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ORIGINAL ARTICLE

Effects of chromium malate on glycometabolism, glycometabolism-related enzyme levels and lipid metabolism in type 2 diabetic rats: A dose–response and curative effects study

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ABSTRACT

Aims/Introduction: The present study was designed to evaluate the effect of chromium malate on glycometabolism, glycometabolism-related enzyme levels and lipid metabolism in type 2 diabetic rats, and dose–response and curative effects.

Materials and Methods: The model of type 2 diabetes rats was developed, and daily treatment with chromium malate was given for 4 weeks. A rat enzyme-linked immunosorbent assay kit was used to assay glycometabolism, glycometabolism-related enzyme levels and lipid metabolism changes.

Results: The results showed that the antihyperglycemic activity increased with administration of chromium malate in a dose–dependent manner. The serum insulin level, insulin resistance index and C-peptide level of the chromium malate groups at a dose of 17.5, 20.0 and 20.8 μg chromium/kg bodyweight were significantly lower than that of the model, chromium trichloride and chromium picolinate groups. The hepatic glycogen, glucose-6-phosphate dehydrogenase and glucokinase levels of the chromium malate groups at a dose of 17.5, 20.0 and 20.8 μg chromium/kg bodyweight were significantly higher than that of the model, chromium trichloride and chromium picolinate groups. Chromium malate at a dose of 20.0 and 20.8 μg chromium/kg bodyweight significantly changed the total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides levels compared with the chromium trichloride and chromium picolinate groups.

Conclusions: The results showed that chromium malate exhibits greater benefits in treating type 2 diabetes, and the curative effect of chromium malate is superior to chromium trichloride and chromium picolinate.

INTRODUCTION

Diabetes mellitus has been identified as one of the three diseases (cardiovascular diseases, cancer and diabetes mellitus) difficult to cure by the World Health Organization. It was mainly divided into type 1 diabetes and type 2 diabetes[1]. Type 2 diabetes, which appears in middle-aged people and the elderly, was the focus of the present research. It accounts for more than 90% of diabetes cases[2]. Furthermore, the age of type 2 diabetes patients showed a downward trend. The occurrence of type 2 diabetes has been associated with genetic and acquired factors[3]. Type 2 diabetes is accompanied by glycometabolism, glycometabolism-related enzymes, lipid metabolism disorders and so on. The complications of type 2 diabetes are coronary heart disease, low learning deficits, low cognitive ability and cardiovascular disease[4–7]. However, type 2 diabetes and its complications

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cannot be cured completely, and prescribed medication and supplements should be given priority[8]. Chromium (Cr^{3+}), magnesium, multivitamins, calcium and aspirin are used as over-the-counter supplements[9–11].

Studies have shown that Cr^{3+} and its complex can decrease fasting blood glucose level[12–15]. Cr can enhance insulin sensitivity and regulate lipid metabolism in diabetes[9,16–19]. Studies have reported that supplemental Cr^{3+} propionate complex can significantly decrease serum insulin levels and increase the insulin sensitivity index. Similar results were reported that Cr^{3+} propionate complex can significantly reduce serum triacylglycerols, total cholesterols and low-density lipoprotein (LDL) cholesterol levels. Chromium supplementation can significantly reduce total cholesterol (TC), triglyceride (TG) and LDL levels in type 2 diabetes patients[19]. Currently, inorganic chromium and organic chromium are two categories of chromium supplements. Inorganic chromium complex, including chromium trichloride and chromium nitrate, have low absorption and high toxicity compared with organic chromium complex[20,21]. Chromium picolinate, chromium nicotinate and chromium yeast are organic chromium complexes, and have been widely used as supplements[22,23]. However, studies have shown that chromium picolinate can result in genotoxicity and cytotoxicity, thus its safety is a concern. The poor solubility and unstable structure of chromium nicotinate and chromium yeast have limited their application, respectively. Therefore, a novel and non-toxic organic chromium complex with antihyperglycemic activity has become an important issue.

Chromium malate is a new type of organic chromium complex that has been synthesized by our research group[24]. Chromium malate was synthesized by chelating Cr^{3+} with L-malic acid, which was selected as the natural ligand. Its chemical formula and molecular weight are $Cr_2C_{12}H_{22}O_{20}$ (or $Cr_2[C_4H_4O_5]_3 \cdot 5H_2O$) and 590.18 g/mol, respectively. Chromium malate can control blood glucose levels in alloxan-induced diabetic mice and does not cause oxidative DNA damage, and was tested as non-toxic in acute and subacute toxicity studies[25]. The dose–response relationship of chromium malate can describe the curative effects in type 2 diabetes and provide a reference for clinical medication. However, few studies on the dose–response relationship in the antihyperglycemic activity of chromium malate have been reported. The relationship between chromium malate intake and the curative effects in type 2 diabetes was examined in the present study. The effect of chromium malate on improving glycometabolism, glycometabolism-related enzymes and lipid metabolism in type 2 diabetic rats was also studied.

METHODS

Ethics Statement

All the experimental procedures were carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans, the EC Directive 86/609/EEC for animal experiments, and were approved by the Jiangsu University Committee on Animal Care and Use. Sprague–Dawley rats were procured from Jiangsu University (license number SYXK [SU] 2013–0036). The Sprague–Dawley rat is not a protected or endangered species. Our experiments complied with the laws and ethical recommendations currently in effect in China where the experiments were carried out.

Materials and Chemicals

The chromium malate was synthesized in our previous study[25]. The raw materials were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Streptozotocin was obtained from Sigma Chemical Co. (St. Louis, MO, USA) Distilled water was used throughout all the experiments. The "glycogen kit" was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Rat enzyme-linked immunosorbent assay (ELISA) kits were purchased from Hefei Bomei Biotechnology Co., Ltd (Hefei, China).

Animals and Diet

A total of 200 Sprague–Dawley male rats with an average weight of 180 ± 10 g were purchased from Jiangsu University

Table 1 | Experiment design and the dose of chromium malate, chromium trichloride and chromium picolinate

Group	Rats	Dose (Cr, μg/kg bw)
Normal control group	Normal rats	–
Model group	Type 2 diabetic rats	–
Chromium trichloride-treated group	Type 2 diabetic rats	20.8
Chromium picolinate-treated group	Type 2 diabetic rats	20.8
Chromium malate-treated group 1	Type 2 diabetic rats	2.5
Chromium malate-treated group 2	Type 2 diabetic rats	5.0
Chromium malate-treated group 3	Type 2 diabetic rats	7.5
Chromium malate-treated group 4	Type 2 diabetic rats	10.0
Chromium malate-treated group 5	Type 2 diabetic rats	12.5
Chromium malate-treated group 6	Type 2 diabetic rats	15.0
Chromium malate-treated group 7	Type 2 diabetic rats	17.5
Chromium malate-treated group 8	Type 2 diabetic rats	20.0
Chromium malate-treated group 9	Type 2 diabetic rats	20.8
Chromium malate control group	Normal rats	20.8

BW, bodyweight; Cr, chromium.

for all the experiments. Before the experiment, the animals were allowed 3 days for environmental and trainer handling acclimatization. The temperatures and relative humidity of the animal house were 24 ± 1°C and 55–60%, respectively. Rats consumed distilled water during the entire experimental period.

Models of Type 2 Diabetes

The models of type 2 diabetes were developed using the method of Li *et al.* and Xu *et al.* with slight modification[26,27]. A high-sugar and high-fat diet was supplied to rats in the first 2 months. Then the rats were injected with streptozotocin at a dose of 30 mg/kg bodyweight through intraperitoneal injection. Fasting blood glucose (FBG) level was measured by a one touch glucometer 3 days later. The oral glucose tolerance test and insulin resistance test were carried out. The FBG level of 33.3 mmol/L > FBG ≥ 11.1 mmol/L, accompanied by oral glucose tolerance and reduced insulin resistance (IR) in rats was taken as a successful induction of type 2 diabetes.

