



Study on hypoglycemic effects of irradiated ginseng adventitious roots

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ABSTRACT

We aimed to explore the effects of the ⁶⁰Co- γ irradiated ginseng adventitious root (GAR) with different radiation doses on the hypoglycemic effects of its extract (GARSE) through in vivo and in vitro experiments. The total saponin of GARSE was increased by 4.50% after 5 kGy irradiation, and the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability was enhanced by 5.10%. At 50 μ g/mL, GARSE irradiated by 5 kGy displayed superior protective effects on human glomerular mesangial cells (HMCs) with high glucose damage. After feeding type 1 diabetes mellitus (T1DM) mice with GARSE irradiated by 5 kGy at 500 mg/kg-BW for 4 weeks, the glucose values was decreased by 16.0% compared with the unirradiated. The Keap1/Nrf2/HO-1 pathway was activated and the oxidative stress was attenuated, which further alleviated T1DM.

Introduction

The number of people with diabetes, one of the most prevalent chronic non-communicable epidemics of the 21st century, reached approximately 463 million worldwide as of 2019, and the prevalence is expected to rise to 578 million by 2030 (Saeedi et al., 2019). Type 1 diabetes mellitus (T1DM) is an autoimmune disease whose pathogenesis includes the abnormal attack of autoimmune antibodies and immune cells on pancreatic islet β -cells, which could cause a reduction or loss of function, thus resulting in abnormally high blood glucose (Szablewski, 2014). On the other hand, type 2 diabetes mellitus (T2DM) is affected by the body's inability to use insulin effectively, with various manifestations, including reduced insulin sensitivity, insulin resistance, and ultimately elevated blood glucose values (Halim and Halim, 2019). T1DM is the main type of diabetes mellitus in children and adolescents, and once being developed, it not only affects the growth and development of patients, but also requires lifelong dependence on medication. However,

none of them can fundamentally restore the islet function, and lifelong medication increases the risk of chronic complications as well (Sikor-skaya et al., 2021). Therefore, it is worthwhile to develop functional foods of medicinal and dietary origin in daily diet to assist the treatment and alleviation of T1DM in adolescents.

Ginseng, as a natural health food, is rich in saponins, polysaccharides, and other active ingredients with pharmacological activities such as antioxidant and hypoglycemic, which could make it a more ideal functional diet to help the treatment of diabetes (Ju et al., 2020). By lowering blood glucose, improving glucose tolerance, increasing insulin sensitivity, improving the body's resistance to oxidative stress, protecting against kidney damage, and regulating blood lipids, Saponins, the main substances in ginseng, play a hypoglycemic role, which can prevent and treat diabetes and its complications (Ratan et al., 2021). Wild ginseng has a long growth cycle, so tissue culture has become a potential way to produce valuable plants for its unique advantages such as high yield, ease of control and short growth period. Ginseng

Abbreviations: GAR, Ginseng Adventitious Root; GARSE, Ginseng Adventitious Root Saponins Extract; HMCs, Human Glomerular Mesangial Cells; STZ, Streptozotocin; OGTT, Oral Glucose Tolerance Test; ABTS, 2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonate); DPPH, 2,2-Diphenyl-1-picrylhydrazyl; PTIO, 2-phenyl-4,4,5,5-tetramethylimidazole-1-oxyl-3-oxide; TC, Total Cholesterol; TG, Triglycerides; GHb, Glycosylated Hemoglobin; DMEM, Dulbecco's Modified Eagle Medium; T-SOD, Total Superoxide Dismutase; MDA, Malondialdehyde; CAT, Catalase; ROS, Reactive Oxygen Species; GSH, Micro Reduced Glutathione; T1DM, Type 1 Diabetes Mellitus; FBS, Fatal Bovine Serum; PBS, Phosphate Buffered Solution; CCK-8, Cell Counting Kit-8; BCA, Bicinchoninic Acid; AGEs, Advanced Glycation End Products.

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adventitious roots (GAR) are induced by the stimulation of external factors, such as hormones and pathogenic microorganisms, which manifests as a regenerative response of the plant (Wang et al., 2020). The plant defense mechanisms of ginsenosides are activated in response to external stimuli to promote their synthesis. Some studies have confirmed that the pharmacological activity and bioactive substance contents of GAR are much higher than that of natural ginseng (Yao et al., 2020). Meanwhile the GAR extract at dosage levels of 250 and 500 mg/kg.BW significantly lowered the blood glucose, total cholesterol and triglyceride content in streptozotocin-induced diabetic rats (Murthy et al., 2014).

In order to avoid microbial contamination of GAR during tissue culture and storage, they need to be sterilized. Food irradiation refers to a safe new low-carbon and energy-saving non-thermal food processing technology, and the process of physical or chemical reaction of food components or microorganisms after food or raw materials are subjected to high-energy and high-penetrating specific doses of non-ionizing radiation (Pan et al., 2021). This process can effectively inhibit metabolism; inactivate microorganisms, viruses and bacteria, achieve food sterilization and extend shelf life, and effectively maintain the nutrient content, freshness and flavor of food (Bisht et al., 2021). $^{60}\text{Co-}\gamma$ ray irradiation is a common method of food irradiation. In 2003; the Codex Alimentarius Commission announced that irradiation doses for any food are safe under current technological conditions (Roberts, 2016). A study confirmed that gamma irradiation can be an effective method for the conversion of the ginsenoside Rb1 to Rg3 (Kim et al., 2012). The combination of mutation technique by γ -irradiation and plant regeneration by tissue cultures may be an effective way to ginseng improvement (Zhang et al., 2014). In addition; the total phenolic contents and total flavonoid contents in raw pine pollen were irradiated by 6 kGy $^{60}\text{Co-}\gamma$ radiation increased by 16.90% and 14.66%; respectively (Cheng et al., 2021). Cherry tomatoes irradiated by 1 kGy $^{60}\text{Co-}\gamma$ radiation exhibited a significant increase in the total carotenoids and vitamin C (Mendes et al., 2020). It can be seen that ginsenosides and other active ingredients were affected by irradiation.

