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The regulatory effect of total flavonoids of *Sedum aizoon* L. on oxidative stress in type 1 diabetic mice

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ABSTRACT

In this study, the optimal extraction conditions for the total flavonoids of *Sedum aizoon* L. (STF) were optimized by response surface methodology. Evaluation of the antioxidant in vitro of STF, and modulatory effects of glucolipid metabolism, and oxidative stress in mice with type 1 diabetes mellitus (T1DM). STF showed good antioxidant capacity in vitro. STF could improve glucolipid metabolism, organ coefficients, and antioxidant stress enzymes in T1DM mice effectively, reduce the damage to liver tissue, and regulate redox imbalance in the organism by modulating the Nrf2/Keap1/ARE signaling pathway. The results of this study could provide a theoretical reference for the application of *Sedum aizoon* L. in the development of auxiliary hypoglycemic functional foods and improvement of diabetes.

1. Introduction

Diabetes mellitus (DM) is a chronic disease. It has seriously endangered people's health. According to the latest report of the International DM Federation (IDF) 2021. There are approximately 537 million people who have DM worldwide. The number will increase to more than 693 million by 2045 (Gómez-Peralta et al., 2020). After two diseases, cardiovascular disease and cancer, DM is now the third "silent killer" in the world. The study found that the T1DM prevalence was between 0.15% and 28.98% among patients with a novel coronavirus (COVID-19) outbreak in 2019 (Nassar et al., 2021). Hyperglycemia has been observed to be an important factor in COVID-19-related complications. Therefore, it is very important for the treatment of T1DM. At present, more and more research is focusing on the treatment and prevention of diabetes through diet (Marunaka et al., 2020).

Sedum aizoon L. is a perennial herb of the genus Sedum in the family Sedum. Also known as Aizoon Stonecrop Herb (Wang et al., 2022). It is a medicinal and edible herb and contains a large number of flavonoids, alkaloids, and phenols. The content of flavonoids ranges from 1.13% to 4.08% (Li et al., 2011, 2017). *Sedum aizoon* L. has antibacterial, anti-inflammatory, hemostatic, and hypotensive effects (Wang et al., 2022; Xu et al., 2015). The study showed that flavonoids have a variety of biological activities, such as the treatment of cardiovascular disease, antibacterial, antioxidant, regulation of immunity, etc (Wang et al., 2020; Xu et al., 2015). Therefore, the study of flavonoids in *Sedum aizoon* L. has a certain value.

Oxidative stress is an important mechanism in the development of DM. It leads to a higher incidence of liver disease and worsening of liver lesions in DM patients. This is mainly attributed to the imbalance between reactive oxygen species and antioxidant defense mechanisms in DM patients (Ighodaro and Pharmacotherapy, 2018). In T1DM, a series of damages are usually generated against β-cells, which in turn induce β-cell death. DM aggravates the level of oxidative stress. Enhanced oxidative stress leads to the development of T1DM by damaging islet β-cells and reducing insulin sensitivity in peripheral tissues. Islet β-cells are sensitive to damage by ROS because of their relatively low content and activity of antioxidant enzymes, which can directly damage islet β-cells and activate many redox-sensitive signaling pathways, mainly TLRs/NF-κB and Nrf2/Keap1/ARE. Then cause the development of T1DM. High-glucose environment leads to excessive β-cell death by directly inducing mitochondrial damage (Giacco and Brownlee, 2012). It can be seen that oxidative stress mainly affects insulin secretion from pancreatic β-cells and causes an imbalance of the redox homeostasis in

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Abbreviations

STF	total flavonoids of Sedum aizoon L.
DM	Diabetes mellitus
T1DM	Type 1 Diabetes Mellitus
IDF	International DM Federation
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulphonate)
STZ	Streptozotocin
TC	Total Cholesterol
TG	Triglyceride;
T-SOD	Total Superoxide Dismutase
GSH	Glutathione Peroxidase
CAT	CATalase
MDA	Malondialdehyde
FBG	Fasting Blood Glucose
OGTT	Oral Glucose Tolerance Test
HE	hematoxylin-eosin
ANOVA	Analysis of variance
BCA	Bicinchoninic Acid

the body. This ultimately leads to exacerbation of T1DM development.

At present, the research on *Sedum aizoon* L. is mostly focused on its cultivation technology and antimicrobials (Liu et al., 2021; Xu et al., 2018). However, there was no optimization of its total flavonoid extraction process, antioxidant activity in vitro and in vivo, and hypoglycemic research. Therefore, in this experiment, the response surface methodology was used to optimize the optimal extraction conditions of total flavonoids (STF) of *Sedum aizoon* L.. To evaluate the antioxidant activity in vitro and the modulation of glucolipid metabolism and oxidative stress of STF in T1DM mice under these extraction conditions.

2. Materials and methods

2.1. Materials

Sedum aizoon L. lyophilized powder was provided from Heilongjiang Dezhao Agricultural Technology Co., Ltd..

The diammonium 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS); 2,2-diphenyl-1-picrylhydrazyl (DPPH); ascorbic acid were purchased from Yuanye Biotechnology Co. (Shanghai, China). Ferric sulfate ($FeSO_4$), potassium persulfate ($K_2S_2O_8$), and all other analytically pure reagents were purchased from Ruiyong Biotechnology Co. (Shanghai, China); Streptozotocin (STZ) (AR, purity≥98%) were purchased from Sigma-Aldrich Trading Co., Ltd, (Shanghai, China); Total Cholesterol (TC), Triglyceride (TG), Total Superoxide Dismutase (T-SOD), Glutathione Peroxidase (GSH), CATalase (CAT), Malondialdehyde (MDA) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China); Nrf2, HO-1, Keap1, NQO1 polyclonal antibodies were purchased from Abcam (Shanghai, China); Anti-β-actin polyclonal antibody were purchased from Boster Biological Technology Co.Ltd. (Wuhan, China).

