Research Paper

Effects of Rutin and Selenomethionine in Selenium-Enriched Tartary Buckwheat Roasted Grain Tea on Glucose and Lipid Metabolism in db/db Mice

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ABSTRACT

Rutin and selenomethionine in selenium-enriched Tartary buckwheat roasted grain tea have certain effects on glucose and lipid metabolism in type II diabetes mellitus, but the mechanism of intervention is still unclear. This study utilized selenium-enriched Tartary buckwheat tea, rutin, and selenomethionine as research materials, with db/db mice serving as the research subjects. Diabetes control (DC), positive control group (PC), rutin group (RC), selenomethionine group (SC), low dose selenium-enriched Tartary buckwheat tea group (TBG-L), medium dose group (TBG-M) and high dose group (TBG-H) were set up. The effects of selenium enriched Tartary buckwheat tea, rutin and selenomethionine on glucose and lipid metabolism in db/db mice were investigated by blood biochemical analysis, liver function and oxidative stress measurement, histopathology and glycogen determination and mRNA expression analysis. Selenium-enriched Tartary buckwheat tea and its active ingredients rutin and selenomethionine can reduce fasting blood glucose and glucose resistance in db/db mice. Dyslipidemia of db/db mice was improved to some extent. The changes of MDA, CAT, T-SOD and GSH-Px enzyme activities in liver also indicate that TBG can improve the oxidative stress of liver and exert liver protection activities. Selenium-enriched Tartary buckwheat tea, rutin and selenomethionine can mediate glycogen storage by increasing glycogen synthesis and inhibiting gluconeogenesis in the liver of diabetic db/db mice, and can effectively mediate the expression of related genes during gluconeogenesis. Among the selenium-enriched Tartary

buckwheat groups, the effect of TBG-H group was the most obvious, which indirectly indicated that rutin and selenomethionine could play synergistic roles in gluconogenesis.

1 INTRODUCTION

Tartary buckwheat [*Fagopyrum tataricum* (L.) Gaertn.] is a kind of food and medicine dual-purpose characteristic multigrain crop (Ren et al., 2015). It is rich in protein, lipids, dietary fiber, digestible starch and other nutrients (Qin, 2012), and also contains rutin, quercetin, γ-aminobutyric acid and other active ingredients that are not available in other food crops (Fabjan et al., 2003). The soil in Ankang City of Shaanxi province is rich in selenium, and the planted Tartary buckwheat in Ankang city could enrich a high selenium content (Gao et al., 2014), reaching 118.04 μg/kg (Niu et al., 2020), which is 20 times higher than that in common Tartary buckwheat (Sun et al., 2005; Park et al., 2002). *Compendium of Materia records* that the Tartary buckwheat has bitter taste, calming cold, beneficial to the strength, the continuation of the spirit, the benefit of the eyes and ears, and the role of widening the intestine and strengthening the stomach. Clinical practice has proved that Tartary buckwheat has beneficial effects on lowering fasting glycemic index and insulin resistance, reducing glycated hemoglobin and glycated serum protein, as well as lowering blood lipid levels. These effects contribute to inhibiting the development of diabetes and its associated complications (Ren et al., 2015; Qin et al., 2012). Diabetes mellitus is a health problem of common concern in modern society, which brings heavy economic burden for society and family. According to data provided by the International Diabetes Federation (IDF), the global prevalence of type 2 diabetes (T2D) is expected to reach 13.5% in 2040 (Zhou et al., 2018).

T2D is widely recognized as a multifaceted metabolic disease. It is observed that over 90% of individuals with diabetes experience insulin deficiency and insulin resistance in target organs due to malfunctioning pancreatic beta cells (Zhang et al., 2017). Complications caused by T2D also increase the harm of this disease, such as blindness, kidney failure, lower limb amputation, heart disease, kidney disease, and liver disease (Zhang et al., 2017). Diabetes is a pressing issue that requires immediate attention. However, certain drugs used for treating T2D are frequently linked to various side effects, including vitamin B12 deficiency, gastrointestinal problems, liver damage, and lactic acidosis (Zhou et al., 2019; Lee et al., 2016; Hu

et al., 2017). Therefore, it is of great significance to explore the effect and the mechanism of dietary intervention of selenium-enriched Tartary buckwheat and its rutin and selenomethionine in the treatment of T2D.

Selenium has the effect of regulating insulin mediated physiological process to reduce blood glucose (Seale et al., 2013). Selenium mainly exists in the form of selenocysteine in mammals, and is involved in the synthesis of selenoprotein, which is used as the main carrier to play the corresponding biological functions (Niu et al., 2022). Plasma GSH-Px (GPx3) and selenoprotein P are the most common selenoproteins in blood, selenoprotein P is the main and best form of selenium in the body, 60% of selenium is distributed in selenoprotein P, and 30% of selenium is distributed in GPx3 (Tan et al., 2016). GPx3 can remove peroxides produced in the physiological or pathological processes of cells and reduce the damage of reactive oxygen species on the body (Zhou et al., 2019). Selenoprotein P is an extracellular glycoprotein, mainly produced by liver cells and secreted into plasma, which is of great significance for selenium transport and storage, and also has antioxidant stress properties (Thiry et al., 2013).