Dose–Response Relationship

Antihyperglycemic Activity

The type 2 diabetic rats were randomly divided into 12 groups of 10 animals. The experiment design, and the dose of chromium malate, chromium trichloride and chromium picolinate are shown in Table 1. Chromium malate was given by oral gavage to rats. Chromium trichloride and chromium picolinate were used as a positive control. The content of Cr in a standard pellet diet is 4.63 μg/kg. The Cr content, which was consumed by rats, is less than 1 μg, therefore, there has no obvious effect on this test. Normal rats were used as experimental animals in a blank control group and chromium malate control group. The type 2 diabetic rats were treated with chromium malate daily by gavage for 4 weeks. FBG and body mass were tested once a week. Blood samples were collected and centrifuged at the end of the experiment. The anticoagulated blood and the blood serum were used for hematological and biochemical analyses.

Glycometabolism and Glycometabolism-Related Enzymes

The serum and liver of rats were collected when the rats were killed. Serum insulin and C-peptide (C-P) were assayed by rat ELISA kits. The IR index was calculated by the following formula:[28]

$$\mathrm{IR} = \mathrm{insulin}/22.5\mathrm{e}^{-\ln \mathrm{FBG}}$$

A glycogen kit was used to assay liver glycogen levels. The livers of rats were homogenized and centrifuged at 5,000 *g* for 10 min at 4°C, and then supernatant was collected. Rat ELISA kits were used to estimate glucose-6-phosphate dehydrogenase (G6PD) and glucokinase (GCK) activity in the liver supernatant.

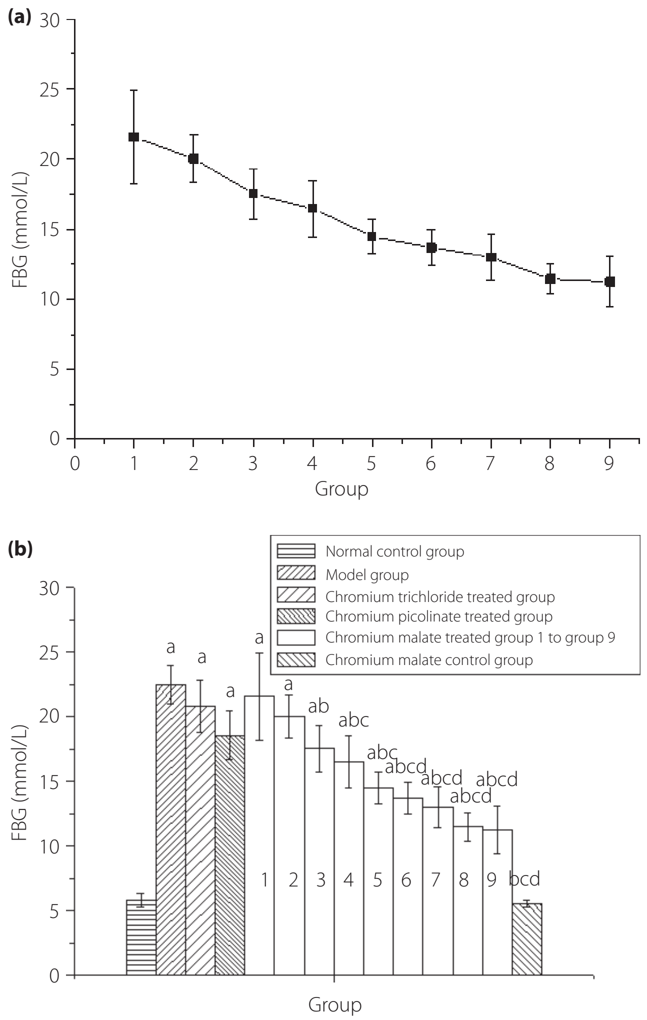

Figure 1 | (a) Dose–response relationship of chromium malate in antihyperglycemic activity in the fourth week. Groups 1–9 are the chromium malate treated groups. (b) The fasting blood glucose (FBG) level changes were treated with chromium malate, chromium trichloride, and chromium picolinate in normal and type 2 diabetic rats. Chromium trichloride and chromium picolinate was used as a positive control. Each value is presented as mean ± standard deviation (n = 10). [a]Significantly different from normal the control group ($P < 0.05$). [b]Significantly different from the model group ($P < 0.05$). [c]Significantly different from the chromium trichloride-treated group ($P < 0.05$). [d]Significantly different from the chromium picolinate-treated group ($P < 0.05$).

Lipid Metabolism

The serum of rats, which was collected in the previous step, was used to measure lipid metabolism analysis. TC, LDL, high-density lipoprotein cholesterol (HDL) and TG levels in blood serum were assayed by a rat reagent kit (Dong'ou Jinma Technology, Co., Ltd., Zhejiang, China).

Cr Content

The Vista-MPX Simultaneous Inductively coupled plasma (ICP) method (Varian, Inc., Palo Alto, CA, USA) was used to