2.2. One-factor experiment and response surface design

The lyophilized powder of *Sedum aizoon* L. with a certain concentration of ethanol solution. According to a certain liquid-material ratio, extraction temperature, and extraction time, extraction was carried out. Centrifugation was followed by alcohol precipitation and spin lyophilization to powder.

The single-factor test was carried out on the concentration of ethanol, the liquid-material ratio, the extraction temperature, and the extraction time during the extraction of total flavonoids. According to the results of the single-factor experiment, response surface experimental design using Design Expert 8.0.6 software. The ethanol concentration, liquid to material ratio, extraction temperature, and extraction time were used as the four experimental factors on the response surface. And according to the analysis method of response surface optimization, the process of extracting STF has four factors and three levels (Wang et al., 2021). The experimental factors and levels are shown in Table A.1. The determination of total flavonoids was carried out according to existing methods (Zhang et al., 2021).

2.3. Antioxidant properties in vitro

Extraction of STF was performed with the optimal extraction process optimized by response surface methodology. Vc was used as the positive control. DPPH·, $ABTS^{+}$·, ·OH, and total antioxidant capacity were used as indicators to analyze their antioxidant activities (Zhu et al., 2021).

2.4. Animal experiment

Male C57BL/6 mice (18–20 g, aged 6–7 weeks) were obtained from Changchun Yisi Laboratory Animal Technology Co., Ltd. All mice were acclimated for 7 days. They had free access to tap water and a diet under conditions of temperature (22 ± 2 °C) and a 12-h light-dark cycle. All animal experiments complied with the Arrival guidelines and carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

2.5. Establishment a mouse model of T1DM

Mice have fasted without water for 12 h (8:00 a.m.-8:00 p.m.). Fasting blood glucose (FBG) was measured and recorded. The mice fasted without water for 12 h the next day (8:00 p.m.-8:00 a.m.). Low-dose (50 mg/kg·BW) intraperitoneal injection of STZ for 4 consecutive days based on weight. High dose (100 mg/kg·BW) intraperitoneal injection of STZ on day 5 (Lu et al., 2022). Resume eating after 1 h of injection. After one week, FBG ≥11.1 mmol/L was considered a successful model. No mice died during modeling. A total of 30 models were successfully built.

Six mice were used as a blank control group (BC). The mice with successful modeling were randomly divided into five groups: negative control group (NC), positive group (PC), low-dose STF group (SL), medium-dose STF group (SM), and high-dose STF group (SH). BC and NC were given 200 mg/kg·BW of saline every day. PC were given 200 mg/kg·BW of metformin every day. SL, SM, and SH were given 50, 100, and 200 mg/kg·BW of STF. All groups were gavaged for 7 weeks.

2.6. Measurement of various indicators in mice

Determine the initial body weight of the mice. After starting the gavage, water intake, food intake, and weight measurement were measured and recorded at 9:00 a.m. every day. The final body weight was determined before the end of the experiment. Blood extraction from the eyeball. The supernatant obtained by centrifugation at 3000 rpm for 10 min is the serum sample, and it is stored at −20 °C for backup. The heart, liver, spleen, and kidney were taken and rinsed with saline. Then weighed and calculated organ coefficients.

The Oral Glucose Tolerance Test (OGTT) was performed on mice before the end of the experiment. The mice have fasted overnight for 12 h. Oral gavage of 40% glucose solution at a dose of 200 mg/kg·BW. Blood was collected from the tail vein at 30, 60, 90, and 120 min to measure blood glucose values (F. Li et al., 2020). Mice were sacrificed by cervical dislocation, and blood and organs were collected. Determination of TC, TG levels, T-SOD, MDA, GSH, and CAT levels.

2.7. HE staining

Immerse liver tissue in a 4% tissue fixative. Then it was paraffin-embedded, sectioned and HE stained. Sections were observed under a 400x microscope and photos were taken (Yuan et al., 2021).

2.8. Western blot analysis

After extraction of mouse liver tissue proteins, protein concentration was determined by the BCA protein concentration assay kit (Nanjing Jiancheng). Boiling to denature the protein. Proteins were separated by 12% SDS-PAGE gel electrophoresis and transferred to a PVDF membrane. Membranes were incubated with primary antibodies (Keap1, Nrf2, HO-1, and NQO1) overnight at 4 °C. The secondary antibody was incubated at room temperature for 2 h after washing 3 times with TBST. Imaging with gel imaging systems after development.

2.9. Statistics analysis

Statistical analysis graphs were done with GraphPad Prism 5.0 software and Design Expert 8.0.6 software. Protein strips were analyzed by Image J software. The data was expressed as mean ± SD. Significant differences between groups were determined by ANOVA. Significant differences between multiple groups were determined by One-Way ANOVA analysis with SPSS 23.0 software, $P < 0.05$ was considered a significant difference.