The close correlation between oxidative stress and T2D suggests that selenium and corresponding selenoproteins have anti-oxidative stress and insulin-like effects. They improve the secretion function of pancreatic β cells, increase the levels of insulin and C-peptide, enhance the content and activity of GSH-Px, and improve the ability to scavenge free radicals. This protection can helps preserve insular tissue and insulin from destruction (Zhao et al., 2019; Qin et al., 2013; Rodrigo et al., 2015). The relationship between selenium and T2D has been widely concerned by scholars. Flavonoids can inhibit α-glucosidase, prevent the occurrence of diabetes, reduce blood glucose level, alleviate the development of diabetes and alleviate diabetes complications (Wu et al., 2021). The mechanism of Tartary buckwheat flavonoids to reduce blood lipids is mainly to inhibit the absorption of triacylglycerol and reduce the level of triacylglycerol after meals (Dzah et al., 2020; Hwang et al., 2019; Almuhayawi et al., 2021). The mechanism of flavonoids to reduce blood glucose is to improve insulin signaling molecules and pathways in the liver, increase glucose

consumption and glycogen production, and assist in insulin regulation to reduce blood glucose content (Chen et al., 2017; Zhu, 2021; Guo et al., 2012; Ruan et al., 2022; Zielińska et al., 2012). The effect mechanism of selenium-enriched Tartary buckwheat tea and its active components selenomethionine and rutin on glucose and lipid metabolism in T2D mellitus is still unclear, and whether there is a synergistic effect needs to be verified.

In this study, a tradition Tartary buckwheat product made from selenium-enriched Tartary buckwheat, was selected as the research material. The feeding effects of different selenium-enriched Tartary buckwheat tea, rutin and selenomethionine on different groups of db/db mice were compared. Blood biochemical analysis, liver function and liver oxidative stress were measured. The effects of selenium-enriched Tartary buckwheat tea, rutin and selenomethionine on glycolipid metabolism in T2D were investigated by histopathology, hepatic glycogen assay and gene expression index, and the study provided theoretical basis and practical reference for selenium-rich Tartary buckwheat products in the adjuvant treatment of T2D mellitus.

2 MATERIALS AND METHODS

2.1 Experimental animals and samples

A total of 84 C57 BL/6 (db/db) species mice were SPF grade healthy males, aged 5 weeks and weighed (30±2) g. They were purchased from Jiangsu Jizhu Pharmaceutical Science and Technology Co., LTD., and kept in the Animal Laboratory of Shaanxi Normal University at an ambient temperature of 23 ± 2 °C and relative humidity of 45%-65%, day and night. After 28 days, the blood, liver, intestines, etc. were slaughtered and frozen for later experiments.

Selenium-enriched Tartary buckwheat tea were planted and processed in Ziyang County, Ankang city, and provided by Lotus Catering Company. Selenium-enriched Tartary buckwheat tea is made from shelled selenium-enriched buckwheat seeds, which have been roasted for 10 minutes at 180 °C. The basic ingredients of selenium-enriched Tartary buckwheat tea are shown in Table 1.

Animal db/db mouse models aged 5 weeks were randomly assigned to 7 groups, namely diabetes control (DC), positive control group (PC), rutin group (RC), selenomethionine group (SC), selenium-enriched Tartary buckwheat tea low dose (TBG-L) group, medium dose (TBG-M) group and high dose group (TBG-H), with 12 mice in each group. Each group was fed according to the feed formula table.

2.2 Feed customization

According to the standard feed, the feed is customized according to the principle of conservation of energy intake and the feed formula table is shown in Table 2.

2.3 Measurements of body weight and liver index

From the beginning of the experiment, the body weight (BW) of each mouse was recorded weekly. After killing the mice, the liver index was calculated according to the formula: liver index = liver weight (g)/body weight (100g).

2.4 Methods of fasting blood glucose and oral glucose tolerance test (OGTT)

The mice were fasted for 12 h at a fixed time every week, and drinking water freely. The blood of the mice was collected from the tail vein and the blood glucose level was measured by glucose meter. After 4 weeks of customized feed treatment, the mice were fasted for 12 h at the same time, and were intragastric with glucose solution at a dose of 2 g/kg. Blood from tail vein was collected and blood glucose was measured at 0, 30, 60 and 120 min.

2.5 Blood collection and biochemical analysis
2.5.1 Serum collection

After 4 w of treatment, the mouse eye was removed immediately after wiping with alcohol cotton swab, and

Table 1. Selenium-enriched Tartary buckwheat Tea Component Content (dry base)

	Moisture (%)	Protein (%)	Flavonoids (%)	Se (μg/kg)	Organic selenium (μg/kg)
Tartary buckwheat roasted grain tea	5.50±0.24	16.11±0.15	1.98±0.08	66.63±0.38	49.60±0.14

Table 2. *Custom feed formula*

AIN-93M (For Maintenance of adult rodents)			DC	PC	SC	RC	TBG-L	TBG-M	TBG-H
Ingredient	**Amount**	g	g	g	g	g	g	g	g
Casein	14.00%	140	140	140	140	140	108	76	44
Dextrinized Cornstarc	15.50%	155	155	155	155	155	155	155	155
Sucrose	10.00%	100	100	100	100	100	100	100	100
Corn Starch	46.57%	465.7	465.7	465.7	465.7	465.7	365.7	225.7	105.7
Alphacel, Non-Nutritive Bulk	5.00%	50	50	50	50	50	50	50	50
Soybean Oil	4.00%	40	40	40	40	40	36	32	28
Mineral Mix	3.50%	35	35	35	35	35	35	35	35
L-Cystine	0.18%	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Vitamin Mix	1.00%	10	10	10	10	10	10	10	10
Choline bitartrate	0.25%	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Tartary buckwheat roasted grain tea	0.00%	0	0	0	0	0	200	400	600
SeMet	0.00%	0	0	0	8.48µg	0	0	0	0
Rutin	0.00%	0	0	0	0	5.6	0	0	0
Metformin hydrochloride	0.00%	0	0	1.05g	0	0	0	0	0
Total	100.00%	1000.0	1000.00	1000.00	1000.00	1000.00	1064.00	1088.00	1132.00
Mass fraction of Tartary buckwheat	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.37	0.53

the blood was collected. After anticoagulation with sodium citrate, the mouse eye was left for 30 min at room temperature, then after centrifuged at 3 000 r/min. The supernatant was obtained, packaged and labeled, and frozen at -80 °C.