However, few studies reported the application of irradiation technology to GAR, thus the value of the contribution of irradiation to the active components of ginseng adventitious roots was underestimated, as well as the effect of irradiation on the hypoglycemic effect of GAR was ignored. Therefore, the aim of this study was to irradiate ginseng adventitious roots with different doses of $^{60}\text{Co-}\gamma$ rays and analyze the changes of the total saponin after irradiation. The *in vitro* and *in vivo* experiments combined with Keap1/Nrf2/HO-1 pathway were adopted to explore the antioxidant and hypoglycemic effects of ginseng adventitious roots, which provides a reference for further development and utilization of ginseng adventitious root resources, and provides new ideas for the application of irradiation technology in GAR.

Materials and methods

Preparation of GARSE with different irradiation doses

GAR was provided by Dalian Academy of Agricultural Sciences, and irradiated by Sichuan Institute of Atomic Energy with $^{60}\text{Co-}\gamma$ ray source. The irradiation doses were set at 0, 5, 10, 15 and 20 kGy. The preparation method of Sun-Ja Kim et al. was referenced and improved by us (Kim et al., 2007). The GAR was crushed and sieved through 80 mesh. The obtained powder was soaked overnight in 70% ethanol at 1:10 (material-to-liquid ratio), and extracted by ultrasound at 100 Hz for 30 min at 60 °C. The supernatant was collected by filtration, and the precipitate was extracted twice. The filtrate was mixed and evaporated to dryness by reduced pressure distillation. Distilled water was added to dissolve it. Then, it was washed for three times with 50 mL ether and extracted three times with 50 mL water-saturated *n*-butanol. The *n*-butanol extract was evaporated to dryness by reduced pressure distillation. Finally it was dissolved in distilled water, and lyophilized. The

GAR saponin extract (GARSE) was obtained.

Determination of total saponins

The total saponin was determined using UV spectrophotometer (Zhang et al., 2012). The standard ginsenoside Re (Yuanye Biotechnology Co. Ltd., Shanghai, China) was dissolved in methanol to prepare a 1 mg/mL reference solution. The reference solution was extracted by gradient, and the solvent was dried to be removed. A total of 0.5% of 1% vanillin solution was added to the solution, and it was immediately cooled with ice water after 15 min of constant temperature water bath at 60 °C. Next, 5 mL 77% sulfuric acid solution was added, and well shaken to measure absorbance at 540 nm and draw standard curve. Subsequently, 50 mg of GARSE was weighed and placed in a 25 mL volumetric flask. Methanol was added to a constant volume until the scale was shaken, and 50 μL was accurately absorbed and dried. Most importantly, the above steps were repeated. The absorbance obtained was calculated in the standard curve.

DPPH activity

Under dark conditions, 3.9432 mg 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (TCI, Shanghai, China) reagent was weighed and dissolved in 100 mL methanol to obtain DPPH reserve solution. Different GARSEs were diluted with 70% ethanol gradient to obtain samples for testing, and 180 μL DPPH reserve solution was added to 20 μL samples. The shaker reacted at 450 rpm for 20 min, and then the absorbance values of each group at 517 nm wavelength were measured. The experiment was repeated three times (Nickel et al., 2016).

ABTS activity

Totally, 180 mg 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) (Yuanye Biotechnology Co. Ltd., Shanghai, China) was dissolved in 50 mL distilled water, and 33 mg potassium persulfate was added. After being mixed, the ABTS reserve solution was prepared by adjusting the OD value of the solution at 415 nm wavelength to 0.70 ± 0.02 at room temperature and dark for 12–24 h. Different GARSEs were diluted with 70% ethanol gradient to obtain samples for testing. Then, 270 μL ABTS reserve solution was added to 30 μL samples, and the absorbance values at 405 nm wavelength were measured after 10 min reaction. The experiment was repeated three times (Hwang et al., 2014).

PTIO activity

First, 0.05 g/L 2-phenyl-4,4,5,5-tetramethylimidazolin-1-oxyl-3-oxide (PTIO) (TCI, Shanghai, China) reserve solution was prepared by dissolving PTIO in PBS. Different GARSEs were diluted with 70% ethanol gradient to obtain samples for testing. Afterwards, 0.2 mL samples were added with 1.8 mL phosphate buffered solution (PBS), and the absorbance values of each group at 557 nm wavelength were measured at 37 °C for 3 h. The experiment was repeated for 3 times (Li et al., 2019).

Cell culture and treatment

Human glomerular mesangial cells (HMCs) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured in dulbecco's modified eagle medium (DMEM) (Gibco Life Technologies, Grand Island, US) medium containing a mixture of 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin Solution (Solarbio, Beijing, China) and placed in a 5% CO_2 cell incubator at 37 °C.

The cells were treated with the gradient DMEM of 10–50 mmol/L glucose for 24 h. Then, the cells cultured with normal DMEM (containing 5.5 mmol/L glucose) were used as controls to determine the high glucose-induced concentration. Normal DMEM was used to dissolve

different GARSE, and the cells were treated with gradient medium of 25–100 µg/mL for 24 h, aiming to determine the drug concentration. The concentrations have been determined by pre-experiments.

Cell grouping and administration

The experiment was divided into 5 groups. Blank control group (BG): DMEM normal medium with glucose content of 5.5 mmol/L; High glucose model group (HG): DMEM high glucose medium with 30 mmol/L glucose content. GARSE intervention group (0–20 kGy) was irradiated by $^{60}\text{Co-}\gamma$ ray.

The GARSE intervention group was all treated with DMEM high glucose medium, with 30 mmol/L glucose content and 50 µg/mL GARSE intervention.

