biochemical evidence of normalization and as such are not evidence of structural hepatoprotection and nephroprotection—such changes in tissue require direct molecular or histological confirmation, as does preservation of the β -cell and metabolic signalling pathways [18].

There are some limitations to be taken into account. Only 6 rats per experimental group, thus limiting the statistical precision and ability to account for biological variation. Although experimental diabetic models are useful for the assessment of metabolic interventions, they cannot fully mimic the complexity of human type 2 diabetes, and findings from animal models must be interpreted with caution [19]. However, only male animals were investigated; the treatment was only 7 weeks, and detailed molecular signalling, long-term safety, and confirmatory tissue-level analyses were not included in the present article; and comparable STZ-based studies also highlight the need for the interpretation of metabolic and oxidative findings in the context of the limitations of the experimental model [20]. Finally, because this study was not a factorial experiment, the combined effect of chromium picolinate and biotin does not formally qualify for a claim of synergy, but rather as a greater or complementary metabolic effect of chromium picolinate combined with biotin, as has been shown in previous studies comparing the effect of chromium picolinate and biotin [9].

CONCLUSION

Chromium picolinate and biotin improved glycaemic control, insulin resistance, lipid metabolism, inflammatory status, oxidative balance, and several secondary biochemical abnormalities in streptozotocin-induced diabetic rats. Both supplements had beneficial effects when taken separately, and the combined supplementation had the greatest overall beneficial effect. The 200 $\mu\text{g/kg}$ chromium picolinate plus 200 $\mu\text{g/kg}$ biotin regimen produced values closest to those of the normal control for several principal metabolic outcomes. These results suggest a complementary metabolic action of chromium picolinate and biotin, but further experimental studies on larger numbers of animals and longer time periods with formal interaction analysis, molecular evaluation, histopathology, and ultimate clinical evaluation would be necessary before translation to human therapy.

DECLARATIONS

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REFERENCES

1. Kurek JM, Król E, Staniek H, Krejpcio Z. Steviol glycoside, L-arginine, and chromium(III) supplementation attenuates abnormalities in glucose metabolism in streptozotocin-induced mildly diabetic rats fed a high-fat diet. *Pharmaceuticals (Basel)*. 2022;15(10):1200. doi:10.3390/ph15101200.
2. Hassan RM, Elsayed M, Kholief TE, Hassanen NHM, Gafer JA, Attia YA. Mitigating effect of single or combined administration of nanoparticles of zinc oxide, chromium oxide, and selenium on genotoxicity and metabolic insult in fructose/streptozotocin diabetic rat model. *Environ Sci Pollut Res Int*. 2021;28(35):48517-48534. doi:10.1007/s11356-021-14089-w.
3. Qi SS, Zheng HX, Jiang H, Yuan LP, Dong LC. Protective effects of chromium picolinate against diabetic-induced renal dysfunction and renal fibrosis in streptozotocin-induced diabetic rats. *Biomolecules*. 2020;10(3):398. doi:10.3390/biom10030398.