2.5.2 Blood biochemical analysis

The frozen animal liver samples were thawed in the -20°C refrigerator for 8 hours, and then transferred to the 4°C refrigerator before subpackaged. During the dissolution process, it is necessary to gently shake evenly and be careful not to cause bubbles and reduce the formation of precipitation. The serum levels of triglyceride (TG) and total cholesterol (TC) in mice were measured by GPO-PAP method after swirling the samples for 15 s with a scroll instrument. The contents of high density lipoprotein (HDL-C) and low density lipoprotein (LDL-C) in serum of mice were directly determined by double reagent method. Serum free fatty acid (FFA) content was quantified using a commercial test kit.

2.6 Measurement of liver function and oxidative stress in the liver

The frozen animal liver samples were thawed in the -20°C refrigerator for 8 hours, and then transferred to the 4°C refrigerator before subpackaged. Serum total protein (TP), albumin (ALB), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured according to the protocol of the corresponding kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). Oxidative stress in the liver was assessed by levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). The procedure follows the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

2.7 Histopathological examination and hepatic glycogen assay

The samples were fixed with 4% (w/v) paraformaldehyde and embedded in paraffin. The specimens were successively sliced to 4 mm and dewaxed. To determine

pathological changes and glycogen content in the liver, sections were stained with hematoxylin and eosin (H&E) and periodate Schiff reagent (PAS), respectively. A slide obtained under the ME21 (MSHOT) microscope (Olympus Corporation, Japan).

The content of hepatic glycogen was determined according to the manufacturer's protocol of the commercial kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

2.8 Quantitative real-time PCR assay

Relative mRNA expression levels of peroxisome proliferator-activated Receptor-γ (PPAR-γ), glucose transporter-4 (GLUT4), phosphoinositol 3-kinase (PI3K), protein kinase B (Akt), glucokinase (GK), and phosphoenyruvate carboxykinase in animal liver samples (PEPCK). The primer sequences are listed in Table 3. The evaluation was conducted by quantitative real-time PCR and sequenced by Sevier Gene Technology Co., LTD. (Wuhan, China).

2.9 Statistical analysis

Data were analyzed with a one-way analysis of variance (ANOVA) followed by LSD multiple comparison

Table 3. Primers used in this study

Gene	Primer direction*	Gene sequence
β-αctin	F	GTGACGTTGACATCCGTAAAGA
β-αctin	R	GCCGGACTCATCGTACTCC
Pparg	F	GGAAGACCACTCGCATTCCTT
Pparg	R	GTAATCAGCAACCATTGGGTCA
Slc2α4	F	GGACCGGATTCCATCCCAC
Slc2α4	R	TCCCAACCATTGAGAAATGATGC
Pik3rl	F	CCCACTACTGTAGCCAACAAC
Pik3rl	R	CGTACCAAAAAGGTCCCATCA
Akt	F	ATGAACGACGTAGCCATTGTG
Akt	R	TTGTAGCCAATAAAGGTGCCAT
Gck	F	TGAGCCGGATGCAGAAGGA
Gck	R	GCAACATCTTTACACTGGCCT
Pck1	F	ATGGGGTGTTTGTAGGAGCA
Pck1	R	CCGAAGTTGTAGCCGAAGAA

*F, Forward primer; R, Reverse primer.

tests. All groups were compared with each other for every parameter. The data were shown as the mean ± SD. Statistical significance was based on $p < 0.05$.

3 RESULT AND DISCUSSION

3.1 Phenotypes on growth of db/db mice during feeding

The changes in body weight and liver index were shown in Fig. 1 (A, B, C).

The final body weight and weight gain of metformin (PC) and selenium-rich Tartary wheat tea groups (TBG-L, TBG-M, TBG-H), selenomethionine groups (SC) and rutin groups (RC) were lower than those of diabetes con-
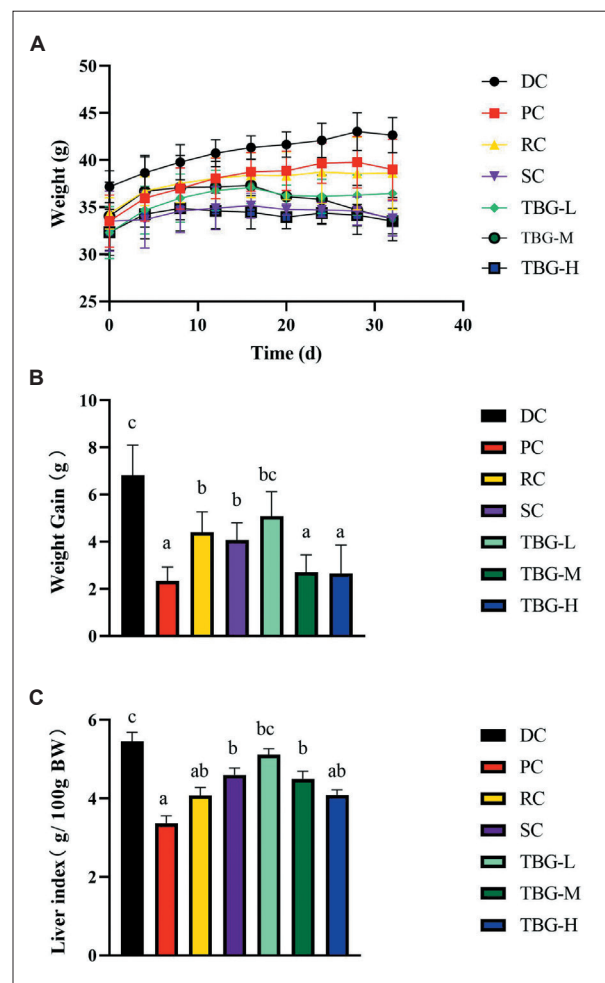

Fig. 1. Changes of body weight and liver index in db/db mice A weight change of db/db mice; B Final weight; C Changes of liver index in db/db mice (p < 0.05)

trol group (DC) (p < 0.05). As shown in Figure 1A, during the 4-week experiment, the weight of mice in all groups increased, which showed a continuous rise from 1 to 20 days, and tended to be stable after 20 days. The body weight of the DC group was the highest in the whole experiment, and the final mean value reached 43.5g. There were certain differences in the final mean value of the other groups, which were PC (37.2g), RC (36.4g), TBG-L (35.7g), SC (34.5g) and TBG-M (33.6g), TBG-H (33.0g).