Cell counting kit-8 (CCK-8) test

The cell density was adjusted to 5×10^4 cells/mL, the cells were seeded into 96-well plates with 100 µL per well, and the control and blank Wells were set at the same time. When the cells were 80% fused, the serum-free medium was replaced, and the cells were starved for 24 h. According to the experimental groups for drug administration, the medium was changed. A total of 10 µL CCK-8 (Beyotime Biotechnology, Shanghai, China) solution was added to each well to avoid light, and the absorbance at 450 nm was determined after incubation for 3 h. The cell viability was expressed as a percentage (Xu et al., 2019).

Animal experiment

The four-week-old male C57BL/6 mice were purchased from Changsheng Biotechnology Company (Liaoning, China). The feeding conditions were as follows: humidity ($55 \pm 5\%$), temperature (24 ± 1) °C, cyclic illumination for 12 h every day, and free drinking water and feeding. Animal care and experiments were in accordance with the guidelines established by the Regulations on the Control of Experimental Animals (Ministry of Science and Technology, China, 2017) and the study was approved by the Medical Ethics Committee, Affiliated Hospital of Yanbian University (Yanbian Hospital).

The mice were randomly divided into 7 groups, with 8 mice in each group. Blank control group (BC) and negative control group (NC) were given distilled water of 500 mg/kg-BW; positive control group (PC) were given 500 mg/kg-BW metformin (Sams Pharmaceutical Co. Ltd, Hebei, China); irradiated groups (IH, IM, IL) were given 5 kGy GARSE of 500, 250, 50 mg/kg-BW, respectively. The non-irradiated group (UI) was given 0 kGy GARSE of 500 mg/kg-BW. The doses administered were set with reference to the experiments of Murthy, H.N. et al (Murthy et al., 2014).

Establishment of a mouse model of T1DM

The mice were fasted overnight and could drink for 12 h. Streptozotocin (STZ) ($\geq 98\%$ HPLC, Sigma, US) was injected intraperitoneally at a low dose (50 mg/kg-BW) for 4 consecutive days, and STZ was injected intraperitoneally at a high dose (100 mg/kg-BW) on the fifth day. However, the blank control group was injected with 0.1 M citric acid sodium citrate buffer solution for sham treatment, and resumed eating 1 h after injection. After one week, the fasting blood glucose level was higher than 11.1 mmol/mL, and the injection dose was modified from the experimental method of ErikoMatsuda et al (Matsuda et al., 2021).

Oral glucose tolerance test (OGTT)

The mice were fasted overnight for 12 h. At the gavage dose of 2 g/kg-BW, a large dose of 40% glucose solution was administered orally for 2 h. Then, the blood glucose level was measured and recorded every 30 min (Li et al., 2020).

Index determination

According to the instructions of the corresponding kit (Jiancheng Institute of Bioengineering, Nanjing, China), the total cholesterol (TC), triglyceride (TG), glycosylated hemoglobin (GHB), total superoxide dismutase (T-SOD), malondialdehyde (MDA), trace reduced glutathione (GSH), catalase (CAT) and reactive oxygen species (ROS) were detected.

HE staining

Part of the kidneys were fixed in 4% paraformaldehyde solution for 24 h. After ethanol gradient dehydration, they were embedded in paraffin. After routine sectioning, they were fixed on the glass slide and dried at 45 °C. Xylene was dewaxed step by step, stained with hematoxylin for 5 min, separated with 1% hydrochloric acid ethanol solution, washed with water, and dyed with eosin for 5 min. After dehydration, xylene was transparently sectioned and sealed with neutral resin (Yuan et al., 2021).

Western blot

A total of 0.2 g of liver tissue was added with 1.5 mL of protein lysate (RIPA lysate: PMSF protease inhibitor: protease inhibitor = 100:1:1), ground on ice, and centrifuged at $12000 \times g$ at 4 °C for 30 min. The supernatant was operated based on the instructions of bicinchoninic acid (BCA) assay protein assay kit. According to 1.5 µg/µL loading amount, the stock solution protein was 5×. The protein loading buffer was mixed in the ratio of 4:1, boiled for 5 min, and sub packed and stored at -20 °C. At constant pressure of 100 V electrophoresis (120 min), and 100 h at constant pressure 100 V (60 min), 5% skim milk powders were sealed for 1 h. TBST was diluted by 2 diluted with dilute solution, two diluted to 2 h at ambient temperature, and then incubated in a dark box with ECL. 1–2 min was added to the gel imaging system and photographed as well.

Statistical method

SPSS 22.0 was used to statistically analyze the test data. The data were expressed in the form of chart and mean \pm standard deviation ($\bar{X} \pm SD$). Other test data were analyzed by one-way ANOVA. $P < 0.05$ represents significant difference, $P > 0.05$ means no significant difference.

Results

Different radiation doses on antioxidant activity

The standard curve of the total saponins was determined as $y = 1.9498x + 0.0018$ $R^2 = 0.9991$. As shown in Fig. 1a, the total saponin decreased in a dose-dependent manner within the radiation dose range of 5 kGy–20 kGy, and the total saponin after 5 kGy $^{60}\text{Co-}\gamma$ radiation treatment was significantly higher than that of other groups ($P < 0.05$). The total saponin was elevated by 4.5% compared with the unirradiated group. Therefore, it is concluded that 5 kGy $^{60}\text{Co-}\gamma$ ray irradiation sterilized GAR could increase the total saponin, but with the increase of the radiation dose, the total saponin loss would be greater.