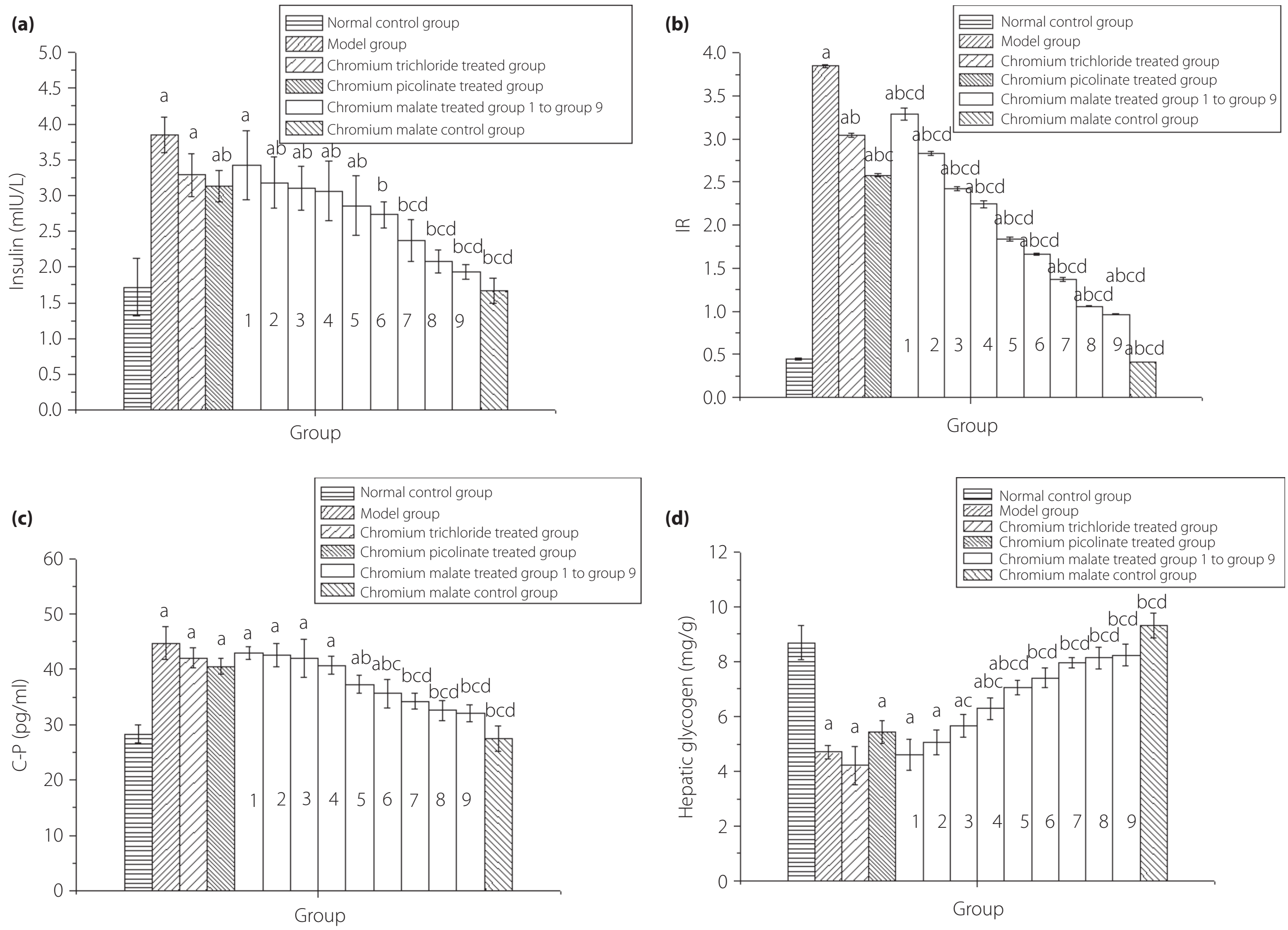

Figure 2 | The changes of (a) serum insulin, (b) IR, (c) C-peptide (C-P), and (d) hepatic glycogen levels in normal rats and type 2 diabetic rats after administration chromium malate, chromium trichloride and chromium picolinate. Chromium trichloride and chromium picolinate were used as positive controls. Each value is presented as mean ± standard deviation (n = 10). [a]Significantly different from normal control group ($P < 0.05$). [b]Significantly different from model group ($P < 0.05$). [c]Significantly different from chromium trichloride-treated group ($P < 0.05$). [d]Significantly different from chromium picolinate-treated group ($P < 0.05$).

measure Cr content in the serum and organs (heart, liver, spleen, lung, kidney, brain) of rats. Cr was determined after wet digestion according to the method described by Wu *et al.*[24] and Sereshti *et al.*[29] The serum and organs of rats were joined in nitric acid and perchloric acid (1:4, v/v) to digestion.

Organ Index

The organs (heart, liver, spleen, lung, kidney, brain) of rats, which were collected in the previous step, were weighed. Relative heart/liver/spleen/lung/kidney/brain weights were expressed as the ratio of the heart/liver/spleen/lung/kidney/brain to bodyweight (mg/g).

Statistical Analysis

Statistical analyses were carried out by the program SPSS 16.0 (Chicago, IL, USA). One-way analysis of variance (ANOVA) was used for data analysis. The Tukey test for multiple comparisons among the groups was carried out to determine significant differences. $P < 0.05$ was considered as statistically significant.

RESULTS

Dose–Response Relationship of Chromium Malate

Antihyperglycemic Activity

The dose–response relationship of chromium malate in antihyperglycemic activity during the fourth week is shown in Figure 1a. It can be observed that the FBG level of the chromium malate-treated groups showed a trend of decline in type 2 diabetic rats after the administration of chromium malate. The antihyperglycemic activity increased with an increase in dose of chromium malate. The FBG level of type 2 diabetic rats remained at 11–12 mmol/L after administration with chromium malate at a dose of 20.0 and 20.8 μg Cr/kg bodyweight. The antihyperglycemic activity of chromium malate shows a dose dependency. The FBG level of chromium malate-treated groups at a dose of 7.5, 10.0, 12.5, 15.0, 17.5, 20.0 and 20.8 μg Cr/kg bodyweight was significantly lower than that of the model group (Figure 1b). It can be observed that chromium trichloride and chromium picolinate can reduce FBG level. The FBG level of the chromium picolinate-treated group and chromium trichloride-treated group was significantly decreased when compared with the model group. From these results, it can be deduced that the FBG level of the chromium malate-treated groups at a dose of 15.0, 17.5, 20.0 and 20.8 μg Cr/kg bodyweight was significantly lower than that of the chromium trichloride-treated group at a dose of 20.8 μg Cr/kg bodyweight (20.82 ± 2.04 mmol/L) and the chromium picolinate-treated group at a dose of 20.0 μg Cr/kg bodyweight (18.57 ± 1.88 mmol/L). The antihyperglycemic activity of chromium malate was better than that of chromium trichloride and chromium picolinate.

Glycometabolism

The changes in serum insulin, IR, C-P and hepatic glycogen levels of normal rats and type 2 diabetic rats after administration of chromium malate, chromium trichloride and chromium picolinate are shown in Figure 2. Chromium malate, chromium trichloride and chromium picolinate can reduce serum insulin, IR and C-P levels in type 2 diabetic rats (Figure 2a–c). The serum insulin, IR and C-P levels of chromium malate-treated groups at a dose of 17.5, 20.0 and 20.8 μg Cr/kg bodyweight was significantly different when compared with the model group, chromium trichloride-treated group and chromium picolinate-treated group. However, the chromium trichloride- and chromium picolinate-treated groups showed no significant change when compared with the model group. The serum insulin, IR and C-P level reduced with an increase in dose of chromium malate. The hepatic glycogen level was increased after administration of chromium malate and chromium picolinate in type 2 diabetic rats (Figure 2d). Chromium trichloride cannot increase the hepatic glycogen level in type 2 diabetic rats. The hepatic glycogen level increased with an increase in dose of chromium malate. The hepatic glycogen level of the chromium malate-treated groups at a dose of 12.5, 15.0, 17.5, 20.0 and 20.8 μg Cr/kg bodyweight was significantly higher than that of the model group, chromium trichloride-treated group and chromium picolinate-treated group. The hepatic glycogen level of the chromium picolinate-treated group showed no significant increase when compared with the model group. The curative effects of chromium malate on the changes of serum insulin, IR, C-P and hepatic glycogen levels are better than those of chromium trichloride and chromium picolinate.

Glycometabolism-Related Enzymes

The changes in G6PD and GCK levels of normal rats and type 2 diabetic rats after administration of chromium malate,

