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EFFECTS OF CHROMIUM PICOLINATE AND BIOTIN SUPPLEMENTATION ON HEMATOLOGICAL AND HEPATORENAL BIOMARKERS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Background: Diabetes mellitus is associated with oxidative and metabolic disturbances that may impair hematological status and induce hepatic and renal dysfunction. Chromium picolinate and biotin have been investigated for their potential metabolic and tissue-protective effects, but their comparative and combined actions on hematological and hepatorenal biomarkers remain insufficiently characterized.

Objective: To evaluate the effects of chromium picolinate and biotin, administered individually and in combination, on hematological, hepatic, and renal biomarkers in streptozotocin-induced diabetic rats.

Methods: Sixty-six adults male Wistar rats were randomized into 11 groups of six animals. Diabetes was induced using a single intraperitoneal dose of streptozotocin (65 mg/kg), and diabetic animals received chromium picolinate, biotin, or corresponding combinations at 100, 150, or 200 µg/kg/day for seven weeks. Red blood cell count, hemoglobin, hematocrit, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, total bilirubin, creatinine, and blood urea were assessed.

Results: Untreated diabetic rats showed reduced erythrocyte indices and albumin with marked elevations in hepatic enzymes, bilirubin, creatinine, and urea. Significant group differences were observed for red blood cell count ($p=0.0003$), hemoglobin ($p=0.0001$), and hematocrit ($p=0.015$). Chromium picolinate at 200 µg/kg produced the highest red blood cell count (8.70 ± 0.55 million/mm³) and hemoglobin (14.97 ± 0.21 g/dL), while the 150 µg/kg combination produced the highest hematocrit ($44.67 \pm 1.16\%$). The 200 µg/kg combination reduced ALT, AST, ALP, creatinine, and urea to 124.40 ± 0.59 U/L, 171.15 ± 0.92 U/L, 127.40 ± 1.70 U/L, 40.62 ± 0.89 µmol/L, and 19.90 ± 0.89 mg/dL, respectively.

Conclusion: Chromium picolinate improved hematological indices, whereas combined chromium picolinate and biotin supplementation produced the greatest overall hepatorenal biochemical recovery.

KEYWORDS: Biotin; chromium picolinate; diabetes mellitus; hematological indices; hepatic biomarkers; renal biomarkers; streptozotocin.

INTRODUCTION

Diabetes mellitus is complex metabolic disorder with biochemical disturbances that go far beyond glucose homeostasis impairment, and are triggered by persistent hyperglycaemia [1]. The high production of ROS, non-enzymatic glycation, mitochondrial dysfunction and activation of pro-inflammatory pathways cause progressive damage to cells in metabolically active tissues [2]. The liver and kidneys are especially vulnerable to the effects due to their central roles in regulating glucose, metabolizing xenobiotics, and in the elimination of nitrogenous waste and maintenance of systemic metabolic homeostasis. Streptozotocin (STZ)-induced diabetes recapitulates some of these abnormalities in the experimental setting, such as biochemical dysfunction in the liver and kidney, oxidative damage and tissue structural changes, and thus serves as a useful means to test interventions aimed at preventing diabetes-related organ injury [3].

Haematological compartment is also affected by systemic effects of experimental diabetes. Hyperglycaemia and oxidative stress are persistent, and can alter the proteins and lipids of the erythrocyte membrane, decrease the deformability and stability of the membrane, and disrupt the survival of erythrocytes [4]. This may then lead to decreases in red blood cell count, haemoglobin concentration and haematocrit, depending on the severity and duration

of diabetes in the experiments. Concurrently, higher levels of circulating alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase may be a sign of liver damage related to diabetes, while high levels of creatinine and urea are typical of decreased kidney function. Experimental findings also suggest that the biochemical abnormalities are not mutually exclusive of histopathological changes in liver and kidneys providing good evidence for the need to assess haematological and hepatorenal outcomes in addition to glycaemic control [6].