As shown in Fig. 1b–d, in the range of 50–4000 µg/mL, the scavenging ability of DPPH, ABTS and PTIO radical of GARSE increased with the increase of the concentration, and stabilized at 4000 µg/mL. Compared with the un-irradiated group, the scavenging ability of DPPH and ABTS radicals was significantly enhanced by 5.1% and 4.1% ($P < 0.05$), respectively, and the scavenging ability of PTIO radicals was slightly enhanced by 0.7% ($P > 0.05$) after the irradiation with $^{60}\text{Co-}\gamma$ rays at 5 kGy. The $^{60}\text{Co-}\gamma$ rays of 10–20 kGy irradiation dose can weaken the scavenging ability of DPPH, ABTS and PTIO free radicals to various degrees, which is negatively correlated with the irradiation dose.

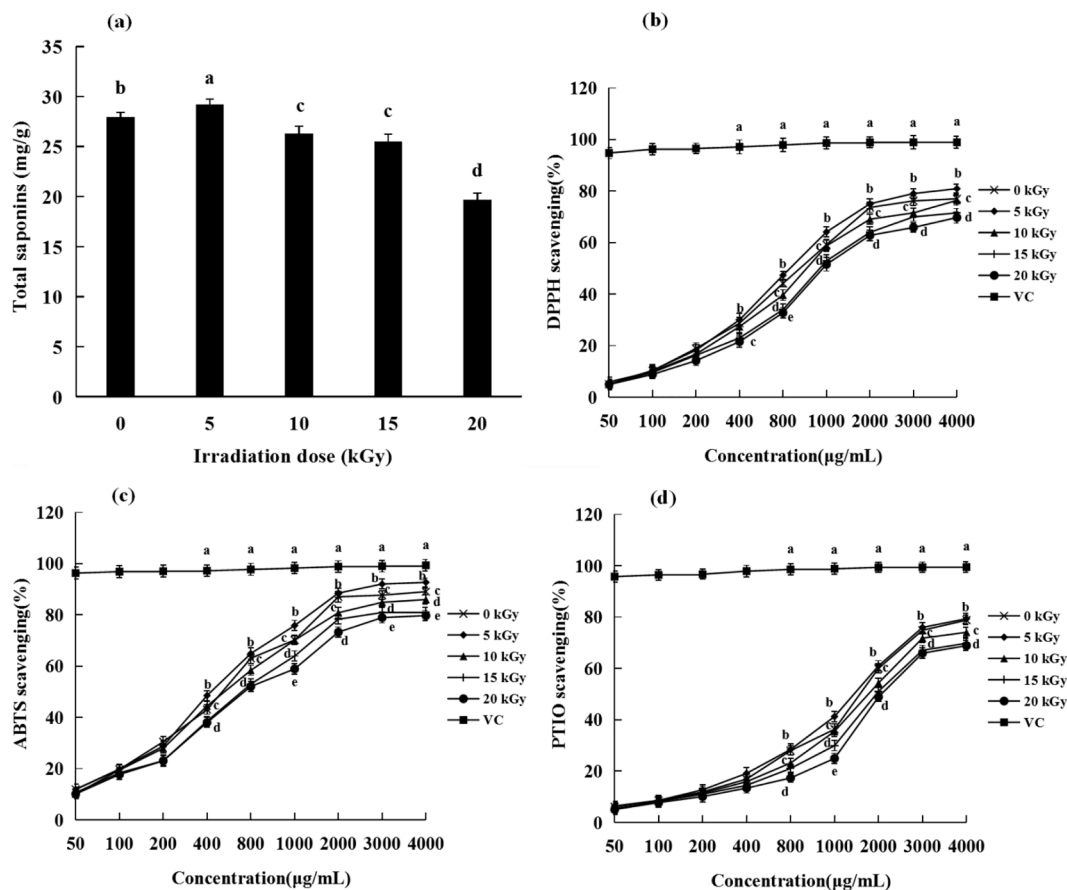


Fig. 1. Different radiation doses on antioxidant activity. Total saponin content (1a), 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging rate(1b), 2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) radical scavenging rate(1c), 2- phenyl-4, 4, 5, 5-tetramethylimidazol-1-oxyl-3-oxide (PTIO) radical scavenging rate (1d). The irradiation doses were selected as 0 kGy, 5 kGy, 10 kGy, 15 kGy, and 20 kGy. The concentration interval of GARSE in DPPH, ABTS and PTIO experiments was set to 50–4000 µg/mL. Different letters indicate significant difference at $p < 0.05$.

The protective effects of different GARSE on HMCs

As shown in Fig. 2a, the survival rate of HMCs reached the highest at 30 mmol/L ($P < 0.05$). Due to the stimulation of high glucose, HMCs would proliferate in a self-limiting manner, so glucose concentration of 30 mmol/L was selected for induction. Fig. 2b illustrates that when the drug concentration was 25–50 µg/mL, the survival rate of HMCs in each group was >90%: At this concentration, GARSE at five irradiation doses of 0–20 kGy had no toxic effects on HMCs. In order to obtain significant results, higher concentrations were selected for the next experiments in the absence of toxicity. Therefore, 50 µg/mL was selected as the dose. Fig. 2c demonstrates that, with the increase of the irradiation dose, the survival rate of HMCs would be higher within the irradiation dose range of 5–20 kGy: The inhibition effects of HMCs proliferation under the action of high glucose would be weaker. Among them, GARSE irradiated by 5 kGy $^{60}\text{Co-}\gamma$ ray had the best inhibitory effects on the self-limiting proliferation of HMCs under high glucose ($P < 0.05$), and there were no significant differences between GARSE and blank control group at 48 h and 72 h ($P > 0.05$). Fig. 2d–h indicates that high glucose stimulation can significantly increase ROS and MDA levels ($P < 0.05$), and significantly decrease T-SOD, CAT and GSH levels ($P < 0.05$). After the treatment of GARSE with different radiation doses, the abnormalities of the above indicators were alleviated to some extent. Among them, 5 kGy GARSE had the best treatment effects. The MDA and ROS levels were reduced by 50.5% and 37.3%. The T-SOD, CAT and GSH levels were increased by 58.4%, 36.9%, 47.9%. This was significantly better than the un-irradiated group and other irradiated groups ($P < 0.05$). With the increase of radiation dose, the therapeutic effects decreased.