3. Results

3.1. Single-factor experimental results

The total flavonoid content was highest when the ethanol concentration was at 90% and showed a decreasing trend at 95% (Fig. 1a), presumably because of the principle of similar solubility. The total flavonoid was most similar to the 90% ethanol polarity, so its content reached its highest at this time. The content reached its highest point when the liquid to material ratio was 45:1 mL/g. Continuing to increase the amount of solvent, the total flavonoid content decreased (Fig. 1b). It is speculated that the reason may be that the flavonoids saturates at 45:1 mL/g. Continuing to increase the liquid-material ratio may lead to the precipitation of other impurities. (Hafsa et al., 2017; Huang et al., 2020). The total flavonoid content reached the maximum when the temperature was 75 °C. When the temperature was greater than 75 °C, the total flavonoid appeared to be degraded (Fig. 1c). In the range of 3–3.5 h, the content of STF increased slowly with the extension of extraction time, and the increasing trend was not obvious after the extraction time exceeded 3.5 h (Fig. 1d). Therefore, from the perspective of extraction effect and energy saving, 90% ethanol concentration, 45:1 mL/g liquid to material ratio, 75 °C and 3.5 h were selected as the best extraction parameters.

3.2. Results of optimizing the STF extraction process by response surface methodology

3.2.1. Experimental design and results

Using the Box-Behnken center combination to investigate the effects of four factors and three levels, namely ethanol concentration (A), liquid-to-solid ratio (B), extraction temperature (C), and extraction time

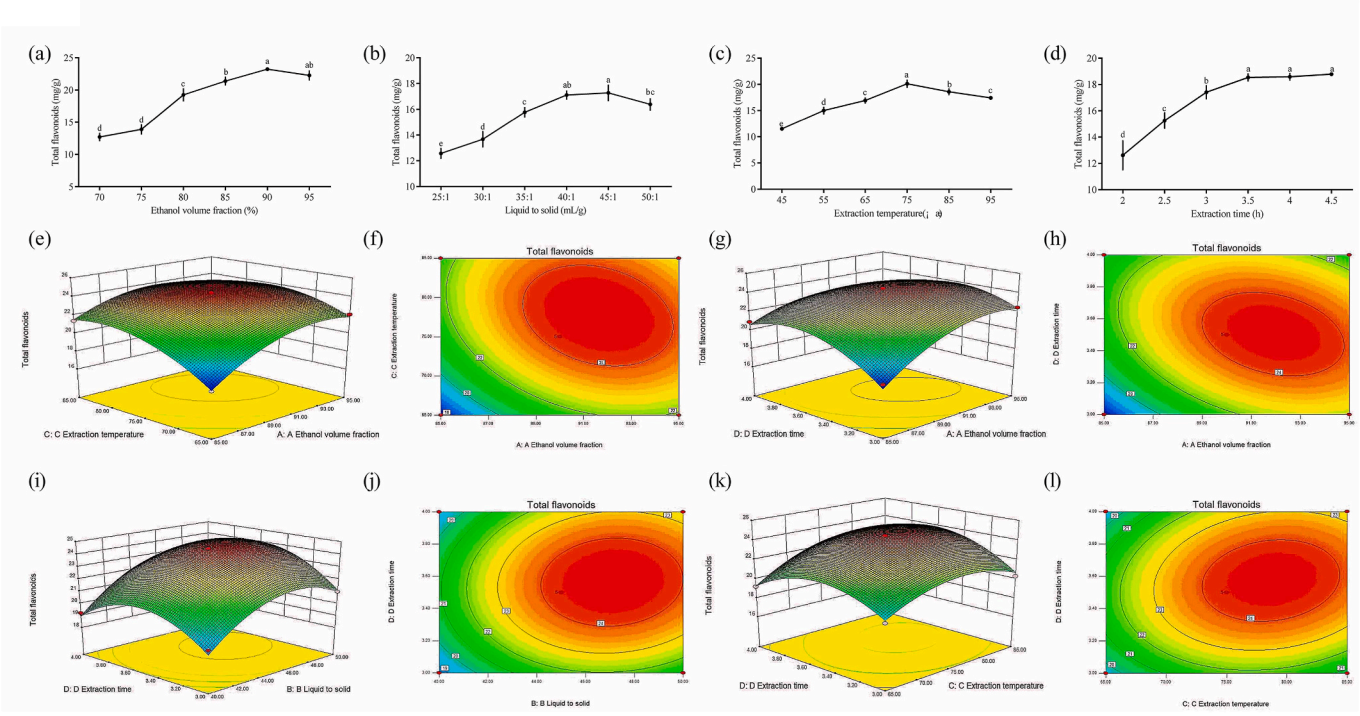

Fig. 1. Optimization of STF extraction process by single factor and response surface methodology. (a) Effect of ethanol concentration on STF; (b) Effect of liquid-solid ratio on STF; (c) Effect of temperature on STF; (d) Effect of time on STF; (e) Response surface map of the effect of ethanol concentration and extraction temperature on STF content; (f) Contour map of the effect of ethanol concentration and extraction temperature on STF content; (g) Response surface map of the effect of ethanol concentration and extraction time on STF content; (h) Contour map of the effect of ethanol concentration and extraction time on STF content; (i) Response surface map of the effect of liquid-to-solid ratio and extraction time on STF content; (j) Contour map of the effect of liquid-to-solid ratio and extraction time on STF content; (k) Response surface map of the effect of extraction temperature and extraction time on STF content; (l) Contour map of the effect of extraction temperature and extraction time on STF content. Different letters indicate significant difference at $P < 0.05$.

(D), on the yield of STF. A total of 29 sets of experiments containing five replicated center combinations were conducted. The experimental design and results are shown in Table A.2. The quadratic regression model between the STF extraction rate response value (Y) and each factor (A, B, C, D) is: $Y = -1208.63 + 15.747\ A + 6.858\ B + 3.826C + 91.182D\text{-}0.0042AB\text{-}0.0153AC0.411AD{+}0.0010BCE{+}0.108BD{+}0.1065C\ D\text{-}0.0686\ A^2\text{-}0.0744\ B^2\text{-}0.0165C^2\text{-}8.4077D^2$.