An organic trivalent chromium complex, chromium picolinate (CrPic) has been the subject of a great deal of experimental interest due to its potential effects on insulin activity and cellular glucose utilization [7]. CrPic increased the insulin receptor substrate-1 phosphorylation and the activity of phosphatidylinositol 3-kinase in insulin-resistant rats, a direct evidence of the ability of chromium to alter the intracellular insulin signalling system [8]. Chromium in STZ-induced diabetic animals has also been found to enhance the activity of the hepatic glycolysis and reduce the activity of the gluconeogenic enzymes as well as normalize the metabolic activity of the hepatic glycogen.Such metabolic effects seem to be reflected in the protection of tissues. The studies conducted in the liver and kidney have shown that chronic administration of CrPic has lowered the increase in serum-liver enzymes, as well as urea and creatinine, and has also mitigated the histological alterations in diabetic liver and kidney; additionally, renal studies have revealed that CrPic has modulated nuclear factor kappa B (NF-κB) and nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathways, implying that chromium administration has reduced inflammatory responses and enhanced antioxidant defense [9].

Biotin is a metabolic intervention that can be mechanistically different, but may be complementary. Biotin serves as a cofactor of various carboxylases that are important for intermediary metabolism of carbohydrates, fatty acids and amino acids [10]. Experimental studies also suggest that its pharmacological effects go beyond the coenzyme role. Biotin has been demonstrated to regulate the transcription of phosphoenolpyruvate carboxykinase in the liver of diabetic rats in the absence of any alteration in the insulin levels, indicating that biotin regulates glucose production in the liver directly. Other experimental studies have shown that biotin is able to regulate the expression of genes related to glucose metabolism in the liver and normalize the impaired glucose tolerance in STZ-induced diabetes. Importantly, there is evidence it can also have an organ-protective effect: biotin treatment reduced renal oxidative damage and improved glomerular and tubular histopathology in STZ-diabetic mice, and thus has a potential nephroprotective effect under sustained hyperglycaemic conditions [11-13].

The combination of CrPic and biotin may have potential due to their partly complementary mechanisms [14]. The main mechanism of action of chromium is probably through the improvement of insulin action and modulation of oxidative and inflammatory pathways, while biotin may impact important enzymatic and transcription factor pathways involved in hepatic glucose metabolism. Sahin et al. showed in an experimental model of high-fat diet combined with STZ that CrPic and biotin has beneficial effects when fed individually or in combination on metabolic abnormalities and modulation of molecular pathways involved in insulin resistance and inflammatory processes [11]. Supplementation resulted in an up-regulation of the peroxisome proliferator-activated receptor gamma and phosphorylated insulin receptor substrate-1, and down-regulation of the NF-κB expression in liver and kidney tissues and was found to be more effective when applied in combination than when administered alone on several metabolic and molecular outcomes [15,16].

However, the protective effects of CrPic and biotin have been mostly studied in terms of glycaemic regulation, insulin sensitivity, lipid profile, oxidative stress or tissue-specific endpoints. Their combined effects on haematological indices and hepatic and renal biochemical markers at the same experimental model have received comparatively less attention in comparison with their individual effects at various doses. Other studies on the CrPic–biotin pairing have also emphasized the importance of examining dose-dependent responses as the dose for metabolic signaling may differ from the dose for haematological and/or organ protection [17,18].

Thus, the present study aimed to evaluate the effect of different doses of chromium picolinate, biotin and a combination of these two nutrients on blood haematology parameters, including red blood cell count, haemoglobin, haematocrit and some liver and kidney parameters in diabetic rats induced by STZ. To assess whether combined supplementation offers wider biochemical protection, the study included haematological indices, which were used in conjunction with the levels of ALT, AST, ALP, Albumin, Total Bilirubin, Creatinine and Urea in the blood. An attenuation in haematological and hepatorenal abnormalities associated with diabetes was hypothesized to be dose-dependent with chromium picolinate and biotin, and this combination would yield the most overall recovery [19,20].

MATERIALS AND METHODS

Study Design and Setting

This randomized controlled animal study was conducted over 18 months, at the University Institute of Diet and Nutritional Sciences in collaboration with the Animal House of the Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan. Animal housing, diabetes induction, supplementation, monitoring, and sample collection were performed at the animal-house facility, while hematological and biochemical analyses were conducted using the laboratory facilities of The University of Lahore.

Ethical Approval
The study protocol was approved by the relevant institutional animal ethics committee of The University of Lahore before commencement of the experiment. All procedures were performed in accordance with accepted standards for laboratory animal care and the ARRIVE 2.0 recommendations. The official ethics approval number should be inserted according to the institutional certificate.