As shown in Fig. 1B, the weight gain of DC group (6.9g) was the largest among all the groups, followed by TBG-L (5.5g), RC (4.8g), SC (4.4g), TBG-M (2.8g), TBG-H (2.7g) and PC (2.3g).

As for the liver index (FIG. 1C), PC showed the smallest value (3.62) among all the groups, and the other groups from small to large were ranked as TBG-H (4.05), RC (4.07), TBG-M (4.25), SC (4.38), TBG-L (5.07), DC (5.36). These results indicated that buckwheat tea, rutin and selenomethionine can effectively intervene in the weight and liver enlargement associated with T2D.

The treatment effect of PC group was the best, and the treatment effect of RC group and SC group was also more significant. Among the selenium-enriched Tartary buckwheat tea intervention groups, the treatment effect of TBG-H group was the best, followed by TBG-M and TBG-L groups. Both selenoprotein and rutin have obvious therapeutic effect on T2D.

3.2 Changes of glucose homeostasis in db/db mice during feeding

As shown in Fig.2A, db/db mice were treated with diet for four weeks and their fasting blood glucose was measured weekly. Fasting blood glucose in DC group increased with the increase of time and continued to increase throughout the cycle, reaching 7.3 mmol/L on day 7 and 17.6 mmol/L on day 28. The blood glucose value of the other groups showed a decreasing trend after reaching the peak value on day 14, and the peaks were successively RC (14.8 mmol/L), SC (14.1 mmol/L), TBG-L (13.9 mmol/L), TBG-M (13.7 mmol/L), TBG-H (12.7 mmol/L), as a sequence. PC (11.8 mmol/L). The effect of PC group and TBG-H group was more obvious, indicating that high dose of selenium-enriched Tartary buckwheat tea can effectively alleviate the increase of blood glucose.
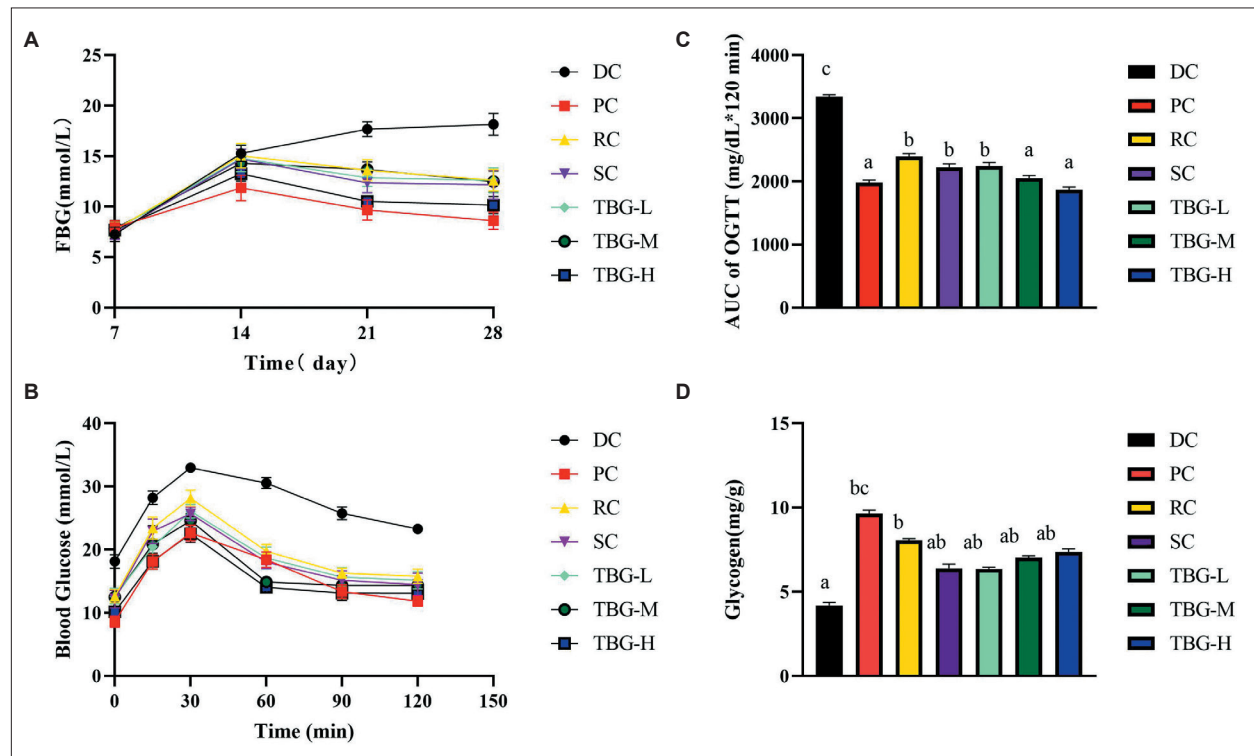

Fig. 2. *Changes of glucose-related indexes during feeding of db/db mice*
A Fasting blood glucose (FBG); B Oral glucose tolerance test (OGTT); C Area under the curve of OGTT; D Hepatic glycogen content (p < 0.05)