The comprehensive results proved that GARSE irradiated by 5 kGy $^{60}\text{Co-}\gamma$ ray had the best ability to resist oxidation and alleviate oxidative stress in vitro. In order to continue to explore the hypoglycemic effects of GARSE irradiated by 5 kGy $^{60}\text{Co-}\gamma$ ray, GARSE irradiated by 5 kGy $^{60}\text{Co-}\gamma$ ray was selected for subsequent animal experiments.

The effects of GARSE on basic indexes of T1DM mice

As shown in Table 1, the food intake and water intake of mice in NC group were significantly higher than those in other groups ($P < 0.05$), while the body weight gain of mice in the NC group were significantly lower than those in other groups ($P < 0.05$), which indicated that diabetes symptoms were extremely obvious. There was no significant difference between IH group and PC group in body weight increment ($P > 0.05$), and tended to BC group. The food intake and water intake of UI group were significantly higher than those of IH group ($P < 0.05$). The results revealed that GARSE irradiated by 5 kGy at dosage levels of 500 mg/kg-BW could significantly restore the metabolism and utilization of glucose in diabetic mice ($P < 0.05$), and then restore the growth of body weight and improve the utilization of food, and the effect was significantly better than GARSE irradiated by 0 kGy at the same dose.

Effects of GARSE on organ coefficients in T1DM mice

According to Table 2, there were no significant differences in heart coefficient and spleen coefficient among all groups ($P > 0.05$), and kidney coefficient and liver coefficient in NC group were significantly higher than those in other groups ($P < 0.05$). At dosage levels of 500

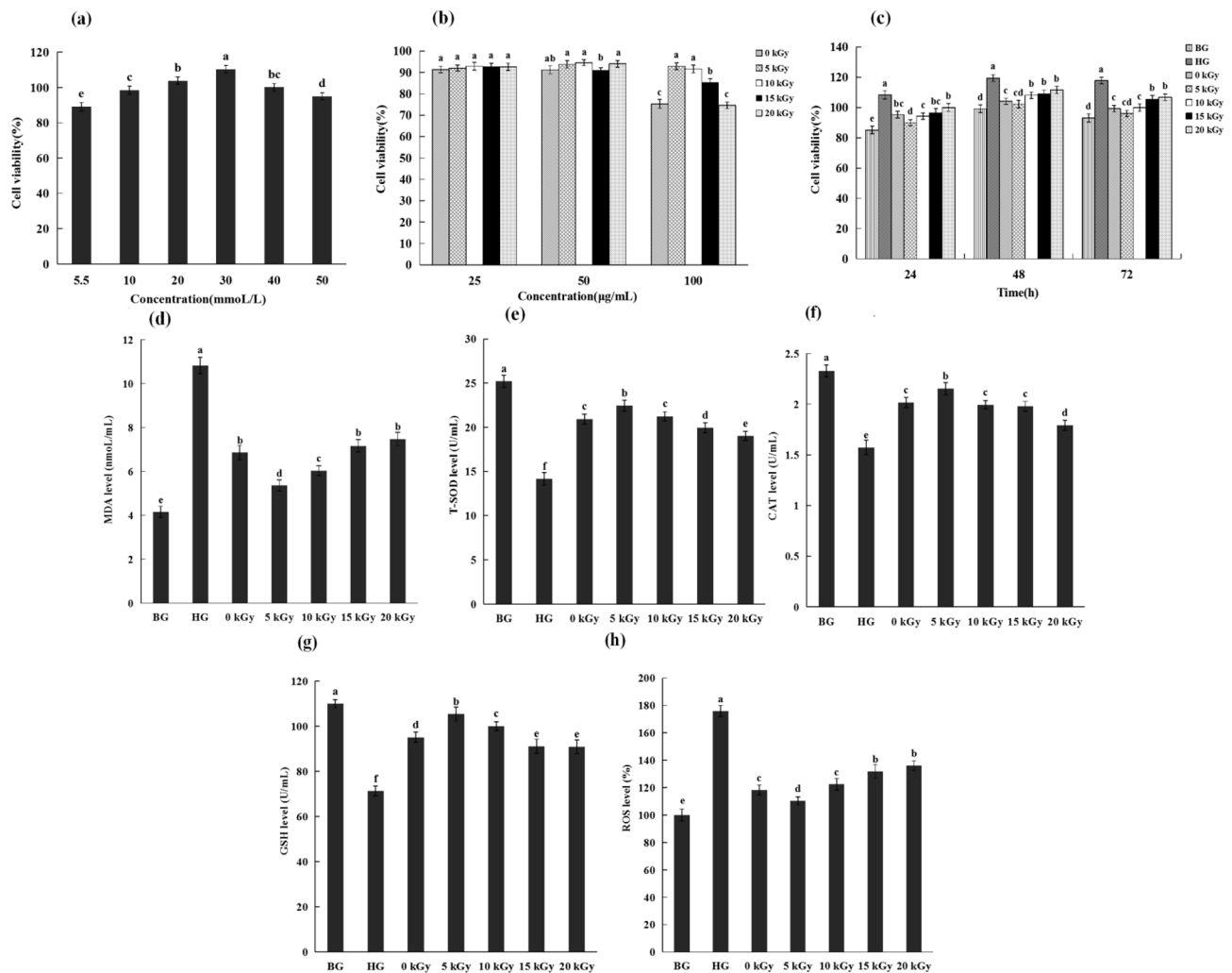


Fig. 2. The protective effects of different GARSE on HMCs. The effects of glucose on HMCs (2a). The effects of different GARSE on HMCs (2b). The effects of different GARSE on HMCs induced by high glucose (2c). The protective effects of different GARSE on Malondialdehyde (MDA), Total Superoxide Dismutase (T-SOD), Catalase (CAT), Micro Reduced Glutathione (GSH), and Reactive Oxygen Species (ROS) induced by high glucose in HMCs (2d–2h). The HMCs of BG were cultured normally. The HMCs of HG were induced by giving 30 mmol/L of glucose. The HMCs in the remaining treatment groups were given 30 mmol/L of glucose and were intervened with different irradiation doses of GARSE at 50 µg/mL (Fig. 2c–2 h). Different letters indicate significant difference at $p < 0.05$.