Experimental Animals and Housing
Sixty-six healthy adult male Wistar rats, aged approximately 8–10 weeks and weighing 180–220 g, were included. Animals were acclimatized for seven days and housed under standard laboratory conditions at 22 ± 2°C, 50–60% relative humidity, and a 12-hour light/dark cycle. Standard rodent chow and clean drinking water were provided ad libitum except during predefined fasting periods.

Experimental Grouping
The rats were randomly allocated into 11 groups of six animals each. Group I served as the non-diabetic control, while Group II served as the untreated diabetic control. Groups III–V received chromium picolinate at 100, 150, and 200 µg/kg/day, respectively. Groups VI–VIII received biotin at 100, 150, and 200 µg/kg/day, respectively. Groups IX–XI received chromium picolinate plus biotin at corresponding doses of 100, 150, and 200 µg/kg/day of each supplement.

Induction and Confirmation of Diabetes
Following a 12-hour overnight fast, diabetes was induced in all groups except the normal control by a single intraperitoneal injection of streptozotocin at 65 mg/kg body weight. Streptozotocin was freshly dissolved in cold 0.1 mol/L citrate buffer at pH 4.5 immediately before administration. The normal control group received the corresponding vehicle.
Fasting blood glucose was measured from tail-vein blood approximately 72 hours after streptozotocin administration. Rats with fasting blood glucose concentrations of ≥250 mg/dL were considered diabetic and retained in the experimental groups.

Supplementation Protocol
Chromium picolinate and biotin were prepared freshly and administered orally by gavage once daily for seven consecutive weeks according to group allocation. Doses were calculated according to individual body weight and adjusted during the study when necessary. Animals in the control groups received an equivalent volume of vehicle. Body weight and general clinical condition were monitored throughout the intervention period.

Blood Collection and Sample Preparation
After the seven weeks of treatment, overnight fasted and anaesthetised animals were terminally bled. Blood for haematological analysis was put into tubes containing EDTA and for biochemical analysis was put into plain tubes. In cases where immediate analysis was not possible, serum was separated from the clotted blood by centrifugation at an approximate 3000 rpm for 10 minutes and kept at −20°C.

Hematological Assessment
Red blood cell count, hemoglobin concentration and hematocrit measurement were performed by standard haematological methods using calibrated automated analyzer of blood of laboratory animals. Results were reported as red blood cell (RBC) count in million/mm³, hemoglobin (Hb) in g/dL and hematocrit (HCT) in percentage.

Liver and Kidney Biochemical Evaluation
Hepatic biochemical status was determined by measuring serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Albumin and Total Bilirubin. Renal biomarkers were determined (serum creatinine and blood urea). The measurements were taken using standard commercial reagents and validated enzymatic or colorimetric methods as per the manufacturers' instructions.

Quality Control and Blinding.
For the laboratory analysis, the samples were coded to reduce the observer bias. Laboratory appliances were calibrated before use and internal quality-control procedures throughout biochemical and haematological testing were observed. Only samples that were grossly hemolyzed or analytically unsuitable as a result of laboratory interference were excluded.

Statistical Analysis
IBM SPSS Statistics version 29.0 was used to analyze the data, which were presented as mean ± standard deviation. The Shapiro-Wilk test was used to check for normality and Levene's test was used to check for homogeneity of

variance. One-way analysis of variance (ANOVA) and Tukey's post hoc test (where appropriate) were used to compare differences between the 11 experimental groups. If parametric assumptions were not met, Welch's analysis of variance or the Kruskal-Wallis test was used. All statistical tests were two-sided with a $p<0.05$ deemed statistically significant.

RESULTS

Haematological Outcomes

Diabetes was induced by streptozotocin and resulted in a significant change in erythrocyte related indices. The untreated diabetic control group had significantly reduced red blood cell (RBC) count, hemoglobin concentration and hematocrit as compared to the non-diabetic control group. When these three parameters were evaluated, they were improved by chromium picolinate and biotin supplementation, which was progressive, but the extent of the response depended on the treatment, dose, and parameter.

There was an increase in the number of RBC in chromium picolinate 200 µg/kg group which was 8.70 ± 0.55 million/mm³ as compared to untreated diabetic rats 6.48 ± 0.47 million/mm³. The same group had the highest concentration of hemoglobin (14.97 ± 0.21 g/dL). The chromium picolinate–biotin combination group given 150 µg/kg of each supplement had the highest hematocrit (44.67 ± 1.16%). The overall differences between groups were statistically significant for RBC count ($p=0.0003$), hemoglobin ($p=0.0001$) and hematocrit ($p=0.015$) (Table 1).