The fasting blood glucose levels and OGTT were carried out through tail vein blood sampling method (Fig.2B). Within 30 min of gastric glucose administration, the blood glucose levels in both the intervention and DC groups increased to a peak value, among which the DC group was higher than all other groups. The peak value of 30 min was lower in PC group and TGB-H group. As shown in Fig.2C, AUC values in PC group and other dietary intervention groups (TBG-L, TBG-M, TBG-H, RC, SC) were significantly lower than those in DC group. These results indicated that selenium-enriched Tartary buckwheat tea, rutin and selenomethionine could alleviate the increase of blood glucose after meals. In selenium-enriched Tartary buckwheat tea group, TBG-H group had the best effect, followed by TBG-M group and TBG-L group, showing a dose-response relationship. Comparing the PC group, RC group and SC group, both SC group and RC group can effectively inhibit glucose tolerance, and the effect of SC group is more significant than that of PC group.

3.3 Changes of blood lipids in db/db mice during feeding

Compared with DC group, the levels of TC, TG and LDL-c in other groups (PC, TBG-L, TBG-M, TBG-H, RC, SC) were significantly increased (p < 0.05), and the level of HDL-c was significantly decreased (p < 0.05), indicating that the dyslipidemia of db/db mice had been improved. In the intervention groups, TC, TG and LDL-c were lower in PC group, RC group, SC group and TBG-H group, while HDL-c was higher.

Compared with DC group, RC group and SC group were more effective in the treatment of dyslipidemia. In the selenium-enriched Tartary buckwheat group, the treatment effect of TBG-H group was the best, followed by TBG-M group and TBG-L group. These results indicate that rutin and selenomethionine can effectively intervene the dyslipidemia of T2D mellitus, and have a certain synergistic effect.

3.4 Changes of liver indexes in db/db mice during feeding

As shown in Fig.3A and B, the concentrations of TP and ALB in db/db mice were significantly increased after metformin administration compared to the DC group. Similar to the PC group, RC group, SC group, and TBG-M group, the TBG-L group successfully restored the reduction of TP and ALB in db/db mice. There was no significant difference between RC group and SC group.

The metformin group and all dietary interventions exerted a protective effect on the liver by improving the increased activity of ALT and AST. There was no significant difference between RC group and SC group. Among selenium-enriched Tartary buckwheat tea group, TBG-H group had the best effect, followed by TBG-M group and TBG-L group.

The occurrence of T2D is associated with increased lipid peroxidation and oxidative stress (Wu et al., 2016). As a significant biomarker of lipid peroxidation, MDA is a toxic byproduct of lipid oxidation. The level of MDA in the liver indirectly reflects the degree of hepatocyte damage (Si et al., 2017; Hu et al., 2017). CAT, T-SOD and GSH-Px are major antioxidant enzymes that play a central role in the body's antioxidant system (Si et al., 2017).

As shown in Fig.3E, compared with DC group, MDA level in PC group, TBG-L group, TBG-M group, TBG-H group, RC group and SC group decreased by 60.3%, 32.5%, 40.6%, 51.6%, 55.9% and 27.8%, respectively.

After severe oxidative stress occurred in db/db mice, the levels of CAT, T-SOD and GSH-Px were significantly increased by dietary intervention, that is, the dietary intervention of selenium-enriched Tartary buckwheat tea at high, medium and low doses, rutin and selenomethionine (Fig.3F, G, H), indicating that the intervention effectively improved the oxidative stress of db/db mice.

3.5 Pathological observation

In order to evaluate the effects of dietary intervention on liver tissue structure and glycogen storage in diabetic db/db mice, H&E staining was used to observe liver histology, and the contents of hepatic glycogen (Fig. 2D) and free fatty acids (Table 4) were simultaneously determined for combined analysis.