The regression analysis and ANOVA for this equation are shown in Table A.3. It can be seen from Table A3 that the quadratic polynomial fit model is extremely significant ($P < 0.0001$). This shows that the experimental design is reliable. The F value is 3.08, indicating that this model is extremely significant. The lack of fit $P = 0.1452 > 0.05$ is not significant. It shows that the model fits the experimental data well within the experimental range. The R^2 value of the fitting coefficient is 0.9807. It can be assumed that the model explains 98.07% of the variation in response values. The significance analysis of each experimental factor showed that: the P values of A, B, C, D, AC, AD, A^2, B^2, C^2, D^2 are less than 0.01. It shows that these factors have a very significant effect on the content of STF. In summary, there is not a simple linear relationship between STF content and extraction conditions. It can be seen that the model can be used to predict the content of STF.

3.2.2. Model accuracy analysis

The residuals and response design of the experimental data are shown in Fig. A1a. There are no abnormal data points in the graph. The experimental value of this experiment is very close to the predicted value (Fig. A1b). All data points are located between extreme values. The values of all leverage points are less than 1, in the center of the sample space (Fig. A1c). There is no valid error that exists in the experimental model (Fig. A1d). There was no significant effect on the regression coefficients in any of the 29 run data sets (H.Y. Shen et al., 2022). Fig. A1e and Fig. A1f shows that the value of Cook'D is in the determined range. Through the above regression diagnostic analysis, it shows that the STF extraction process model established in this experiment has high accuracy. It can be used to extract STF.

3.2.3. Response surface map and contour analysis

There is no significant interaction effect between AB and BC. And its interaction diagrams tend to be circular, so the interaction diagrams of AB and BC are not listed in this paper. It shows that the interaction between AC and AD is extremely significant (Fig. 1e-l). The effect of BD on STF content is more significant. But the interaction of BD was not significant. The effect of CD on STF was significant, and the interaction of CD was more significant.

3.2.4. Validation experiments

The optimum conditions were analyzed as 91.35% ethanol concentration, 47.06:1 liquid to material ratio, 78.04 °C extraction temperature, and 3.55 h extraction time, and the content of STF was 24.97 mg/g under these conditions. Five parallel verification tests were performed using the optimal process parameters. The test result was measured at 25.03 ± 0.08 mg/g. The difference with the analysis value of the built model is small, indicating that the model's analysis is satisfactory.

3.3. Antioxidant activity in vitro

It can be seen from Fig. 2a–c, the clearance of DPPH·, $ABTS^{+}$· and ·OH of STF increased with increasing concentration. At the concentration of 2.0 mg/mL, the clearance rate of DPPH· of STF and VC were 85.94 ± 0.35% and 98.76 ± 0.33%. The clearance rate of ·OH were 75.30 ± 1.28% and 99.56 ± 0.24% respectively. At a concentration of 0.3 mg/mL, the clearance rate of $ABTS^{+}$· were 96.21 ± 0.48% and 98.99 ± 0.67% respectively. Fig. 2d shows the total antioxidant capacity of STF is positively correlated with the concentration range. The FRAP values of STF and Vc were up to 0.225 ± 0.03 and 0.242 ± 0.02 mmol/L in the measurement range. It can be seen that STF has good antioxidant activity and total antioxidant capabilities.

3.4. Effect of STF on basal indices in mice

At the 8th week of the experiment (the 7th week of gavage), the initial final body weight, total food and water intake, body weight gain, and food titer of mice in each group before and after the experiment