Table 1. Hematological indices across experimental groups

Experimental group	RBC count (million/mm³)	Hemoglobin (g/dL)	Hematocrit (%)
Normal control	8.32 ± 0.38	14.42 ± 0.36	43.10 ± 1.42
Diabetic control	6.48 ± 0.47	11.71 ± 0.44	35.83 ± 1.58
CrPic 100 µg/kg	7.25 ± 0.51	12.65 ± 0.39	38.60 ± 1.33
CrPic 150 µg/kg	8.02 ± 0.42	13.88 ± 0.31	41.90 ± 1.21
CrPic 200 µg/kg	**8.70 ± 0.55**	**14.97 ± 0.21**	44.20 ± 1.28
Biotin 100 µg/kg	6.95 ± 0.46	12.21 ± 0.34	37.20 ± 1.41
Biotin 150 µg/kg	7.41 ± 0.49	12.86 ± 0.37	39.10 ± 1.25
Biotin 200 µg/kg	7.85 ± 0.44	13.56 ± 0.32	41.07 ± 1.17
Combination 100 µg/kg	7.62 ± 0.43	13.18 ± 0.33	40.23 ± 1.31
Combination 150 µg/kg	8.33 ± 0.39	14.25 ± 0.28	**44.67 ± 1.16**
Combination 200 µg/kg	8.48 ± 0.41	14.52 ± 0.25	44.15 ± 1.12
Overall p-value	**0.0003**	**0.0001**	**0.015**

Values are presented as mean ± standard deviation; n=6 per group. CrPic, chromium picolinate.

Hepatic Biochemical Outcomes

Marked hepatic biochemical alterations were observed following induction of diabetes. The diabetic control group demonstrated substantially higher serum ALT, AST, ALP, and total bilirubin concentrations, together with a reduction in serum albumin, compared with the normal control group. Progressive improvement was observed with increasing doses of both chromium picolinate and biotin, while combination treatment generally produced the most pronounced recovery.

The untreated diabetic group showed ALT, AST, and ALP activities of 195.50 ± 3.20 U/L, 260.40 ± 4.10 U/L, and 190.50 ± 3.80 U/L, respectively. In comparison, administration of chromium picolinate plus biotin at 200 µg/kg reduced these values to 124.40 ± 0.59 U/L, 171.15 ± 0.92 U/L, and 127.40 ± 1.70 U/L, respectively, approaching the concentrations observed in the normal control group.

A similar pattern was evident for albumin and bilirubin. Serum albumin decreased to 2.91 ± 0.15 g/dL in untreated diabetic rats but increased progressively with supplementation, reaching 4.12 ± 0.10 g/dL in the high-dose combination group. Total bilirubin decreased from 0.78 ± 0.08 mg/dL in diabetic controls to 0.33 ± 0.03 mg/dL in animals receiving the 200 µg/kg combination. Overall group differences for all hepatic biomarkers were statistically significant ($p<0.001$) (Table 2).

Table 2. Hepatic biochemical parameters across experimental groups

Experimental group	ALT (U/L)	AST (U/L)	ALP (U/L)	Albumin (g/dL)	Total bilirubin (mg/dL)
Normal control	122.80 ± 1.50	168.20 ± 2.40	125.60 ± 2.10	4.30 ± 0.12	0.29 ± 0.04
Diabetic control	195.50 ± 3.20	260.40 ± 4.10	190.50 ± 3.80	2.91 ± 0.15	0.78 ± 0.08
CrPic 100 µg/kg	174.60 ± 2.70	237.20 ± 3.60	170.80 ± 3.20	3.20 ± 0.13	0.65 ± 0.06