As shown in Fig.5, diabetic db/db mice in the DC group exhibited significant hepatic steatosis and hepatocyte necrosis, characterized by hepatocyte hypertrophy, cytoplasmic vacuolation, and loss of nucleus. The PC group showed effective improvement, and the liver morphology was similar to that of normal mice. After 4 weeks of treatment with selenium-enriched Tartary buckwheat tea, rutin and selenomethionine diet, the liver lesions were improved to varying degrees. In contrast, both hepatocyte hypertrophy and hepatic lipid accumulation were significantly reduced in RC group compared with SC, TBG-L, TBG-M, and TBG-H groups. Excess inflow of free fatty acids (NEFA) is an important cause of triglyceride accumulation in the liver and is associated with insulin re-
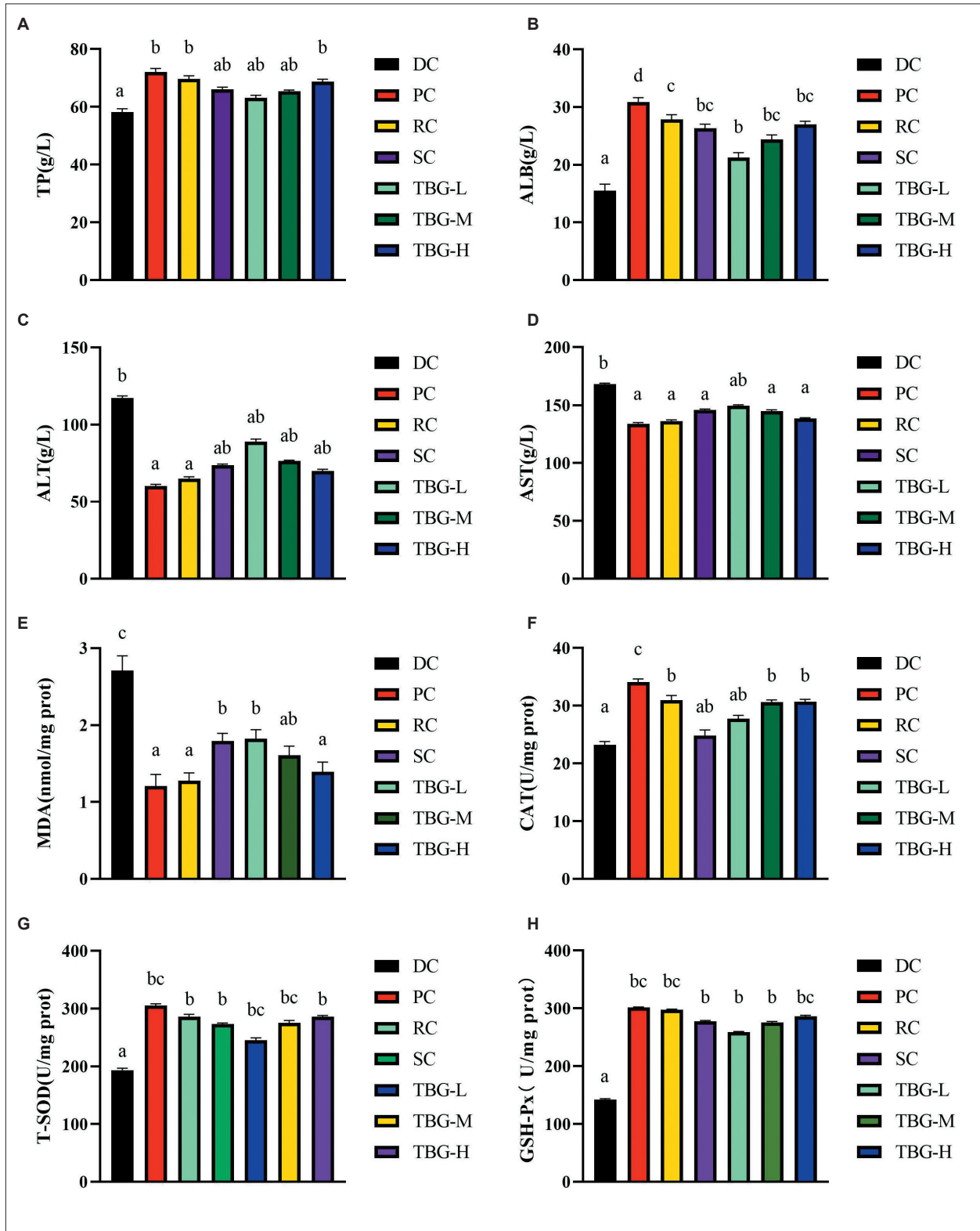
Fig. 3. *Effects of feeding on liver function and oxidative stress in db/db mice*
A TP level; B ALB level; C the activity of ALT; D AST activity; E MDA level; F CAT; G T-SOD; H GSH-Px (p < 0.05)

Table 4. *Effects of Tartary buckwheat tea, rutin and selenomethionine on blood lipid in db/db mice*

Groups	TC	TG	HDL-c	LDL-c	NEFA
DC	4.94±0.15a	1.95±0.09a	1.24±0.12e	1.17±0.05a	4.67±0.21a
PC	2.01±0.22f	1.34±0.06c	2.58±0.07a	0.48±0.09c	2.89±0.17d
RC	2.78±0.17de	1.41±0.11c	2.47±0.06a	0.59±0.03c	3.02±0.15d
SC	2.91±0.11d	1.55±0.08bc	2.11±0.11b	0.73±0.11b	3.36±0.15c
TBG-L	4.37±0.18b	1.67±0.13b	1.79±0.12d	0.85±0.03b	4.04±0.11b
TBG-M	3.69±0.16c	1.51±0.15bc	1.96±0.09bc	0.71±0.08b	3.83±0.13b
TBG-G	2.81±0.17d	1.42±0.08c	2.06±0.14b	0.66±0.06b	3.67±0.24c

Note: Different letters in the same row indicate the significant difference among groups ($p < 0.05$)

sistance (Hu et al., 2011). As can be seen from the changes of NEFA level in mice from different groups, metformin was significantly reduced after administration, 35.33% in RC group, 28.05% in SC group, 13.49% in TBG-L group and 17.99% in TBG-M group, respectively. TBG-H group decreased the NEFA by 21.41%, compared with DC group.