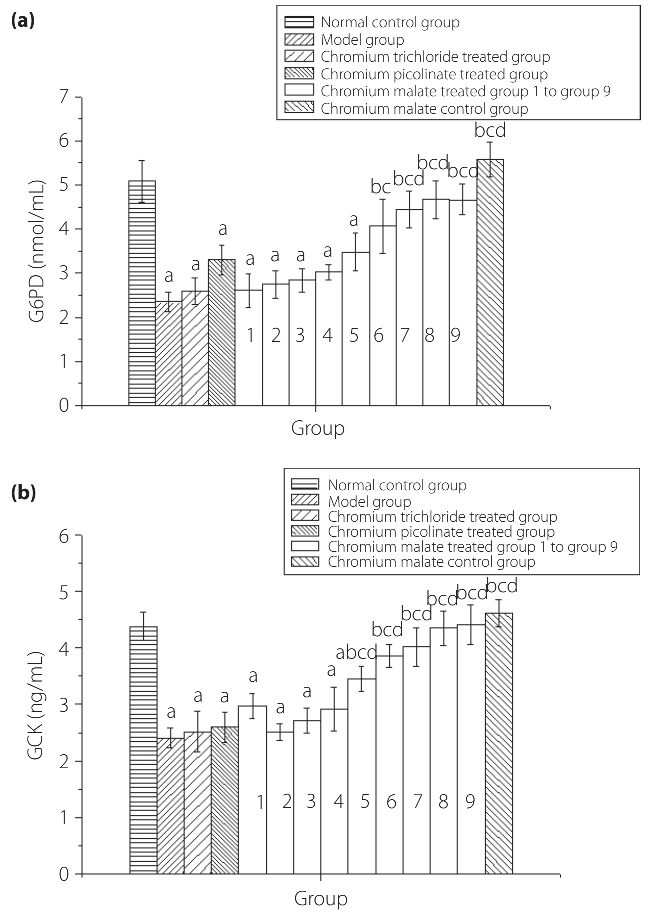

Figure 3 | The changes of (a) glucose-6-phosphate dehydrogenase (G6PD) and (b) glucokinase (GCK) levels in normal rats and type 2 diabetic rats after administration of chromium malate, chromium trichloride and chromium picolinate. Chromium trichloride and chromium picolinate were used as positive controls. Each value is presented as mean ± SD (n = 10). [a]Significantly different from normal control group ($P < 0.05$). [b]Significantly different from model group ($P < 0.05$). [c]Significantly different from chromium trichloride-treated group ($P < 0.05$). [d]Significantly different from chromium picolinate-treated group ($P < 0.05$).

chromium trichloride and chromium picolinate are shown in Figure 3. Chromium trichloride and chromium picolinate can increase the content of G6PD and GCK in type 2 diabetic rats. However, the G6PD and GCK levels of chromium trichloride-treated group and chromium picolinate-treated group showed no significantly increase when compared with model group. The results indicated that chromium malate can increase the G6PD and GCK level in type 2 diabetic rats. The G6PD and GCK levels increased with an increase in dose of chromium malate. The G6PD level of the chromium malate-treated groups at a dose of 17.5, 20.0 and 20.8 μg Cr/kg bodyweight was significantly increased when compared with the model group, chromium trichloride-treated group and chromium picolinate-treated group. The GCK level of the chromium

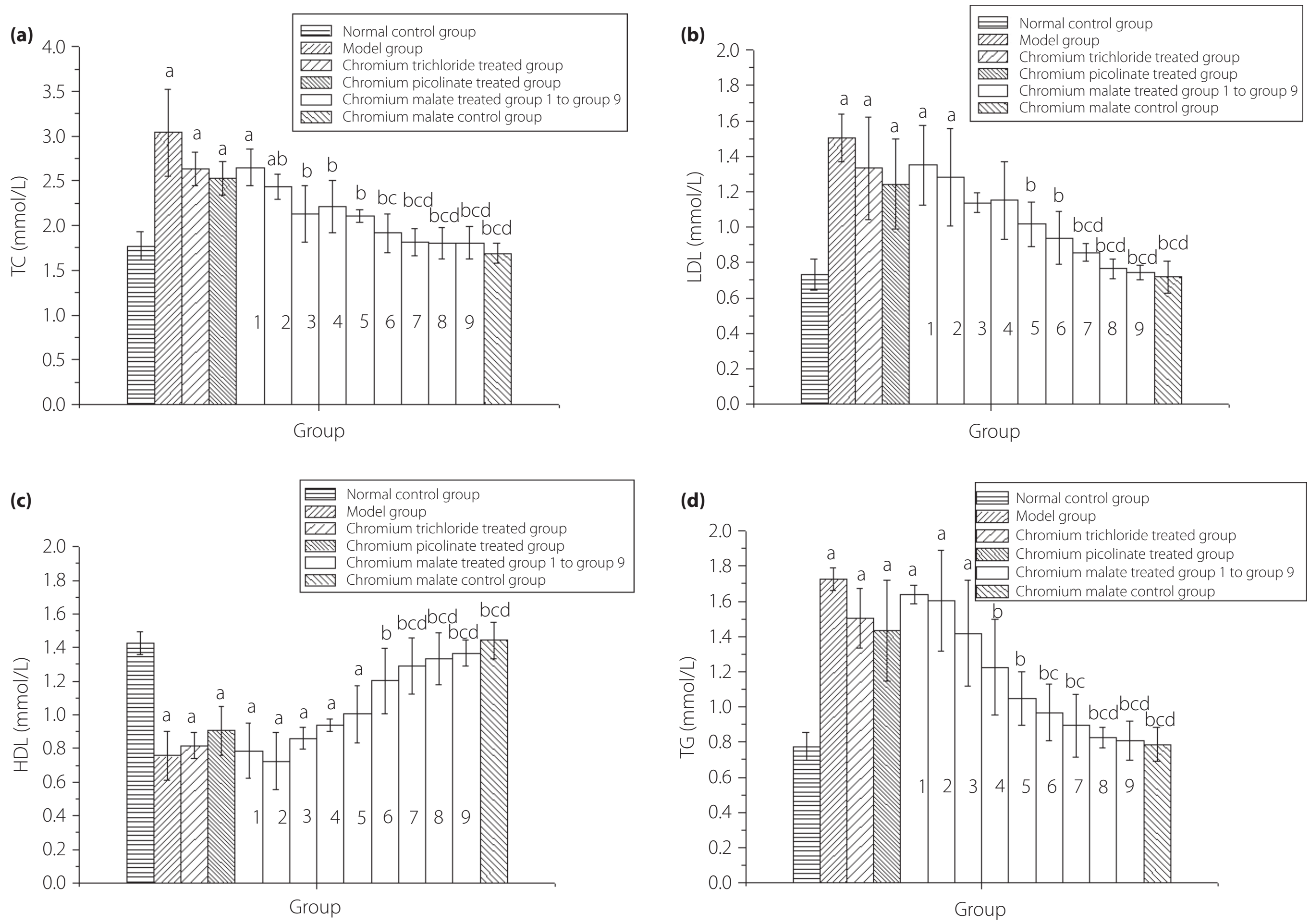

Figure 4 | The changes of serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and triglycerides (TG) in normal rats and type 2 diabetic rats after administration chromium malate, chromium trichloride and chromium picolinate. Chromium trichloride and chromium picolinate were used as positive controls. Each value was presented as mean ± SD (n = 10). [a]Significantly different from normal control group ($P < 0.05$). [b]Significantly different from model group ($P < 0.05$). [c]Significantly different from chromium trichloride-treated group ($P < 0.05$). [d]Significantly different from chromium picolinate-treated group ($P < 0.05$).

malate-treated groups at a dose of 12.5, 15.0, 17.5, 20.0 and 20.8 μg Cr/kg bodyweight was significantly increased when compared with the model group, chromium trichloride-treated group and chromium picolinate-treated group. The curative effects of chromium malate on increased G6PD and GCK levels are better than those of chromium trichloride and chromium picolinate.

Lipid Metabolism

The changes in TC, LDL, HDL and TG levels of normal rats and type 2 diabetic rats after administration of chromium malate, chromium trichloride and chromium picolinate are shown in Figure 4. Chromium trichloride and chromium picolinate can reduce serum TC, LDL and TG levels, and increase serum HDL levels in type 2 diabetic rats. However, chromium trichloride and chromium picolinate cannot significantly reduce serum TC, LDL and TG levels, or increase serum HDL levels when compared with the model group. The results showed that chromium malate can reduce the serum TC, LDL and TG levels, and increase serum HDL levels. The TC, LDL and TG levels reduced with an increase in dose of chromium malate. Serum HDL levels increased with an increase in dose of chromium malate. The TC, HDL and TG levels of the chromium malate-treated groups at a dose of 17.5, 20.0 and 20.8 μg Cr/kg bodyweight significantly changed when compared with the model group, chromium trichloride-treated group and chromium picolinate-treated group. The LDL level of chromium malate-treated groups at a dose of 20.0 and 20.8 μg Cr/kg bodyweight was significantly changed when compared with the model group, chromium trichloride-treated group and chromium picolinate-treated group. The curative effects of chromium malate on reducing TC, LDL and TG levels, and increasing HDL levels are better than those of chromium trichloride and chromium picolinate.