CrPic 150 µg/kg	153.80 ± 2.10	210.60 ± 2.90	151.40 ± 2.70	3.51 ± 0.11	0.52 ± 0.05
CrPic 200 µg/kg	135.70 ± 1.60	187.50 ± 2.20	137.60 ± 2.30	3.82 ± 0.12	0.42 ± 0.04
Biotin 100 µg/kg	181.60 ± 2.90	244.40 ± 3.30	176.20 ± 3.00	3.18 ± 0.14	0.67 ± 0.06
Biotin 150 µg/kg	163.40 ± 2.40	218.70 ± 3.00	156.70 ± 2.80	3.48 ± 0.13	0.54 ± 0.05
Biotin 200 µg/kg	144.80 ± 1.90	195.50 ± 2.50	141.60 ± 2.50	3.76 ± 0.11	0.45 ± 0.04
Combination 100 µg/kg	154.70 ± 2.20	207.80 ± 2.80	149.80 ± 2.60	3.65 ± 0.12	0.49 ± 0.04
Combination 150 µg/kg	135.90 ± 1.50	184.80 ± 2.10	135.20 ± 2.00	3.94 ± 0.11	0.39 ± 0.03
Combination 200 µg/kg	**124.40 ± 0.59**	**171.15 ± 0.92**	**127.40 ± 1.70**	**4.12 ± 0.10**	**0.33 ± 0.03**
Overall p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Values are presented as mean ± standard deviation; n=6 per group. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; CrPic, chromium picolinate.

Renal Biochemical Outcomes

Renal biochemical impairment was evident in the untreated diabetic group, which demonstrated substantially increased serum creatinine and blood urea concentrations compared with non-diabetic controls. Serum creatinine increased from 41.80 ± 1.40 µmol/L in normal controls to 78.60 ± 3.20 µmol/L in untreated diabetic rats, while blood urea increased from 19.20 ± 1.00 mg/dL to 47.40 ± 2.60 mg/dL.

Both supplements produced dose-related reductions in renal biomarkers, with a stronger overall response in the combination groups. The 200 µg/kg chromium picolinate–biotin combination produced the lowest creatinine (40.62 ± 0.89 µmol/L) and urea (19.90 ± 0.89 mg/dL) concentrations among diabetic treatment groups. These values were comparable with those of the normal control group. Overall between-group differences in creatinine and urea were statistically significant ($p<0.001$) (Table 3).

Table 3. Renal biochemical parameters across experimental groups

Experimental group	Creatinine (µmol/L)	Blood urea (mg/dL)
Normal control	41.80 ± 1.40	19.20 ± 1.00
Diabetic control	78.60 ± 3.20	47.40 ± 2.60
CrPic 100 µg/kg	69.50 ± 2.80	39.80 ± 2.10
CrPic 150 µg/kg	59.20 ± 2.20	32.80 ± 1.80
CrPic 200 µg/kg	50.40 ± 1.70	27.10 ± 1.40
Biotin 100 µg/kg	70.10 ± 2.60	40.50 ± 2.20
Biotin 150 µg/kg	61.20 ± 2.10	34.10 ± 1.80
Biotin 200 µg/kg	52.30 ± 1.80	28.20 ± 1.50
Combination 100 µg/kg	57.60 ± 2.00	31.20 ± 1.70
Combination 150 µg/kg	47.70 ± 1.50	23.90 ± 1.20
Combination 200 µg/kg	**40.62 ± 0.89**	**19.90 ± 0.89**
Overall p-value	**<0.001**	**<0.001**

Values are presented as mean ± standard deviation; n=6 per group.

Overall Treatment Pattern

Taken together, the findings demonstrated a consistent dose-dependent improvement in diabetes-associated hematological and hepatorenal abnormalities. Chromium picolinate at 200 µg/kg produced the strongest improvement in RBC count and hemoglobin, whereas the intermediate combination dose produced the highest hematocrit. In contrast, the high-dose chromium picolinate–biotin combination showed the most comprehensive hepatic and renal biochemical recovery. The progressive decline in ALT, AST, and ALP across the combination-treatment groups is illustrated in Figure 1, with enzyme activities at the highest combined dose approaching the non-diabetic control profile.