Table 1
Basic indicators of mice (n = 8).

	Weight gain (g)	Food intake (g)	Water consumption (g)
BC	4.97 ± 0.87 ^a	568.23 ^g	651.92 ^g
NC	1.70 ± 0.37 ^d	1273.66 ^a	4525.87 ^a
PC	3.76 ± 0.54 ^b	743.96 ^f	1997.16 ^f
IH	3.30 ± 0.30 ^b	880.59 ^e	2575.82 ^e
IM	2.49 ± 0.37 ^c	1038.43 ^c	3207.25 ^c
IL	1.98 ± 0.19 ^d	1169.73 ^b	4009.64 ^b
UI	2.65 ± 0.43 ^c	1027.64 ^d	2979.63 ^d

Different letters indicate significant difference at $p < 0.05$.

mg/kg-BW, the liver and kidney organ coefficients in IH group were without significant difference, compared with those in PC group ($P > 0.05$), but were significantly lower than those in UI group ($P < 0.05$). The results gave the information that STZ-induced T1DM in mice would not affect the heart and spleen within 5 weeks of disease, and GARSE would not cause side effects. However, diabetes would lead to fat infiltration and blood viscosity in the liver, thus resulting in hepatomegaly. At dosage levels of 500 mg/kg-BW, GARSE irradiated by 5 kGy had the best effects in alleviating significant liver and kidney, which was significantly better than GARSE irradiated by 0 kGy at the same dose (P

Table 2
Organ coefficient of mice (n = 8).

	Heart (%)	Liver (%)	Spleen (%)	Kidney (%)
BC	0.64 ± 0.09 ^a	4.06 ± 0.07 ^c	0.28 ± 0.05 ^a	1.29 ± 0.06 ^d
NC	0.65 ± 0.05 ^a	6.74 ± 0.09 ^a	0.30 ± 0.05 ^a	2.10 ± 0.10 ^a
PC	0.67 ± 0.07 ^a	4.80 ± 0.09 ^d	0.29 ± 0.03 ^a	1.57 ± 0.05 ^c
IH	0.67 ± 0.07 ^a	4.97 ± 0.12 ^d	0.32 ± 0.08 ^a	1.62 ± 0.05 ^c
IM	0.70 ± 0.03 ^a	6.12 ± 0.08 ^c	0.35 ± 0.05 ^a	1.80 ± 0.03 ^b
IL	0.67 ± 0.03 ^a	6.40 ± 0.13 ^b	0.30 ± 0.09 ^a	1.99 ± 0.08 ^a
UI	0.71 ± 0.05 ^a	5.94 ± 0.15 ^c	0.33 ± 0.08 ^a	1.78 ± 0.05 ^b

Different letters indicate significant difference at $p < 0.05$.

< 0.05), and the therapeutic effects were directly proportional to the dose concentration.

The protective effects of GARSE on T1DM mice

Based on Fig. 3a, IH group had obviously hypoglycemic effects similar to metformin in the second week of administration. After 4 weeks of administration, the blood glucose in IH group was similar to that in PC group, and significantly lower than that in IM, IL and UI groups ($P < 0.05$). The blood glucose value of IH group was 16.0% lower