Table 2 | Chromium content of normal rats and type 2 diabetic rats after administration of chromium malate, chromium trichloride and chromium picolinate, and changes in serum and organs

Group	Parameter						
	μg/mL	μg/g					
	Serum	Heart	Liver	Spleen	Lung	Kidney	Brain
Normal control group	15.28 ± 1.06	6.29 ± 2.01	5.97 ± 2.12	10.45 ± 2.49	14.54 ± 2.01	9.32 ± 2.27	9.98 ± 2.27
Model group	10.01 ± 1.05†	5.91 ± 1.38	5.88 ± 1.24	9.74 ± 2.75	13.98 ± 2.92	7.58 ± 2.50	8.59 ± 2.13
Chromium trichloride-treated group	12.03 ± 1.13†	5.93 ± 2.17	6.01 ± 2.19	11.00 ± 3.62	13.06 ± 4.30	8.64 ± 3.06	9.46 ± 3.27
Chromium picolinate-treated group	17.14 ± 1.51‡§	7.12 ± 4.28	5.84 ± 1.87	11.97 ± 4.19	14.56 ± 4.31	10.37 ± 3.24	10.58 ± 3.25
Chromium malate-treated group 1	10.47 ± 1.27†¶	5.30 ± 2.23	5.84 ± 2.58	9.86 ± 2.59	13.91 ± 2.67	7.93 ± 2.48	8.51 ± 2.02
Chromium malate-treated group 2	10.94 ± 1.47†¶	5.72 ± 2.14	5.97 ± 1.98	9.92 ± 3.01	13.98 ± 2.64	8.55 ± 2.30	8.69 ± 2.85
Chromium malate-treated group 3	11.01 ± 1.05†¶	6.02 ± 2.01	5.91 ± 2.24	10.06 ± 3.21	13.98 ± 3.82	8.99 ± 3.01	8.82 ± 3.02
Chromium malate-treated group 4	11.25 ± 1.34†¶	6.18 ± 2.26	5.95 ± 2.33	10.58 ± 3.51	14.12 ± 2.52	9.49 ± 2.15	9.03 ± 2.34
Chromium malate-treated group 5	13.77 ± 1. 29¶	6.50 ± 2.12	5.97 ± 2.56	11.36 ± 3.90	14.15 ± 3.92	10.01 ± 2.59	9.20 ± 3.09
Chromium malate-treated group 6	16.42 ± 1.65‡§	6.63 ± 2.48	6.00 ± 3.53	13.36 ± 5.04	14.22 ± 4.43	10.48 ± 3.43	10.04 ± 3.21
Chromium malate-treated group 7	17.04 ± 1.53‡§	7.16 ± 3.47	6.00 ± 2.62	14.55 ± 3.37	14.39 ± 3.21	10.76 ± 3.41	10.21 ± 3.35
Chromium malate-treated group 8	17.68 ± 1.35‡§	7.91 ± 3.14	6.03 ± 2.21	15.58 ± 4.53	14.59 ± 4.03	11.00 ± 3.62	10.34 ± 3.41
Chromium malate-treated group 9	17.98 ± 1.17‡§	8.09 ± 3.54	6.06 ± 1.29	16.09 ± 5.21	14.60 ± 5.01	11.02 ± 4.23	10.82 ± 4.05
Chromium malate control group	15.45 ± 1.24‡§	6.39 ± 2.10	5.97 ± 2.67	12.31 ± 4.53	14.41 ± 4.86	10.69 ± 4.41	11.26 ± 4.16

Data is presented as mean ± standard deviation ($n = 10$). Chromium trichloride and chromium picolinate were used as positive controls. †Significantly different from normal control group ($P < 0.05$). ‡Significantly different from model group ($P < 0.05$). §Significantly different from chromium trichloride-treated group ($P < 0.05$). ¶Significantly different from chromium picolinate-treated group ($P < 0.05$).

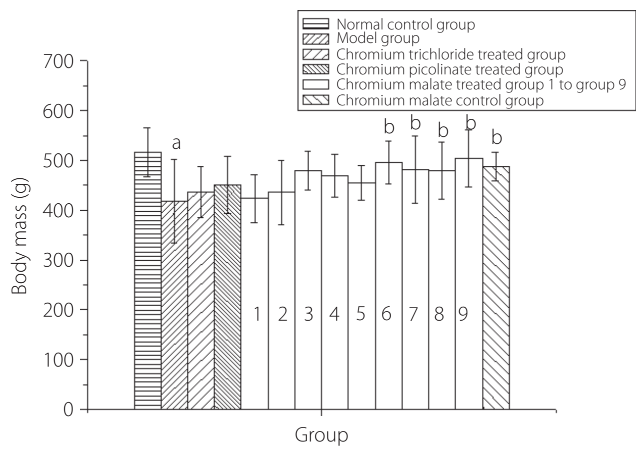

Figure 5 | The dose–response relationship of chromium malate in the body mass of normal and type 2 diabetic rats in the fourth week. Chromium trichloride and chromium picolinate were used as positive controls. Each value was presented as mean ± SD ($n = 10$). [a]Significantly different from normal control group ($P < 0.05$). [b]Significantly different from model group ($P < 0.05$). [c]Significantly different from chromium trichloride-treated group ($P < 0.05$). [d]Significantly different from chromium picolinate-treated group ($P < 0.05$).

Cr Content

The changes in Cr content in the serum and organs (heart, liver, spleen, lung, kidney, brain) of normal rats and type 2 diabetic rats after administration chromium malate, chromium trichloride and chromium picolinate are shown in Table 2. The serum Cr content of the normal control group was significantly higher than that of the model group. The results showed that type 2 diabetes can reduce serum Cr content, and chromium malate, chromium trichloride and chromium picolinate can increase the serum Cr content in type 2 diabetic rats. The serum Cr content of the chromium malate-treated group at a dose of 15.0, 17.5, 20.0 and 20.8 μg Cr/kg bodyweight, and the chromium picolinate-treated group was significantly increased when compared with the chromium trichloride-treated group. The results showed that the absorption of chromium malate and chromium picolinate was higher than that of chromium trichloride, and the absorption rate of chromium malate was the same as chromium picolinate. The Cr content in organs (heart, liver, spleen, lung, kidney, brain) of the model group showed no significant decrease when compared with the normal control group, chromium malate-treated group, chromium trichloride-treated group and chromium picolinate-treated group. The Cr content of the chromium malate-treated group at a dose of 20.8 μg Cr/kg bodyweight is spleen > lung > kidney > brain > heart > liver.