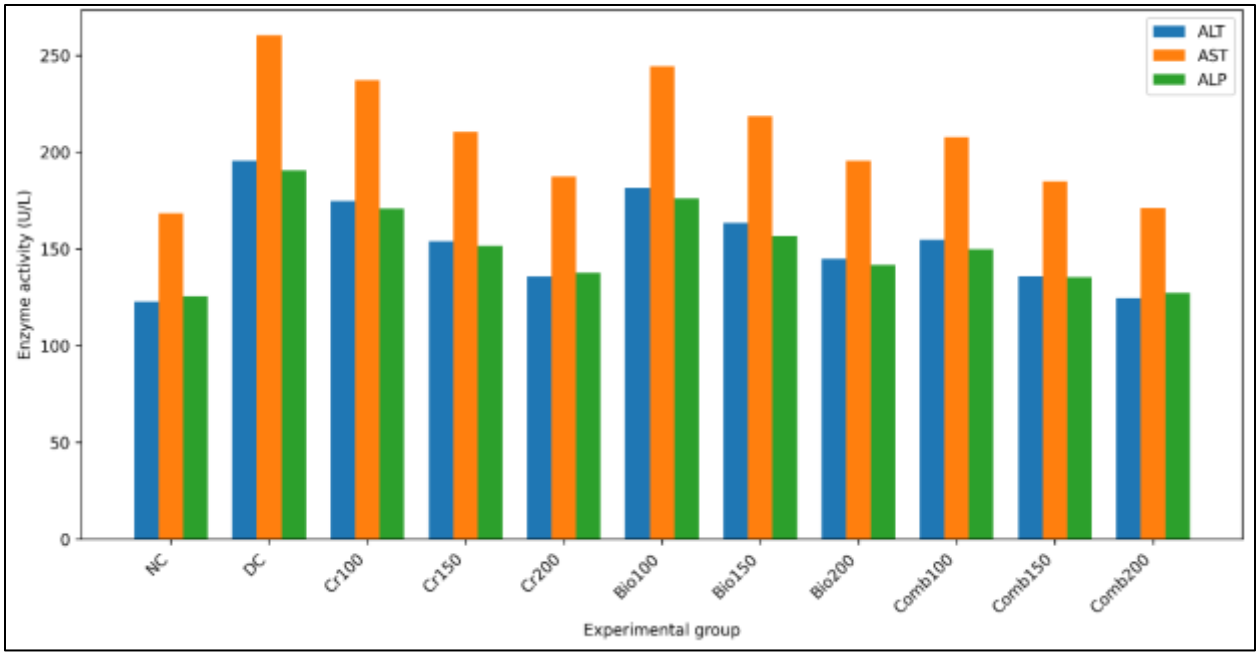

Figure 1. Hepatic enzyme profile across experimental groups

Figure 1 demonstrates the elevation of ALT, AST, and ALP following streptozotocin-induced diabetes and the progressive reduction in enzyme activities after chromium picolinate, biotin, and combined supplementation. The most pronounced normalization was observed in the chromium picolinate–biotin 200 µg/kg group.

DISCUSSION

In the present study, diabetes induced by streptozotocin caused significant alteration in hematological, biochemical parameters of liver and kidney and administration of chromium picolinate and biotin prevented these changes in a dose-dependent manner [1]. Three main results were found. First, untreated diabetic rats exhibited a marked decrease in the red blood cell count, hemoglobin and hematocrit, which indicated a poor status of the erythrocyte related indices. Second, there were also significant increases in Alanine Aminotransferase, Aspartate Aminotransferase, Alkaline Phosphatase, Total Bilirubin, Creatinine and Blood Urea with a significant decrease in Serum Albumin, consistent with biochemical hepatic and renal injury. Third, chromium picolinate resulted in the most effective recovery of selected hematological indices, while combined chromium picolinate and biotin supplementation, especially using 200 µg/kg, resulted in the most complete recovery of the hepatic and renal biomarkers [2,3].

The decrease in red blood cell number, hemoglobin and hematocrit in diabetic rats without treatment is in line with the systemic effects of chronic hyperglycaemia [4]. Diabetes is associated with oxidative stress, non-enzymatic glycation, membrane lipid peroxidation and changes in erythrocyte deformability and survival. These processes may also make erythrocytes fragile, and reduce the life span of circulating erythrocytes. Moreover, renal dysfunction due to diabetes can cause problems in the control of erythropoiesis and thus cause further decrease in hemoglobin levels. Hematological changes such as described here have been found in experimental diabetic models, which agrees with the concept that changes of erythrocyte indices are an important systemic aspect of the prolonged metabolic disorder [5,6].

Chromium picolinate had a noteworthy hematological response. The highest dose of chromium showed the highest number of RBCs and concentration of hemoglobin content in all treated groups [7]. It is possible that this result is due to the fact that chromium has the ability to enhance insulin related glucose utilization and decrease the oxidative burden that is due to chronic hyperglycemia. Chromium has been found to increase insulin receptor substrate signaling and phosphatidylinositol 3-kinase activity, which may help cells handle glucose better and decrease metabolic stress, experiments have shown [5]. Chromium supplementation has also been linked to decreases in oxidative damage and inflammation in diabetic models [1,3]. Chromium may have indirect beneficial effects on the integrity and survival of the erythrocytes by improving the systemic metabolic environment [8,9].