3.6 Glucose and lipids related gene expression

The liver plays a central role in maintaining blood glucose homeostasis. This function is closely related to two major processes: one is postprandial glycogen conversion and glucose uptake, and the other is fasting glucose production and glycogen and gluconeogenesis (Postic, 2004). As a key regulator of glucose homeostasis, PPAR-γ has been reported to promote the recovery of insulin sensitivity and has been suggested for the treatment of T2D (Fiévet et al., 2006). Activation of PPAR-γ can increase transcription of transporters such as GLUT4 and increase glucose uptake by the liver (Staels, 2005). The PI3K/Akt signaling pathway is a major insulin effector and has been reported to be involved in hepatic glycogen synthesis (Schultze et al., 2013; Zhu et al., 2016). PI3K is an insulin signaling intermediate, and its activation leads to Akt activation. Activated Akt can mediate glu-
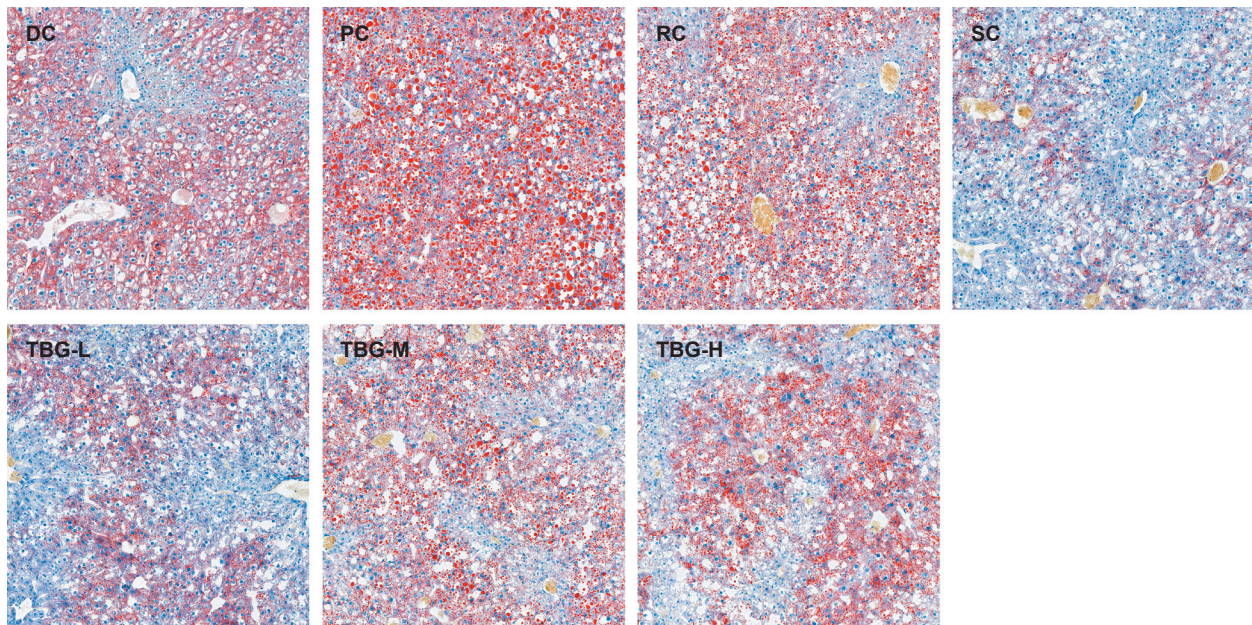

Fig.4. *Changes of liver histological structure in db/db mice during feeding*

cose metabolism in the liver, for example by stimulating glucose uptake and inhibiting gluconeogenesis (Manning et al., 2007). As the first enzyme in the glycogen synthesis pathway, GK promotes glucose phosphorylation and subsequently initiates glycogen synthesis (Agius et al., 2008). PEPCK is a rate-limiting enzyme in gluconeogenesis, which can be indirectly inhibited by activating Akt (Schultze et al., 2012). Down-regulation of PEPCK in db/db mice has been found to increase glycogen content in the liver (Zhou et al., 2015).

The mRNA expressions of Pparg, Slc2a4, Pik3r1, Akt and Gck were the lowest in the liver of DC group, and the expression of Pck1 was the highest. As shown in Figure 5A and B, dietary intervention (selenium enriched Tartary buckwheat tea, rutin, and selenomethionine) significantly increased the expression of Pparg and Slc2a4 (60S except) in the liver of db/db mice (p< 0.05), which may have a beneficial effect on increasing glucose uptake. Figure 5C and D showed increased expressions of Pik3r1 and Akt in the dietary intervention group
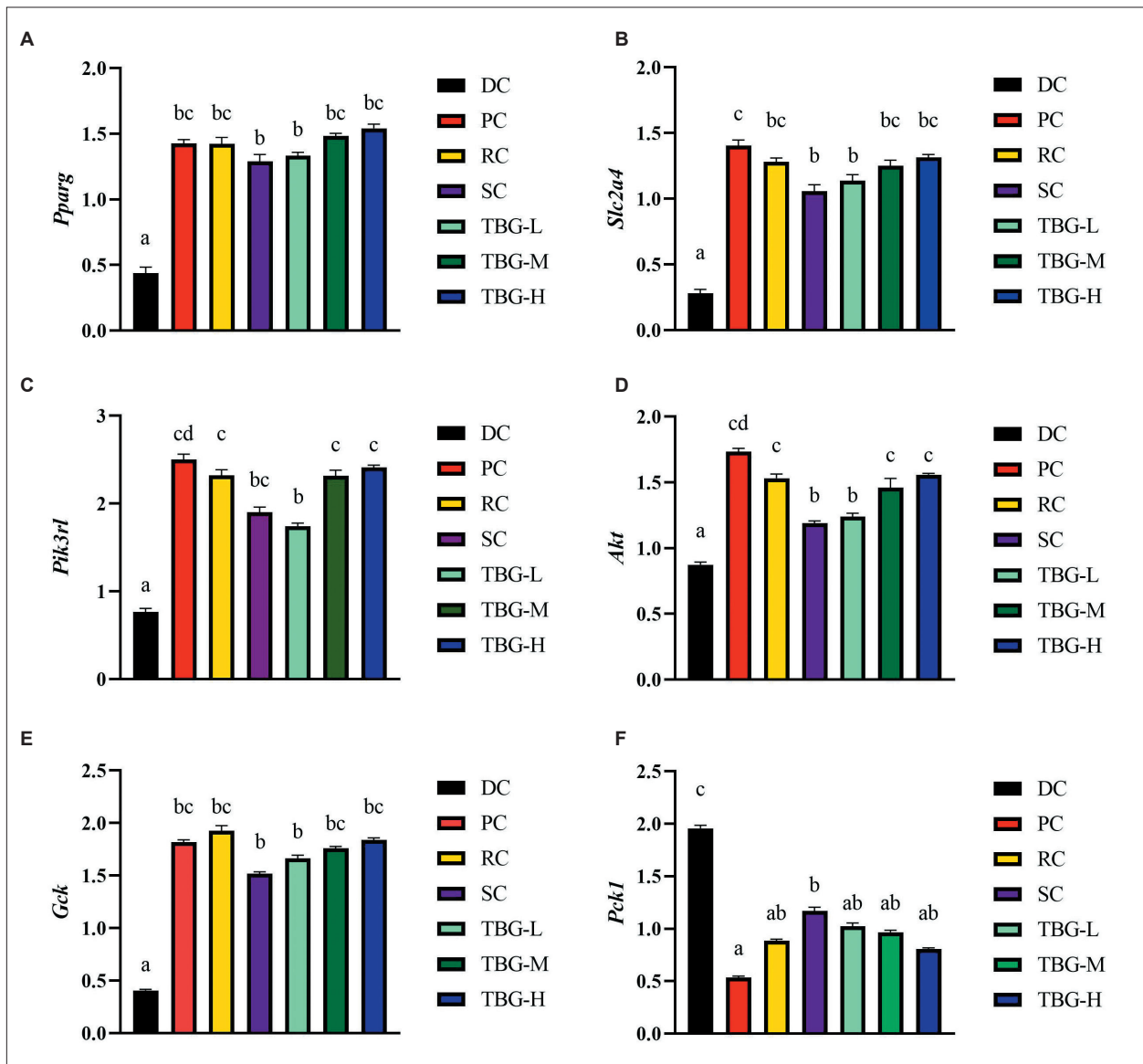