Dose–Response Relationship of Chromium Malate in Body Mass

The dose–response relationship of chromium malate in the body mass of normal rats and type 2 diabetic rats in the fourth week is shown in Figure 5. It can be observed that the body mass of type 2 diabetic rats increased with an increase in chromium malate. The body mass of the chromium malate-treated groups at a dose of 15.0, 17.5, 20.0 and 20.8 μg Cr/kg

Table 3 | Biochemical analyses in normal rats and type 2 diabetic rats after administration of chromium malate, chromium trichloride and chromium picolinate

Group	Parameter					
	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TP (g/L)	BUN (mmol/L)	Crea (μmol/L)
Normal control group	45.28 ± 6.36	95.29 ± 11.72	155.57 ± 28.95	67.92 ± 5.46	4.45 ± 0.77	33.90 ± 7.12
Model group	41.11 ± 5.11	121.53 ± 34.38	242.83 ± 30.42†	70.24 ± 1.17	5.82 ± 0.58†	45.85 ± 9.05
Chromium trichloride-treated group	47.13 ± 8.89	123.63 ± 17.48	243.33 ± 29.91†	71.00 ± 2.16	5.90 ± 0.13	41.65 ± 6.20
Chromium picolinate-treated group	40.41 ± 5.21	120.30 ± 14.28	224.20 ± 37.88†	69.77 ± 1.96	5.67 ± 0.36	37.88 ± 4.21
Chromium malate-treated group 1	44.71 ± 7.17	124.77 ± 19.32	231.00 ± 31.85†	69.66 ± 2.35	6.11 ± 0.74†	43.80 ± 4.88
Chromium malate-treated group 2	45.83 ± 7.17	120.78 ± 29.41	234.67 ± 20.98†	71.00 ± 2.65	5.99 ± 0.76†	43.33 ± 4.20
Chromium malate-treated group 3	49.25 ± 5.08	102.72 ± 11.36	230.00 ± 28.76†	68.76 ± 2.13	5.88 ± 0.82†	40.37 ± 3.67
Chromium malate-treated group 4	46.50 ± 9.99	105.78 ± 26.27	214.75 ± 21.36	69.78 ± 1.78	5.81 ± 0.25	40.05 ± 5.63
Chromium malate-treated group 5	47.70 ± 9.82	110.00 ± 12.27	211.75 ± 13.65	68.83 ± 2.04	5.57 ± 0.39	35.18 ± 2.43
Chromium malate-treated group 6	46.42 ± 6.55	106.03 ± 18.45	211.67 ± 19.35	68.33 ± 3.06	5.62 ± 0.44	35.86 ± 3.13
Chromium malate-treated group 7	45.84 ± 7.39	107.56 ± 10.47	206.33 ± 26.26	68.50 ± 1.73	5.52 ± 1.12	36.78 ± 6.94
Chromium malate-treated group 8	49.30 ± 7.55	100.83 ± 14.53	201.75 ± 18.01	68.67 ± 2.03	5.65 ± 0.22	35.58 ± 3.70
Chromium malate-treated group 9	48.58 ± 7.02	103.38 ± 15.45	196.00 ± 19.29	68.25 ± 4.44	5.20 ± 0.42	34.10 ± 3.29
Chromium malate control group	48.15 ± 7.45	93.33 ± 10.25	152.92 ± 17.60‡§¶	67.74 ± 3.94	4.01 ± 0.68‡	29.78 ± 1.74

Data is presented as mean ± standard deviation (n = 10). Chromium trichloride and chromium picolinate were used as positive controls. ALT, alanine transaminase; ALP, alkaline phosphatase; AST, aspartate transaminase; BUN, blood urea nitrogen; Crea, creatinine; TP, total protein. †Significantly different from normal control group ($P < 0.05$). ‡Significantly different from model group ($P < 0.05$). §Significantly different from chromium trichloride-treated group ($P < 0.05$). ¶Significantly different from chromium picolinate-treated group ($P < 0.05$).

bodyweight showed a significant increase when compared with the model group. However, there was no significant increase when compared with the chromium trichloride-treated group and chromium picolinate-treated group. Chromium trichloride and chromium picolinate can increase the body mass of type 2 diabetic rats. However, there was no significant change when compared with the model group.

Biochemical and Hematological Analyses

The biochemical and hematological analyses of chromium malate by oral gavage to normal rats and type 2 diabetic rats are shown in Tables 3 and 4, respectively. The results showed that type 2 diabetes can lead to alkaline phosphatase and blood urea nitrogen rising in type 2 diabetic rats. Chromium malate, chromium trichloride and chromium picolinate can reduce this biochemical index. However, there was no significant change when compared with the model group. The results of hematological analyses are similar to the results of biochemical analyses. These results showed that chromium malate did not significantly affect the biochemical and hematological index.

Change of Absolute and Relative Organ Weights in Rats

The changes in absolute and relative organ weights (heart, liver, spleen, lung, kidney, brain) of normal rats and type 2 diabetic rats after administration of chromium malate, chromium trichloride and chromium picolinate are shown in Table 5. Chromium malate, chromium trichloride and chromium picolinate can reduce the absolute and relative organ weights. However, there was no significant change when compared with the model group. These results showed that chromium malate did not have a significant effect on absolute and relative organ weights in rats.

DISCUSSION

Diabetes mellitus is a chronic disease affecting millions of people globally and resulting in significant death rates each year. Type 2 diabetes is one type of diabetes mellitus and is found all over the world including China. It is also characterized by glycometabolism disorders, glycometabolism-related enzymes disorders and lipid metabolism disorders. Its can increase the risk of developing learning and memory deficits, diabetic nephropathy, atherosclerotic arterial disease, and so on[6,30]. Type 2 diabetic rats showed significant hyperglycemia in the present study. The FBG level of type 2 diabetic rats was 22.46 ± 1.47 mmol/L and significantly higher than that of normal rats (5.82 ± 0.53 mmol/L).

Cr is an important micronutrient that can reduce the FBG level in diabetes mellitus. Similar results were reported by Pattar *et al.*[31], who found that chromium picolinate can enhance glucose metabolism; however, the safety and dose problems of chromium picolinate have been a concern. Chromium malate reduced FBG levels in type 2 diabetic rats after administration of chromium malate in the present study. Hepatic glycogen is composed of glucose and stored in the liver. Its accumulation is promoted by hyperglycemia and insulin, and in diabetes patients it is often accompanied by lower hepatic glycogen level[32]. The results of the present study showed that the hepatic glycogen levels of type 2 diabetic rats were significantly lower

Table 4 | Hematological analyses in normal rats and type 2 diabetic rats after administration of chromium malate, chromium trichloride and chromium picolinate