Interestingly, the combination of intermediate dose resulted in the highest HCT value, not the highest dose. The difference between the two higher combination groups was small, but this trend indicates that response may not be linear with supplementation dose with regard to hematological responses [10]. Plateau effects are commonly observed in biological systems when they are close to normal physiological levels. The observation could thus suggest that the dose necessary to achieve a maximal recovery of erythrocytes is different from that necessary for optimal biochemical protection of liver or kidney [11].

The liver changes in untreated diabetic animals were extensive. A rise in ALT and AST activities is compatible with loss of hepatocellular membrane integrity and leakage of the hepatocellular enzymes into the circulation, whereas elevated ALP and bilirubin indicate a more extensive hepatobiliary dysfunction [12]. The decrease in albumin may be the result of diminished protein synthesis by the liver or altered protein turnover or the result of systemic catabolism caused by uncontrolled diabetes. It is well known that experimental hyperglycemia leads to enhanced oxidative stress, mitochondrial dysfunction, lipid accumulation and inflammatory signaling within the liver that may lead to progressive hepatocellular injury [1,2].

There was a significant improvement in the hepatic biochemical profile with chromium picolinate, and the recovery was increased with increasing levels of chromium. Elevated levels of hepatic enzymes were also found to be reduced and histopathological changes were found to be reduced by chromium supplementation in previous experimental studies in streptozotocin-induced diabetes [13]. Sundaram et al. found that chromium administration enhanced the hepatic glucose metabolism by enhancing the glycolytic pathway, suppressing the gluconeogenic pathway and restoring the hepatic glycogen metabolism [6]. Such effects could lower the metabolic stress placed on the hepatocyte and could be a possible reason for the progressive decrease of ALT, AST and ALP seen in the present study [14].

Biotin also showed beneficial effects on hepatic biochemical parameters and the effects were less marked than the equivalent combined treatments. Biotin is a cofactor for several carboxylases which are essential for glucose, fatty-acid and amino-acid metabolism, and may affect the transcriptional regulation of enzymes of liver metabolism [15]. In animal models of diabetes, it has been shown that biotin downregulates the expression of hepatic phosphoenolpyruvate carboxykinase and enhances glucose tolerance [7,8]. These mechanisms may limit excessive hepatic glucose production and reduce metabolic stress, thereby contributing to the improvements in liver-associated biochemical markers observed in the current experiment [16].

Rats fed the highest combined dose of chromium picolinate and biotin had the best hepatic recovery. The ALT, AST and ALP levels in this group were similar to that of normal controls and there was a significant increase in albumin and decrease in bilirubin concentration [17]. This indicates complementary action of chromium and biotin, not merely a redundancy of effect. Chromium seems to work mainly by improving insulin-related signaling and regulating oxidative and inflammatory pathways, while biotin directly influences key metabolic enzymes and transcriptional pathways in the liver regulating glucose metabolism [18].

The experimental results of Sahin et al. confirmed this interpretation, showing that supplementation with combined chromium picolinate and biotin resulted in improvement in metabolic abnormalities and alteration of the molecular pathway linked to insulin resistance and inflammation in diabetic rats [11]. Their study revealed an up-regulation of peroxisome proliferator-activated receptor gamma expression and phospho-insulin receptor substrate-1 and down-regulation of nuclear factor kappa B. These results offer a feasible molecular mechanism for the improved biochemical recovery seen in combination supplementation in the current study [19].

Another clear effect of diabetes induced by streptozotocin was renal biochemical changes. The serum creatinine and blood urea levels significantly increased in untreated diabetic rats, which could be interpreted as a decrease in renal filtration and changes in the metabolism of nitrogenous substances. Prolonged hyperglycemia induces renal oxidative stress, inflammatory activation, glomerular hemodynamics and progressive renal structure damage. These mechanisms can eventually cause reduced renal clearance, and an increase of creatinine and urea in the bloodstream [18-20].