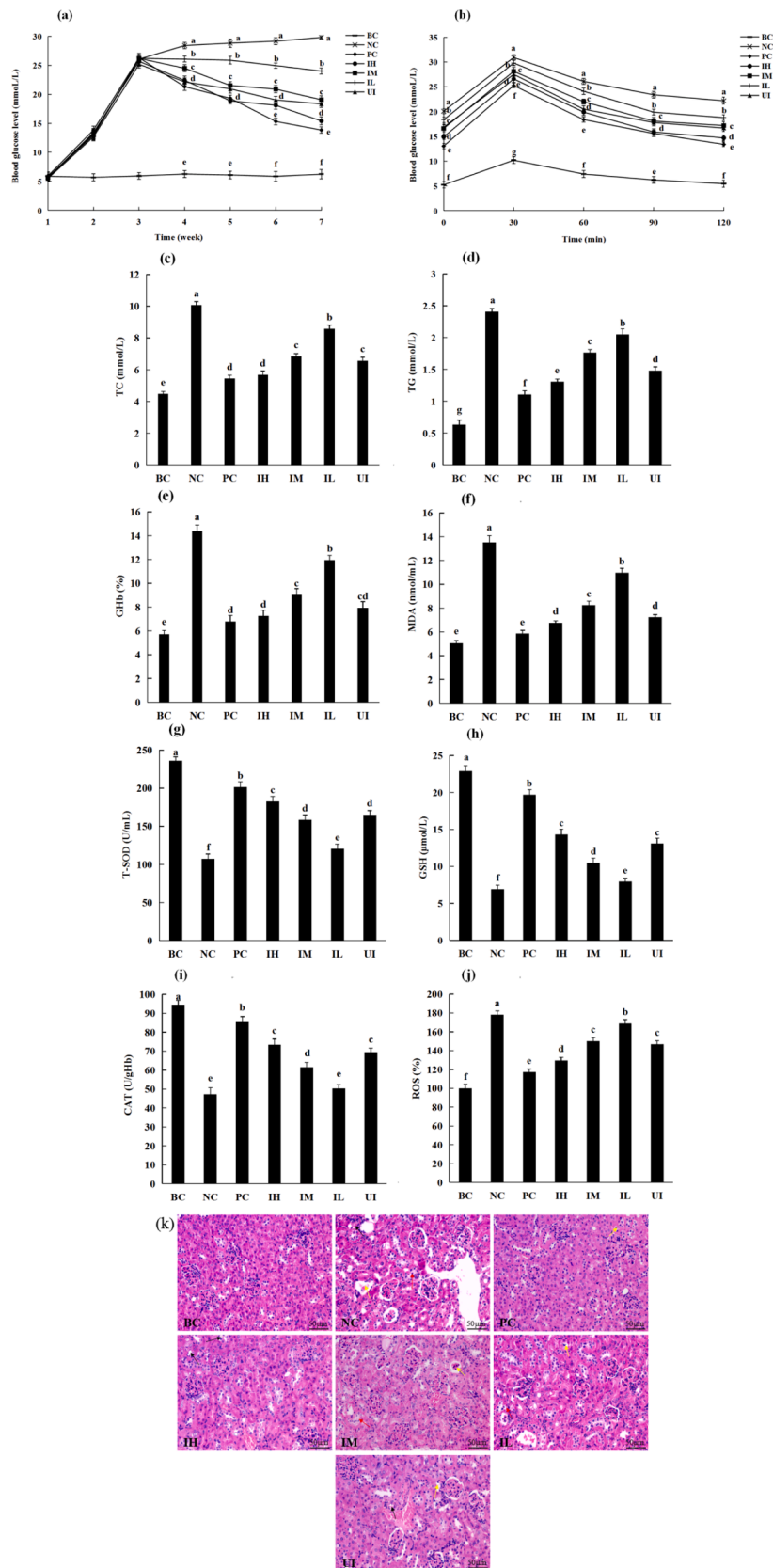


Fig. 3. The protective effects of GARSE on T1DM mice (n = 8). The effects of GARSE on blood glucose in T1DM mice (3a). The effects of GARSE on glucose tolerance in T1DM mice (3b). The effects of GARSE on Total Cholesterol (TC), Triglycerides (TG), Glycosylated Hemoglobin (GHb), Malondialdehyde (MDA), Total Superoxide Dismutase (T-SOD), Micro Reduced Glutathione (GSH), Catalase (CAT), Reactive Oxygen Species (ROS) in T1DM mice (3c-3j), HE staining of kidney, and 200×(3 k). The mice with IH, IM and IL were given 500 mg/kg-BW, 250 mg/kg BW and 50 mg/kg-BW of 5 kGy irradiated GARSE. The mice with UI were given 500 mg/k-BW of unirradiated GARSE. The mice with PC were given 500 mg/kg-BW of metformin. The mice with BC and NC were given distilled water. Different letters indicate significant difference at $p < 0.05$.

than that of the UI group. It indicates that the hypoglycemic effect of GARSE was elevated after being irradiated by 5 kGy at dosage levels of 500 mg/kg-BW.

Fig. 3b displays that after a one-time oral administration of large dose of glucose, the blood glucose values of each group returned to the

initial level at 120 min. IH group tended to PC group, while UI group tended to IM group. The results suggested that the glucose tolerance level of STZ-induced injury was improved under the intervention of GARSE. Moreover, the effects of GARSE irradiated by 5 kGy at dosage levels of 500 mg/kg-BW were closest to metformin, and significantly