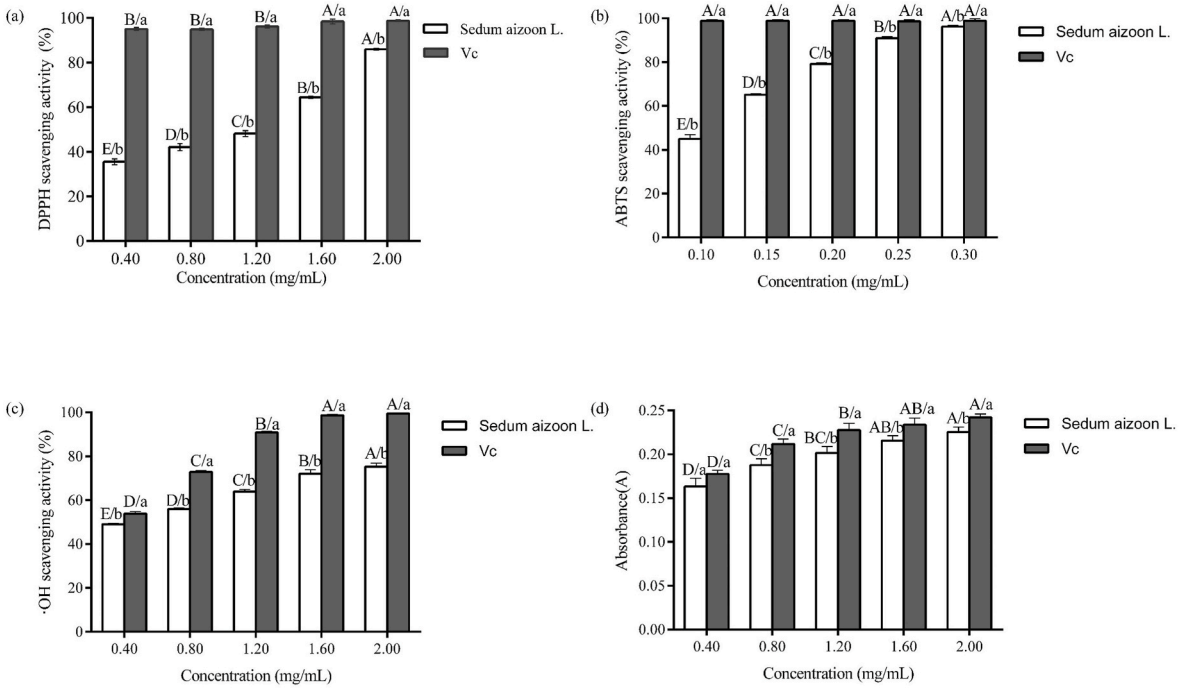

Fig. 2. Antioxidative activity of STF in vitro (a) Scavenging ability of STF to DPPH·; (b) Scavenging ability of STF to $ABTS^{+}$·; (c) Scavenging ability of STF to OH; (d) Total resistance of STF Oxidative capacity. Different letters indicate significant difference at $P < 0.05$.

were counted throughout the experimental period. As shown in Fig. 3a–d, before the end of the experiment, the body weight of mice in PC, SH, SM and SL groups were significantly higher than that in NC group ($P < 0.05$). Compared with the NC group, the total water intake and total food intake in the treatment group were reduced to different degrees. The weight gain of the NC group was significantly lower than that of the other groups ($P < 0.05$). There is a dose dependence of food potency in the SL, SM, and SH groups. This indicates that STF can significantly restore body weight gain in T1DM mice. Improve food utilization in T1DM mice effectively. Both the body weight and the dietary status of the mice improved. As shown in Fig. 3e, before the mice were molded, there was no significant difference in FBG among all groups ($P > 0.05$). The FBG of the model group reached its highest in the third week. After 7 weeks of STF gavage, the FBG in the PC group and SH group were significantly lower than those in the NC group ($P < 0.05$). As can be seen in Fig. 3f and g, compared with the BC group. The levels of OGTT significantly decreased in the NC group. The area under the curve increased significantly. It indicated that STF could regulate glucose metabolism in T1DM mice.

3.5. Effect of STF on organ coefficients in mice

The effect of STF on the organ coefficient of mice is shown in Table 1. It indicated liver and kidney enlargement in the NC group because of the significantly higher coefficients for both liver and kidney in the NC group. While the two coefficients in the drug-treated group decreased significantly. The state of its organs has been significantly relieved.

3.6. Effects of STF on lipid metabolism, oxidative stress and liver pathology in mice

The main risk factors for complications in T1DM patients are high levels of TC and TG. In the NC group, the levels of TC and TG were significantly increased ($P < 0.05$). However, STF can significantly reduce the levels of TC and TG ($P < 0.05$) (Fig. 4a and Fig. 4b). This indicates that STF can improve abnormal lipid metabolism in T1DM mice.

The islet β-cell damage in mice is due to STZ induction. Eventually, leading to DM. Oxidative stress is caused by excess free radicals in the body, resulting in damage to the antioxidant defense system. The production of reactive oxygen species reduces the levels of antioxidant enzymes such as SOD, GSH, and CAT, and induces oxidative stress. The structure and function of the cells are altered (Wu et al., 2021). MDA is a

Table 1
The effect of STF on organ coefficient of mice(n = 6) (%).

group\project (w/w, %)	Heart	liver	spleen	kidney
BC	0.65 ± 0.03^{a}	4.24 ± 0.08^{d}	0.21 ± 0.01^{a}	1.24 ± 0.01^{e}
NC	0.56 ± 0.01^{cd}	5.92 ± 0.07^{a}	0.19 ± 0.00^{a}	1.74 ± 0.02^{a}
PC	0.53 ± 0.01^{d}	5.53 ± 0.11^{c}	0.21 ± 0.01^{a}	1.47 ± 0.03^{d}
SL	0.62 ± 0.03^{ab}	5.78 ± 0.08^{b}	0.19 ± 0.01^{a}	1.62 ± 0.02^{b}
SM	0.62 ± 0.02^{ab}	5.59 ± 0.07^{c}	0.21 ± 0.01^{a}	1.61 ± 0.01^{b}
SH	0.60 ± 0.02^{bc}	5.57 ± 0.07^{c}	0.18 ± 0.01^{a}	1.53 ± 0.02^{c}

Note: Different letters indicate significant difference at $P < 0.05$.

product of lipid peroxidation. It can be seen from Fig. 4c–f that compared with the BC group, the activities of T-SOD, GSH, and CAT in the NC group were significantly decreased ($P < 0.05$). It indicates that the liver of T1DM mice is subjected to some oxidative damage. Compared with the NC group, the activities of T-SOD, GSH, and CAT in the PC group, SH group, and SM group were significantly increased ($P < 0.05$). However, the MDA content was significantly reduced ($P < 0.05$). It shows that STF can alleviate the level of lipid peroxidation to a certain extent, and reduce free radical production in the body to enhance the body's antioxidant capacity.

The results of HE staining of liver tissue are shown in Fig. 4g. Hepatocytes in the normal group of mice showed a radial distribution. And the nucleus is complete and round. Some nuclei shrink and disappear with the STZ induction. A large number of lipid droplets appear in hepatocytes. A small amount of particle degeneration after drug intervention. The liver cords are more neatly arranged and the fat vacuoles are significantly reduced. It indicates that STF has a protective effect on the liver of diabetic mice.