Renal biochemical abnormalities were decreased with both chromium picolinate and biotin, with the greatest response seen with combination supplementation. The most effective combined treatment lowered both the creatinine and urea levels to those of the non-diabetic control group. Earlier, it was reported that chromium could modulate nuclear factor kappaB and Nrf2 pathways that play a crucial role in anti-inflammatory and antioxidant effects, therefore exerting its protective role against diabetic renal dysfunction [3]. Other studies have shown a decrease in the levels of creatinine and urea and an improvement in the renal histopathological changes after chromium picolinate administration [1].

Biotin might provide extra renal protection via its actions on intermediary metabolism and oxidative stress. Biotin has been found to decrease renal oxidative injury and enhance morphology of glomerulus and tubules in streptozotocin-induced diabetic models in experimental studies [8]. It is therefore not unexpected that the greater response seen when biotin was given with chromium picolinate suggests that a combined effect of biotin on multiple pathways, including modulation of glucose metabolism, oxidative defense and inflammatory pathways, may be more beneficial for providing broader renal protection than focusing on one pathway alone [9].

It is also noteworthy that the overall dose-response pattern is present. The majority of haematological and biochemical parameters improved gradually with the escalation of the supplements level, especially in chromium and combination groups [10]. However, there were variations in optimum doses as demonstrated by the maximal hematocrit response at the intermediate combination dose. These findings suggest that dose-response relations for micronutrient supplementation may be end point specific and that it is not assumed that higher doses necessarily lead to better effects for all physiological variables [11].

An important strength of the present study is the simultaneous assessment of haematological, hepatic and renal parameters. Previous experimental studies have studied mainly glycemic control, oxidative stress or a single target

organ [12]. The present study assessed several systemic effects in the same experimental model, thus offering a wider view of the biological effects of chromium picolinate and biotin supplementation. Comparative assessment of individual and combined treatment patterns was also possible in the present study because separate graded-dose groups were included and combination groups evaluated separately.

However, there are some caveats that need to be taken into account. Some between-group estimates may not be as precise because the number of animals in each group was relatively small. Male rats only were used and the results therefore would not reflect any sex differences in response to treatment. Circulating hematological and biochemical biomarkers were targeted, and there was no histopathological evaluation of hepatic or renal tissues [14-16]. Also, there was no direct assessment of oxidative stress, inflammatory cytokines, insulin levels, glycated proteins or intracellular signaling pathways. Thus, while the biochemical patterns observed are consistent with antioxidant, metabolic and anti-inflammatory mechanisms, these cannot be directly proven from the present data [17].

In the future, it is recommended to perform biochemical analysis, liver and kidney histopathology, oxidative-stress parameters (malondialdehyde, superoxide dismutase, catalase and glutathione) and inflammatory markers (tumor necrosis factor-α and interleukin-6). Furthermore, evaluation of insulin receptor signaling, NF-κB, Nrf2, and peroxisome proliferator-activated receptor (PPAR) pathways would further elucidate the molecular mechanism of the apparent chromium–biotin interaction. Longer duration studies and experiments incorporating both sexes would also be useful for establishing how persistent and generalizable the observed effects are [18-20].

CONCLUSION

There was significant decrease in the haematological indices and marked biochemical dysfunctions were observed in the liver and kidneys of male Wistar rats induced with streptozotocin-induced diabetes. The most significant increase in red blood cell count and hemoglobin concentration was obtained with chromium picolinate supplementation, especially in 200 µg/kg group, and the most complete recovery of the liver and kidney biomarkers was obtained with chromium picolinate and biotin supplementation. The high dose combination showed a significant decrease in the levels of ALT, AST, ALP, creatinine and blood urea and increase in the levels of albumin and bilirubin, towards the levels of the non-diabetic controls. These results indicate that chromium picolinate and biotin have a complementary protective effect on diabetes associated systemic biochemical injury and that combined supplementation of these two was more beneficial in terms of protecting against diabetes-associated hepatorenal injury than supplementation with either individually. Evaluation of the underlying mechanism(s) by incorporation of histopathological, oxidative, inflammatory and molecular endpoints in future studies will be essential to confirm the mechanism(s) and establish the optimal dose-response relationship prior to consideration of translational implications.

Declarations

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Conflict of Interest: The authors declare no conflicts of interest.

Data Availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Author Contributions: HA conceived and designed the study. AAK contributed to experimental methodology and data interpretation. MA contributed to statistical analysis and critical review. AR contributed to laboratory assessment and manuscript preparation. All authors reviewed and approved the final manuscript.

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