Group	Parameter						
	WBC (10^9/L)	LYMPH (10^9/L)†	MNCC (10^9/L)	NCC (10^9/L)	RBC (10^9/L)	HCT (%)	MCV (fL)
Normal control group	8.04 ± 1.99	8.15 ± 2.11	0.85 ± 0.49	1.40 ± 0.18	8.71 ± 0.10	46.76 ± 4.38	55.00 ± 1.71
Model group	13.45 ± 1.93	5.87 ± 0.94	0.62 ± 0.21	2.42 ± 0.26	9.61 ± 0.31	52.66 ± 1.43	57.33 ± 1.75
Chromium trichloride-treated group	12.41 ± 1.61	5.27 ± 1.53[a]	0.61 ± 0.20	2.07 ± 0.40	9.63 ± 0.16	52.73 ± 3.69	57.25 ± 2.06
Chromium picolinate-treated group	11.96 ± 1.43	6.83 ± 0.95	0.66 ± 0.38	2.26 ± 0.25	9.31 ± 0.92	50.50 ± 3.56	57.50 ± 1.91
Chromium malate-treated group 1	12.72 ± 1.87	5.84 ± 0.56	0.58 ± 0.18	2.20 ± 0.16	9.27 ± 0.16	52.58 ± 4.97	57.50 ± 1.73
Chromium malate-treated group 2	13.03 ± 1.07	6.20 ± 0.72	0.58 ± 0.11	2.31 ± 0.31	9.32 ± 0.18	51.87 ± 1.90	57.00 ± 3.00
Chromium malate-treated group 3	11.29 ± 4.54	6.84 ± 1.92	0.65 ± 0.23	1.87 ± 0.15	8.47 ± 0.39	49.35 ± 1.96	56.17 ± 1.47
Chromium malate treated group 4	10.83 ± 2.30	6.72 ± 1.88	0.73 ± 0.11	1.92 ± 0.34	8.35 ± 0.45	49.58 ± 2.44	56.33 ± 1.97
Chromium malate-treated group 5	10.84 ± 2.22	6.33 ± 0.58	0.78 ± 0.23	1.89 ± 0.16	8.99 ± 0.02	49.70 ± 1.31	56.00 ± 2.65
Chromium malate-treated group 6	10.62 ± 1.64	7.00 ± 1.00	0.74 ± 0.22	1.89 ± 0.10	8.79 ± 0.23	49.97 ± 1.14	55.50 ± 1.52
Chromium malate-treated group 7	10.06 ± 1.69	7.95 ± 1.59	0.78 ± 0.44	1.76 ± 0.55	8.74 ± 0.09	49.58 ± 3.14	55.83 ± 1.72
Chromium malate-treated group 8	10.08 ± 1.71	7.68 ± 1.48	0.75 ± 0.16	1.61 ± 0.18	8.77 ± 0.65	47.80 ± 2.34	55.20 ± 1.84
Chromium malate-treated group 9	9.40 ± 1.59	7.47 ± 1.02	0.75 ± 0.12	1.59 ± 0.37	8.72 ± 0.26	47.70 ± 2.55	55.50 ± 2.74
Chromium malate control group	8.07 ± 1.65	9.61 ± 2.03	0.88 ± 0.26	1.19 ± 0.12	8.72 ± 0.23	46.98 ± 1.97	55.20 ± 1.84

Group	Parameter							
	Hb (g/L)	MHbC (Pg)	MCHC (g/L)	PLT (10^9/L)	PDW (fL)	MPV (fL)	PCT (%)	RDW (fL)
Normal control group	159.69 ± 16.79	17.63 ± 1.15	315.60 ± 24.72	852.67 ± 37.61	13.65 ± 2.45	8.43 ± 1.39	0.69 ± 0.14	14.89 ± 1.05
Model group	167.50 ± 12.26	19.65 ± 1.42	336.33 ± 28.15	968.33 ± 86.07	15.91 ± 1.46	10.80 ± 0.74	1.33 ± 0.26	16.50 ± 1.36
Chromium trichloride-treated group	165.67 ± 14.04	19.05 ± 2.76	335.67 ± 24.73	964.00 ± 83.72	16.30 ± 2.85	10.33 ± 0.84	1.28 ± 0.27	16.73 ± 1.64
Chromium picolinate-treated group	162.75 ± 12.42	19.43 ± 1.42	324.00 ± 21.87	947.72 ± 95.79	15.79 ± 2.34	10.05 ± 1.25	1.13 ± 0.10	16.41 ± 1.04
Chromium malate-treated group 1	167.00 ± 15.66	19.44 ± 1.39	342.00 ± 25.57	965.50 ± 29.18	15.88 ± 1.59	10.64 ± 1.47	1.31 ± 0.38	16.35 ± 1.67
Chromium malate-treated group 2	168.50 ± 12.12	19.55 ± 1.21	339.00 ± 25.29	964.25 ± 71.57	15.70 ± 1.85	10.60 ± 1.28	1.31 ± 0.19	16.40 ± 1.70
Chromium malate-treated group 3	166.25 ± 14.35	19.52 ± 1.34	327.33 ± 24.16	947.00 ± 51.07	15.63 ± 1.33	10.37 ± 1.29	1.22 ± 0.18	16.13 ± 1.72
Chromium malate-treated group 4	165.20 ± 13.03	19.33 ± 1.57	324.67 ± 21.53	942.00 ± 41.55	15.78 ± 1.10	10.28 ± 0.86	1.09 ± 0.11	16.07 ± 1.70
Chromium malate-treated group 5	165.20 ± 13.70	18.43 ± 1.37	322.14 ± 28.03	938.50 ± 28.99	14.97 ± 2.68	9.67 ± 1.21	0.97 ± 0.12	15.72 ± 1.42
Chromium malate-treated group 6	162.17 ± 16.01	18.23 ± 1.67	322.50 ± 23.79	928.50 ± 30.42	14.81 ± 1.01	9.43 ± 1.21	0.89 ± 0.15	15.95 ± 1.07
Chromium malate-treated group 7	162.30 ± 16.11	18.26 ± 1.07	321.70 ± 13.17	892.67 ± 56.45	14.67 ± 1.47	9.09 ± 1.95	0.87 ± 0.39	15.76 ± 1.85
Chromium malate-treated group 8	161.60 ± 13.97	18.30 ± 1.17	320.75 ± 23.86	889.67 ± 61.33	14.50 ± 1.71	8.87 ± 1.62	0.76 ± 0.47	15.64 ± 1.39
Chromium malate-treated group 9	161.38 ± 13.29	18.57 ± 1.06	320.00 ± 24.24	874.75 ± 33.86	14.47 ± 2.67	8.73 ± 1.95	0.76 ± 0.19	15.44 ± 1.29
Chromium malate control group	158.40 ± 17.12	17.47 ± 1.59	317.25 ± 24.79	795.00 ± 24.68	13.73 ± 1.95	8.25 ± 1.69	0.61 ± 0.15	14.49 ± 1.51

Data is presented as mean ± standard deviation (n = 10). Chromium trichloride and chromium picolinate were used as positive controls. Hb, hemoglobin; HCT, hemataocrit; LYMPH, lymphocyte count; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MHbC, mean hemoglobin content; MNCC, mononuclear cell count; MPV, platelet mean volume; NCC, neutral cell count; PCT, platelet deposited; PDW, platelet distribution width; PLT, platelet count; RBC, red blood cell; RDW, red cell distribution width; WBC, white blood cell. †Significantly different from normal control group (P < 0.05).

Table 5 | The changes of absolute and relative organ weights in normal rats and type 2 diabetic rats after administration chromium malate, chromium trichloride and chromium picolinate

Absolute organ weight Group	Parameter					
	Heart	Liver	Spleen	Lung	Kidney	Brain
Normal control group	1.46 ± 0.19	15.07 ± 2.11	0.77 ± 0.14	1.94 ± 0.31	2.48 ± 0.41	1.59 ± 0.28
Model group	1.41 ± 0.21	16.38 ± 2.80	0.76 ± 0.25	2.03 ± 0.35	3.77 ± 0.32†	1.74 ± 0.16
Chromium trichloride-treated group	1.37 ± 0.12	14.43 ± 1.78	0.54 ± 0.11	1.98 ± 0.11	3.70 ± 0.36†	1.82 ± 0.14
Chromium picolinate-treated group	1.36 ± 0.15	16.31 ± 3.06	0.82 ± 0.25	1.91 ± 0.32	3.53 ± 0.36†	1.76 ± 0.18
Chromium malate-treated group 1	1.37 ± 0.14	17.40 ± 2.42	0.90 ± 0.27	2.05 ± 0.17	3.58 ± 0.30†	1.66 ± 0.16
Chromium malate-treated group 2	1.47 ± 0.19	16.36 ± 2.21	0.65 ± 0.11	2.19 ± 0.44	3.58 ± 0.43†	1.82 ± 0.17
Chromium malate-treated group 3	1.33 ± 0.16	16.32 ± 0.79	0.83 ± 0.05	2.00 ± 0.36	3.51 ± 0.22†	1.50 ± 0.15
Chromium malate-treated group 4	1.44 ± 0.14	17.98 ± 1.75	0.86 ± 0.13	2.03 ± 0.11	3.33 ± 0.66†	1.84 ± 0.17
Chromium malate-treated group 5	1.44 ± 0.25	14.33 ± 1.14	0.57 ± 0.07	2.07 ± 0.28	3.50 ± 0.13†	1.77 ± 0.21
Chromium malate-treated group 6	1.41 ± 0.23	15.68 ± 2.19	0.59 ± 0.14	2.01 ± 0.19	3.40 ± 0.08	1.82 ± 0.18
Chromium malate-treated group 7	1.35 ± 0.21	16.53 ± 2.48	0.77 ± 0.27	2.09 ± 0.39	3.25 ± 0.37	1.73 ± 0.19
Chromium malate-treated group 8	1.37 ± 0.10	15.21 ± 1.98	0.67 ± 0.24	2.01 ± 0.13	3.07 ± 0.76	1.79 ± 0.19
Chromium malate-treated group 9	1.27 ± 0.11	16.11 ± 2.87	0.87 ± 0.16	1.81 ± 0.28	3.02 ± 0.57	1.76 ± 0.15
Chromium malate control group	1.54 ± 0.33	15.05 ± 1.52	0.88 ± 0.15	2.07 ± 0.26	2.41 ± 0.21‡§¶	1.82 ± 0.28