3.7. Effects of STF on the expression of HO-1, NQO1, Keap1 and Nrf2

HO-1 is a stress-responsive enzyme. It can produce antioxidants and relieve oxidative stress (Xiong et al., 2022). As a member of endogenous enzymes, NQO1 is the downstream target protein of Nrf2. In the experiment, we found that the relative expressions of HO-1, NQO1 and Nrf2 proteins were significantly elevated in the SM, SH, and PC groups

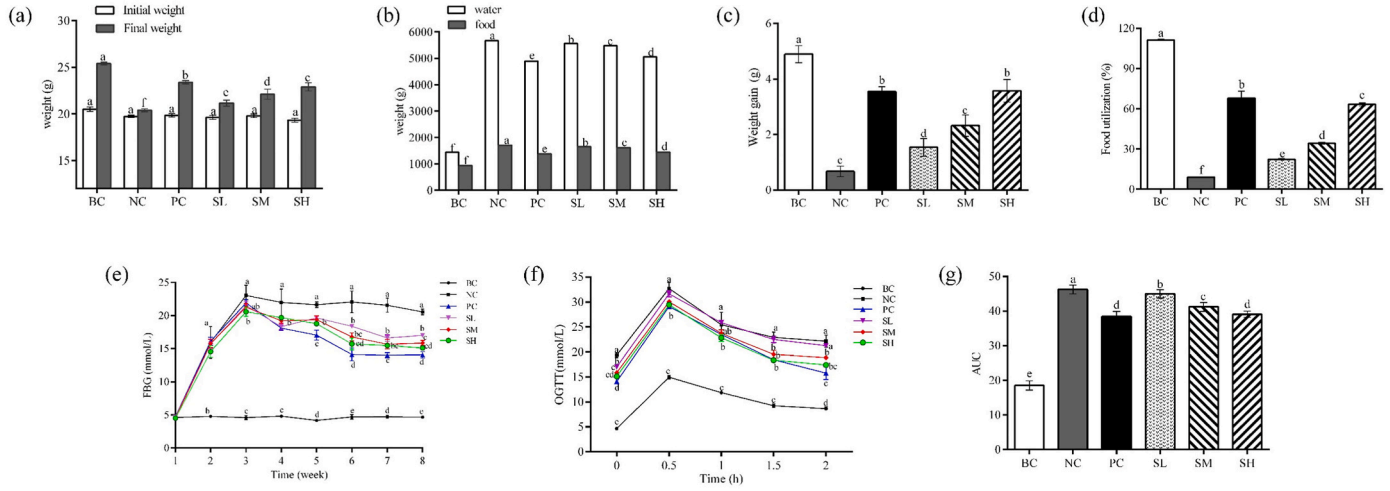

Fig. 3. The effect of STF on basic indicators of T1DM mice (n = 6) (a) Body weight; (b) Food and water intake; (c) Body weight gain; (d) Food titer; (e) FBG (fasting blood glucose); (f) OGTT (Oral Glucose Tolerance Test); (g) Area under the OGTT curve. BC, blank control group; NC, negative control group; PC, positive group; SL, low-dose STF group; SM, medium-dose STF group; SH, high-dose STF group. Different letters indicate significant difference at $P < 0.05$.