Fig. 3. *Effects of feeding on liver function and oxidative stress in db/db mice*
A TP level; B ALB level; C the activity of ALT; D AST activity; E MDA level; F CAT; G T-SOD; H GSH-Px (p < 0.05)

(Tartary buckwheat tea, rutin, and selenomethionine), suggesting that glucose metabolism may be mediated through the PI3K/Akt signaling pathway. Compared with the DC group, the relative mRNA expression of Gck in PC, RC, SC, TBG-L, TBG-M and TBG-H groups was increased by 3.21 times, 3.35 times, 2.97 times, 3.01 times, 3.11 times and 3.26 times, respectively (Figure 5E). As shown in Fig. 5F, PC, RC, SC, TBG-L, TBG-M, and TBG-H reversed the increased expression of Pck1 in the DC group. These results suggest that selenium-enriched Tartary buckwheat tea, along with rutin and selenomethionine, can mediate glycogen storage by increasing glycogen synthesis and inhibiting gluconogenesis in the liver of diabetic db/db mice.

Compared with DC group, all indexes in PC group, PC group and SC group effectively mediated the expression of related genes during gluconeogenesis. Among the selenium-enriched Tartary buckwheat groups, the effect of TBG-H group was the most obvious, which indirectly indicated that rutin and selenomethionine could play synergistic roles in gluconogenesis.

4 CONCLUSION

Different doses of selenium-enriched Tartary buckwheat tea and its active components rutin and selenomethionine have certain therapeutic effects on diabetic db/db mice. Feeding selenium-enriched Tartary buckwheat tea can improve the hyperglycemia, insulin resistance and hyperlipidemia of db/db mice. MDA levels in PC group, TBG-L group, TBG-M group, TBG-H group, RC group and SC group were decreased. The levels of CAT, T-SOD and GSH-Px in db/db mice were significantly increased after dietary intervention of selenium-enriched Tartary buckwheat tea with high, medium and low dose, rutin and selenomethionine, and the oxidative stress of db/db mice was improved. In addition, liver histopathological observation and hepatic glycogen measurement by H&E staining showed that selenium-enriched Tartary buckwheat tea, rutin and selenomethionine increased hepatic glycogen content in db/db mice. Analysis of mRNA expression related to glucose metabolism showed that selenium-enriched Tartary buckwheat tea can increase glycogen synthesis and inhibit gluconeogenesis, thereby mediating glycogen storage in the liver of diabetic db/db mice. TBG has a significant dose-response relationship for DM intervention in db/db mice according to all above parameters.

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IZVLEČEK

Učinek rutina in selenometionina v s selenom obogateni praženi tatarski ajdovi kaši na glukozo in metabolizem lipidov pri db/db miših

Vpliv rutina in selenometionina v s selenom obogatenem praženem čaju iz zrn tatarske ajde na glukozo in lipidni metabolizem pri diabetes II še ni raziskan. Pri tej raziskavi je bil uporabljen s selenom obogaten čaj tatarske ajde kot material za raziskavo in db/db miši kot poskusne živali. Kontrolna skupina z diabetesom (DC), pozitivna kontrolna skupina (PC), skupina z rutinom (RC), skupina s selenometioninom (SC), skupina z nizkim odmerkom selena obogatenim tatarskim ajdovim čajem (TBG-L), skupina s srednjim odmerkom (TBG-M) in skupina z visokim odmerkom (TBG-H) so bile vključene v poskus. Vpliv s selenom obogatenega tatarskega ajdovega čaja, rutina in selenometionina na glukozo in metabolizem lipidov so spremljali v db/db miših z biokemičnimi analizami krvi, analizo funkcije jeter, merjenjem oksidativnega stresa, spremljanjem histopatologije in določanjem glikogena ter ekspresije mRNK. S selenom obogaten čaj tatarske ajde in aktivni sestavini rutin in selenometionin vplivajo na znižanje vsebnosti glukoze in glukozne odpornosti pri db/db miših. Dislipidemija je bila delno izboljšana. Spremembe encimskih aktivnosti MDA, CAT, T-SOD in GSH-Px

v jetrih so nakazovale, da lahko TBG omili oksidativni stres jeter in pokaže aktivnosti zaščite jeter. S selenom obogaten čaj tatarske ajde, rutin in selenometionin lahko posredujejo k povečanju skladiščenega glikogena in zavirajo glukoneogenezo v jetrih diabetičnih db/db miši. Prav tako lahko učinkovito posredujejo k ekspresiji genov med glukoneogenezo. V skupini s selenom obogatene tatarske ajde je pri skupini TBG-H najbolj izrazit posreden vpliv na to, da lahko imata rutin in selenometionin pri glukoneogenezi sinergističen vpliv.