4. Staniek HZ, Król E, Wójciak RW. The interactive effect of high doses of chromium(III) and different iron(III) levels on the carbohydrate status, lipid profile, and selected biochemical parameters in female Wistar rats. *Nutrients*. 2020;12(10):3070. doi:10.3390/nu12103070.
5. Turgut M, Cinar V, Pala R, Tuzcu M, Orhan C, Telceken H, et al. Biotin and chromium histidinate improve glucose metabolism and protein expression levels of IRS-1, PPAR- γ , and NF- κ B in exercise-trained rats. *J Int Soc Sports Nutr*. 2018;15(1):45. doi:10.1186/s12970-018-0249-4.
6. Moreira R, Martins AD, Alves MG, Pereira ML, Oliveira PF. A comprehensive review of the impact of chromium picolinate on testicular steroidogenesis and antioxidant balance. *Antioxidants (Basel)*. 2023;12(8):1572. doi:10.3390/antiox12081572.
7. Bohórquez-Medina AL, Bohórquez-Medina SL, Benites-Zapata VA. Biological markers of insulin sensitivity links with dietary antioxidant. In: Patel VB, Preedy VR, editors. *Biomarkers in diabetes*. Cham: Springer International Publishing; 2022. p. 217-238. doi:10.1007/978-3-031-08014-2_10.
8. Krejpcio Z. Effects of chromium(III) supplementation on rodent models of insulin resistance and diabetes. In: Vincent JB, editor. *The nutritional biochemistry of chromium (III)*. 2nd ed. Amsterdam: Elsevier; 2019. p. 195-218. doi:10.1016/B978-0-444-64121-2.00006-4.
9. Orhan C, Kucuk O, Tuzcu M, Sahin N, Komorowski JR, Sahin K. Effect of supplementing chromium histidinate and picolinate complexes along with biotin on insulin sensitivity and related metabolic indices in rats fed a high-fat diet. *Food Sci Nutr*. 2019;7(1):183-194. doi:10.1002/fsn3.851.
10. Pala R, Sari MA, Erten F, Er B, Tuzcu M, Orhan C, et al. The effects of chromium picolinate on glucose and lipid metabolism in running rats. *J Trace Elem Med Biol*. 2020;58:126434. doi:10.1016/j.jtemb.2019.126434.
11. Ngala RA, Awe MA, Nsiah P. The effects of plasma chromium on lipid profile, glucose metabolism and cardiovascular risk in type 2 diabetes mellitus: a case-control study. *PLoS One*. 2018;13(7):e0197977. doi:10.1371/journal.pone.0197977.
12. Yardim M, Aydin S, Kuloglu T. Effect of chromium and L-carnosine use on oxidative stress markers in an experimental model of gestational diabetes mellitus. *J Med Sci*. 2022;25(4):37-46. doi:10.33883/jms.v25i4.1219.
13. Jamilian M, Foroozand F, Kavossian E, Kia M, Aghadavod E, Amirani E, et al. Effects of chromium and carnitine co-supplementation on body weight and metabolic profiles in overweight and obese women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. *Biol Trace Elem Res*. 2020;193(2):334-341. doi:10.1007/s12011-019-01720-8.
14. Akhtar A, Dhaliwal J, Saroj P, Uniyal A, Bishnoi M, Sah SP. Chromium picolinate attenuates cognitive deficit in the ICV-STZ rat paradigm of sporadic Alzheimer's-like dementia via targeting the neuroinflammatory and IRS-1/PI3K/AKT/GSK-3 β pathways. *Inflammopharmacology*. 2020;28(2):385-400. doi:10.1007/s10787-019-00681-7.
15. Jamilian M, Zadeh Modarres S, Amiri Siavashani M, Karimi M, Mafi A, Ostadmohammadi V, et al. The influences of chromium supplementation on glycemic control, markers of cardiometabolic risk, and oxidative stress in infertile women with polycystic ovary syndrome who were candidates for in vitro fertilization: a randomized, double-blind, placebo-controlled trial. *Biol Trace Elem Res*. 2018;185(1):48-55. doi:10.1007/s12011-017-1236-3.
16. Iheagwam FN, Iheagwam OT, Onuoha MK, Ogunlana OO, Chinedu SN. *Terminalia catappa* aqueous leaf extract reverses insulin resistance, improves glucose transport and activates PI3K/AKT signalling in high-fat/streptozotocin-induced diabetic rats. *Sci Rep*. 2022;12(1):10711. doi:10.1038/s41598-022-15114-9.
17. Sahin K, Sahin E, Orhan C, Er B, Akoglan B, Ozercan IH, et al. The impact of magnesium biotinate and arginine silicate complexes on metabolic dysfunctions, antioxidant activity, inflammation, and neuromodulation in high-fat diet-fed rats. *Clin Exp Med*. 2024;24(1):176. doi:10.1007/s10238-024-01434-9.
18. Lee M, Li H, Zhao H, Suo M, Liu D. Effects of hydroxysafflor yellow A on the PI3K/AKT pathway and apoptosis of pancreatic β -cells in type 2 diabetes mellitus rats. *Diabetes Metab Syndr Obes*. 2020;13:1097-1107. doi:10.2147/DMSO.S246381.
19. Fakharzadeh S, Kalanaky S, Hafizi M, Nazaran MH, Zardooz H. DIBc, a nanochelating-based nano metal-organic framework, shows antidiabetic effects in high-fat diet- and streptozotocin-induced diabetic rats. *Int J Nanomedicine*. 2019;14:2145-2156. doi:10.2147/IJN.S196050.
20. Dhaliwal J, Dhaliwal N, Akhtar A, Kuhad A, Chopra K. Tetramethylpyrazine attenuates cognitive impairment via suppressing oxidative stress, neuroinflammation, and apoptosis in type 2 diabetic rats. *Neurochem Res*. 2022;47(8):2431-2444. doi:10.1007/s11064-022-03640-x.