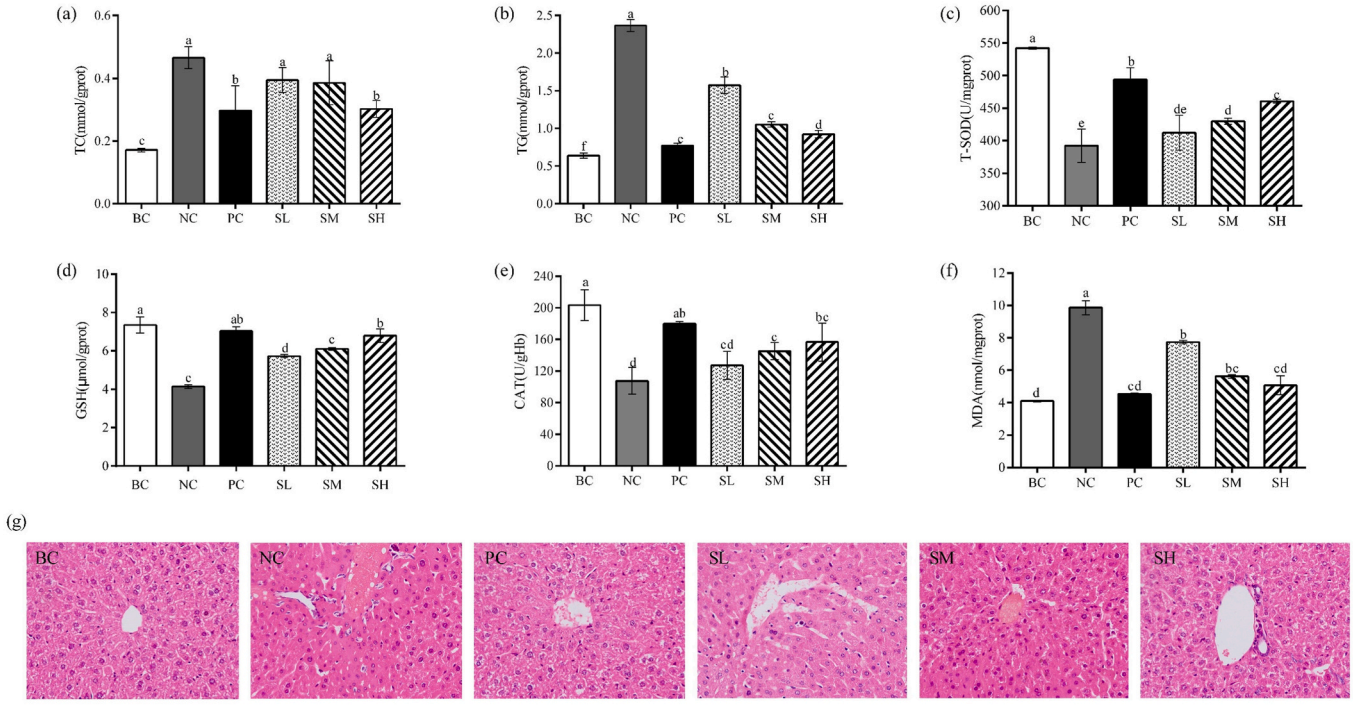

Fig. 4. Effects of STF on lipid metabolism, oxidative stress and liver histopathology in T1DM mice (n = 6) (a) Content of TC; (b) Content of TG; (c) Content of T-SOD; (d) Content of GSH; (e) Content of CAT; (f) Content of MDA; (g) HE staining of liver. BC, blank control group; NC, negative control group; PC, positive group; SL, low-dose STF group; SM, medium-dose STF group; SH, high-dose STF group. Different letters indicate significant difference at $P < 0.05$.

compared with the NC group ($P < 0.05$). The expression of Keap1 in the SH group was significantly decreased ($P < 0.05$), and there was no significant difference with the BC group ($P > 0.05$) (Fig. 5a–e). HO-1 and NQO1 are downstream target proteins regulated by Nrf2. The expression levels of HO-1, NQO1, and other antioxidant proteins are important indicators to judge whether the Nrf2 system pathway is activated in vivo and the degree of activation (Li et al., 2019, 2020; S. Li et al., 2020). In the experiment, the levels of HO-1 and NQO1 in the livers of mice increased significantly when treated with STF. This indicates that the Nrf2 pathway is activated. It indicates that the Nrf2 pathway is activated, thereby regulating the expression of downstream proteins.

4. Discussion

DM is a complex disease. T1DM is the most common autoimmune disease in the world. The risk of developing DM is mainly determined by genetic and environmental factors (Carlos et al., 2017; Teng et al., 2016). The concept of "medicine and food homology" is explained in ancient books: "Eat as food on an empty stomach, and eat as medicine for the patient." (Liu et al., 2018) Compared with modern western medicine, "Medicinal food" herbs can regulate the body. It is beneficial for absorption, also has a health care function, and non-toxic side effects.

Studies have found that *Sedum aizoon* L. contains polysaccharides, flavonoids, gallic acid, and other phenolic acids. It has anti-bacterial, anti-inflammatory, anti-swelling, and detoxifying effects (Liu et al.,

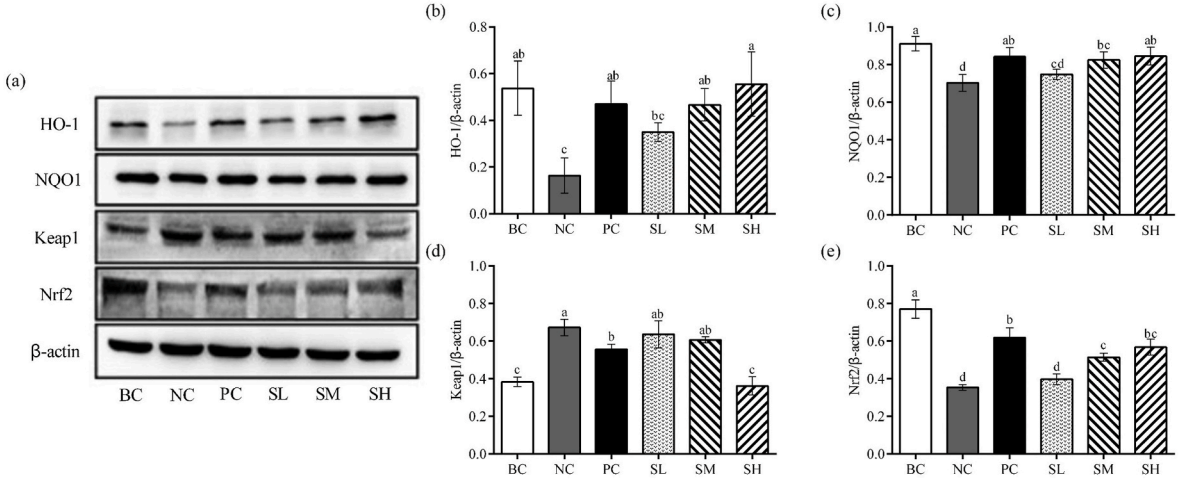

Fig. 5. Effects of STF on Nrf2/Keap1/ARE protein expression (n = 6) (a) Bar graph; (b) Expression of HO-1; (c) Expression of NQO1; (d) Expression of Keap1; (e) Expression of Nrf2. BC, blank control group; NC, negative control group; PC, positive group; SL, low-dose STF group; SM, medium-dose STF group; SH, high-dose STF group. Different letters indicate significant difference at $P < 0.05$.