Relative organ weight Group	Parameter					
	Relative heart weight	Relative liver weight	Relative spleen weight	Relative lung weight	Relative kidney weight	Relative brain weight
Normal control group	0.0028 ± 0.00039	0.029 ± 0.0043	0.0015 ± 0.00029	0.0038 ± 0.00063	0.0048 ± 0.00084	0.0031 ± 0.00057
Model group	0.0034 ± 0.00025	0.039 ± 0.0053	0.0018 ± 0.00030	0.0049 ± 0.00042	0.0090 ± 0.00038†	0.0042 ± 0.00019
Chromium trichloride-treated group	0.0034 ± 0.00024	0.036 ± 0.0035	0.0013 ± 0.00022	0.0049 ± 0.00022	0.0092 ± 0.00071	0.0045 ± 0.00028
Chromium picolinate-treated group	0.0031 ± 0.00026	0.037 ± 0.0053	0.0019 ± 0.00043	0.0044 ± 0.00056	0.0081 ± 0.00063	0.0040 ± 0.00031
Chromium malate-treated group 1	0.0032 ± 0.00029	0.041 ± 0.0051	0.0021 ± 0.00057	0.0048 ± 0.00036	0.0085 ± 0.00063†	0.0039 ± 0.00034
Chromium malate-treated group 2	0.0034 ± 0.00041	0.038 ± 0.0034	0.0015 ± 0.00017	0.0050 ± 0.00068	0.0082 ± 0.00066†	0.0042 ± 0.00026
Chromium malate-treated group 3	0.0028 ± 0.00033	0.034 ± 0.0020	0.0017 ± 0.00013	0.0042 ± 0.00092	0.0073 ± 0.00056†	0.0031 ± 0.00038
Chromium malate-treated group 4	0.0031 ± 0.00032	0.038 ± 0.0041	0.0018 ± 0.00030	0.0043 ± 0.00026	0.0071 ± 0.00015†	0.0039 ± 0.00040
Chromium malate-treated group 5	0.0032 ± 0.00071	0.031 ± 0.0033	0.0012 ± 0.00020	0.0045 ± 0.00080	0.0077 ± 0.00037†	0.0039 ± 0.00060
Chromium malate-treated group 6	0.0028 ± 0.00053	0.032 ± 0.0051	0.0012 ± 0.00032	0.0040 ± 0.00044	0.0069 ± 0.00019	0.0037 ± 0.00042
Chromium malate-treated group 7	0.0028 ± 0.00027	0.035 ± 0.0032	0.0016 ± 0.00035	0.0044 ± 0.00050	0.0069 ± 0.00048	0.0037 ± 0.00024
Chromium malate-treated group 8	0.0029 ± 0.00017	0.032 ± 0.0035	0.0014 ± 0.00042	0.0042 ± 0.00023	0.0064 ± 0.00013	0.0037 ± 0.00033
Chromium malate-treated group 9	0.0025 ± 0.00019	0.032 ± 0.0050	0.0017 ± 0.00028	0.0036 ± 0.00049	0.0060 ± 0.00099	0.0035 ± 0.00026
Chromium malate-control group	0.0032 ± 0.00012	0.031 ± 0.0053	0.0018 ± 0.00052	0.0042 ± 0.00091	0.0049 ± 0.00073‡§¶	0.0037 ± 0.00098

Data is presented as mean ± standard deviation (n = 10). Chromium trichloride and chromium picolinate was used as a positive control. †Significantly different from normal control group ($P < 0.05$). ‡Significantly different from model group ($P < 0.05$). §Significantly different from chromium trichloride-treated group ($P < 0.05$). ¶Significantly different from chromium picolinate-treated group ($P < 0.05$).

than that of normal rats. Insulin is a type of protein hormone and is secreted from pancreatic β-cells to control the blood glucose level[33]. It is a marker in the diagnosis and monitoring of type 2 diabetes, and is often used to control postprandial glucose levels in diabetics. Type 2 diabetes is also associated with insulin resistance and higher IR[34]. C-P is a small polypeptide produced and secreted from pancreatic β-cells during insulin synthesis. The concentration of C-P, which was secreted by islet β-cells, is equal to the insulin in the blood[35]. It is an important index for detection of insulin[36]. The results of the present study showed that the insulin, IR and C-P levels of type 2 diabetic rats were significantly higher than that of normal rats, and the results were similar to those in the literature. G6PD and GCK are key enzymes in glucose metabolism, and exist in the liver. The present results showed that the G6PD and GCK levels of type 2 diabetic rats were significantly lower than that of normal rats. Serum lipids, which include TC, LDL, HDL and TG, in type 2 diabetic rats changed significantly compared with the normal rats. Serum TC, LDL and TG levels in type 2 diabetic rats were significantly higher than that of normal rats. Serum HDL was significantly lower than that of normal rats. Król *et al.*[18] found that chromium (III) propionate complex can significantly reduce serum TG, TC and LDL levels. Sharma *et al.*[19] reported that chromium supplementation can significantly reduce TC, TG and LDL levels in type 2 diabetes. Sahin *et al.*[37] reported that chromium picolinate (80 μg/kg bodyweight per day) can significantly reduce TC and TG when compared with type 2 diabetes mellitus. In conclusion, chromium malate shows beneficial effects against type 2 diabetes-induced glycometabolism, glycometabolism-related enzymes and lipid metabolism disorders. The antihyperglycemic activity of chromium malate is superior to chromium trichloride and chromium picolinate.

The loss of body mass often accompanies type 2 diabetes. Rodbard *et al.*[38] reported that body mass control is a recommended treatment goal in type 2 diabetes patients: any weight gain of diabetes is associated with anti-diabetic therapy. Chromium malate can maintain the body mass of type 2 diabetic rats, whereas chromium trichloride and chromium picolinate cannot. Studies have shown that diabetes can cause biochemical and hematological changes[39,40], and the results were similar to the present results. However, chromium malate has no significant effect on the biochemical and hematological index.

In conclusion, chromium malate shows greater benefits in treating type 2 diabetes, and can improve glycometabolism, glycometabolism-related enzymes and lipid metabolism in type 2 diabetic rats. The dose–response relationship of chromium malate showed that antihyperglycemic activity was increased with administration of chromium malate in a dose–dependent manner.

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