2017). Flavonoids are polyphenolic compounds. It is important in protecting plants from UV energy, microbial and oxidative stress. Numerous studies have shown that flavonoids have good antioxidant and free radical scavenging abilities (N. Shen et al., 2022). Studies have found that flavonoids can protect islet β cells by resisting oxidative stress and preventing apoptosis (Xya et al., 2021). Mohamed S found that flavonoids in basil extract can ease insulin levels in diabetic animals and hypoglycemic (Mohan et al., 2014). It can attenuate liver and kidney dysfunction, inflammatory and apoptotic responses, and improve lipid peroxidation at the same time. The analysis of hypoglycemic kinetics in the study by Yuan et al. showed that the flavonoid extract of water chestnut has a good hypoglycemic effect in in vitro experiments (Yuan et al., 2022). It shows a significant inhibitory effect on both α-amylase and α-glucosidase. At present, there are few analyses of the active ingredients and biological active ingredients of *Sedum aizoon* L.. In particular, there is less in-depth research on the flavonoids of *Sedum aizoon* L., and the potential mechanism of hypoglycemic activity of its flavonoids is still unclear.

Oxidation reaction in the whole life process. Excessive oxidative stress is a key to deepening DM symptoms. Therefore, it can prevent the development of DM by resisting it effectively (An et al., 2018). Liver damage induced by diabetes can be reversed by inhibiting oxidative stress (Agunloye and Oboh, 2021; Chen et al., 2020). The excess ROS will deplete biological processes and damage the cell's mitochondrial system. And reduced the activity of oxidative enzymes in cells ultimately. This may lead to an increase in MDA content. And reduced the total antioxidant capacity of the body (Zhou et al., 2019). On the other hand, MDA would cause a cross-linking reaction, and disrupt the cell structure. Eventually, abnormal metabolism in the body leads to cell death. GSH, SOD, and CAT are the key enzymes for scavenging reactive oxygen species (Gao et al., 2018). It has been suggested that the mechanism of protection against diabetes may through upregulated SOD and GSH enzyme activity in the liver and inhibition of MDA activity (Chen et al., 2019). The results of this experiment show that STF can increase the activities of CAT, T-SOD, and GSH in T1DM mice, and decrease MDA production. Immediately reduce the levels of TC and TG. This suggests that STF can enhance antioxidant enzyme activity and reduce oxidative stress.

The liver is an important site of lipid metabolism in the body. If there is a disorder of lipid metabolism, if there is no timely treatment, steatosis will appear in the early stage, and there will be fat vacuoles in the liver. Liver fibrosis and hepatitis will further develop. In severe cases, deterioration, such as cirrhosis or liver cancer (Klover et al., 2004). The results of this experiment show that with the high-dose STF intervention, there is a small amount of granular degeneration in the livers of mice, the fat vacuoles are significantly reduced, and the liver damage is alleviated. It can indirectly indicate that STF has a protective effect on the liver of diabetic mice.

Activation of Nrf2/Keap1 signaling is a key cellular defense mechanism for managing oxidative stress. In DM, the accumulation of ROS leads to the degradation of the body's antioxidant defense system. ARE is a specific DNA sequence in the promoter region of the Nrf2 target genes. It is required for Nrf2 binding and gene induction (Baird et al., 2020). Keap1 is a component of the Cullin3-based ubiquitin E3 linker that functions as a substrate articulator. HO-1 and NQO1 are the downstream target proteins of Nrf2. Up-regulation through transcription factor binding to the ARE in response to oxidative damage. In a study by Song et al. shows that the mRNA and protein levels of Nrf2 and HO-1 in diabetic rats will be upregulated by blueberry anthocyanins gavage (Yu et al., 2016). Nrf2/HO-1 signaling pathway will be activated, and retinal oxidative stress in diabetic rats will be inhibited. It is well known that the Nrf2 transcription factor is tightly regulated by Keap1 in the cytoplasm. To counteract oxidative stress, Nrf2 dissociates from Keap1 and then translocates to the nucleus, where it binds sequentially to the ARE to regulate enhancer regions. Liu's study indicates that reduction of oxidative stress in rats is associated with activation of the Keap1/Nrf2 signaling pathway (Liu et al., 2022). Therefore, the effect of STF treatment in reducing oxidative damage in this study may be mediated through activation of Nrf2/Keap1 signaling. Its intervention can differentially reverse the increase in Keap1 expression induced by STZ. Simultaneously increased the expression of nuclear Nrf2 in cells, and stimulate the nuclear translocation of Nrf2, up-regulate the protein expression of HO-1 and NQO1, and promote the recovery of antioxidant enzymes. It was consistent with previous studies by others (Kaludercic and Di, 2020; Qin et al., 2019).

5. Conclusion

The results of this study showed that the improvement of oxidoreductase levels in vivo in a dose-dependent manner after STF intervention. It also restores the antioxidant defense system in the liver. The recovery of GSH, SOD, and CAT and the reduction of MDA are important evidence of the protective effect of STF. The histopathological evaluation further confirmed that the symptoms of oxidative stress in T1DM improved significantly, which was induced by STZ through STF treatment. *Sedum aizoon* L. is a medicinal and edible herb without toxicity. In addition to the administration of drugs for the conventional treatment of DM, STF supplementation can be a new strategy to against hyperglycemia. It is worth mentioning that using STF is a promising approach to preventing and treating DM. It can be a drug with the ability to both assist in hypoglycemia and reduce the level of oxidative stress in the body caused by DM.

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CRediT authorship contribution statement

Xin Qi: Data curation, Writing – review & editing. **Xin-tong Lu:** Writing – original draft, Visualization, Investigation. **Xi-han Sun:** Conceptualization, Methodology, Software, Validation. **Cheng-bi Cui:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2022.06.010.

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