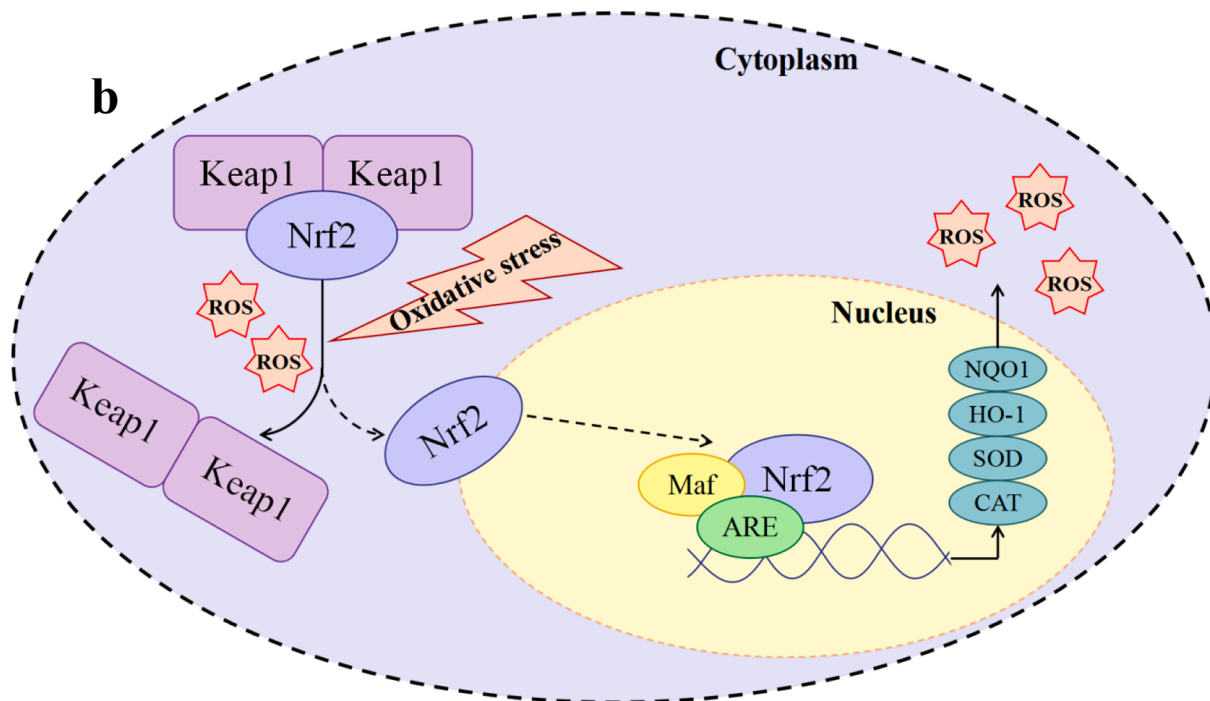
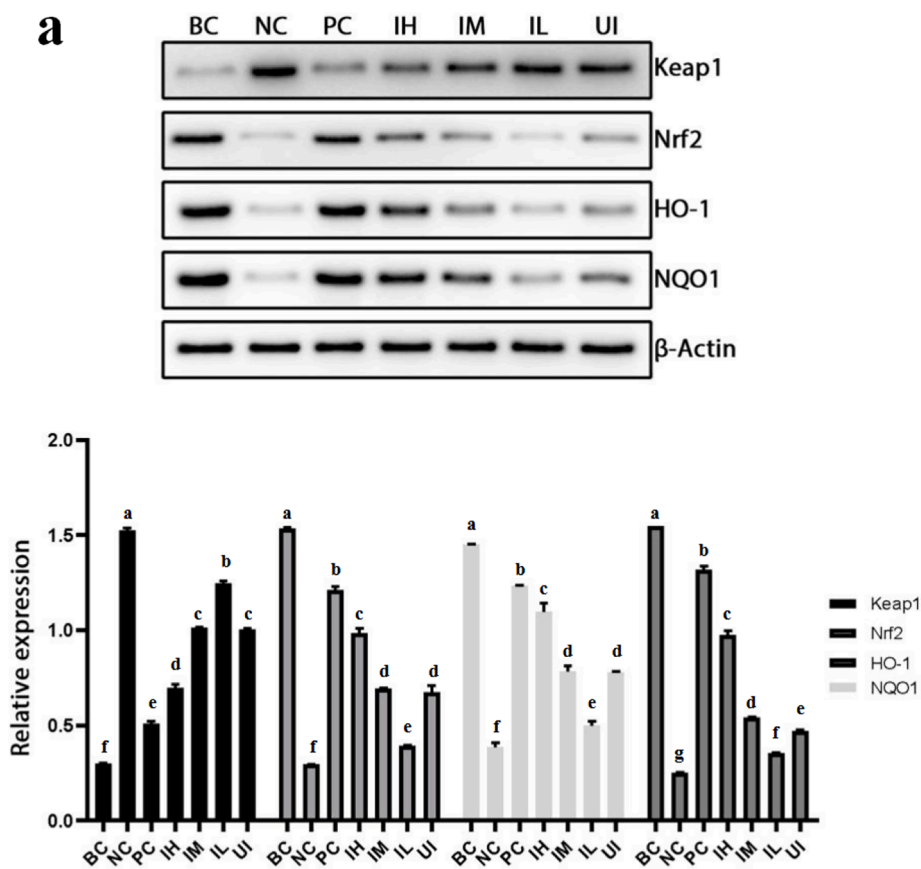


Fig. 4. Irradiated GARSE on Protein Expression in Keap1/Nrf2/HO-1 Pathway (n = 8). Western blot (4a), Keap1/Nrf2/HO-1 pathway diagram 4b. Different letters indicate significant difference at $p < 0.05$.

better than that of irradiated by 0 kGy at the same dose ($P < 0.05$).

Fig. 3c-e showed that the levels of TC, TG and GHb in NC group were significantly higher than those in other groups ($P < 0.05$). The levels of TC, TG and GHb were inversely proportional to the dose concentration after being treated with 5 kGy irradiated GARSE. Among them the contents of TC and GHb in IH group were similar to those in PC group ($P > 0.05$). The levels of TC, TG and GHb in the IH group were decreased by 13.6%, 11.6% and 8.4% compared with the UI group. The results illustrated that at dosage levels of 500 mg/kg-BW, GARSE irradiated by 5 kGy enhanced the ability to alleviate the abnormalities of TC, TG and GHb blood indices in T1DM mice compared with unirradiated ones.

According to Fig. 3f-j, the ROS and MDA contents in NC group were significantly higher than those in other groups ($P < 0.05$), while T-SOD, GSH and CAT contents were significantly lower than those in other groups ($P < 0.05$). The effect of GARSE treated with 5 kGy irradiation on the above indices in T1DM mice was dose-dependent. Among them, the index content of IH group was most close to that of PC group. The levels of ROS and MDA in the IH group decreased by 11.8% and 6.5% compared with the UI group, and the levels of T-SOD, GSH and CAT increased by 10.5%, 9.4% and 5.7%, respectively. It was shown that GARSE irradiated by 5 kGy at dosage levels of 500 mg/kg-BW showed better control of the above indices content than unirradiated. The antioxidant capacity was further enhanced.

Fig. 3k displays the HE staining results of mouse kidney sections. The yellow arrow shows the necrosis of renal tubular epithelial cells and glomerular atrophy. The red arrow marks the epithelial cells of the renal tubules that are edematous with vacuolated cytoplasm. The black arrow is locally seen with mild steatosis. It is obvious that the renal disease of NC group is serious, and all the administration groups can alleviate the lesion, and is proportional to the dose. It was observed that kidney lesions induced by STZ of T1DM mice was improved with the increase of the administered dose after 4 weeks of treatment with GARSE irradiated by 5 kGy.

Irradiated GARSE on protein expression in Keap1/Nrf2/HO-1 pathway

Fig. 4a indicates that after STZ induction, the protein level of Keap1 increased significantly, and the protein level of Nrf2, HO-1 and NQO1 decreased remarkably ($P < 0.05$). After T1DM mice were treated with the GARSE which irradiated by 5 kGy, the protein levels of Keap1 decreased; the protein levels of Nrf2, HO-1 and NQO1 increased. The above effects were dose-dependent. The Keap1/Nrf2/HO-1 pathway was activated. The results in the IH group were closer to those in the PC group. The protein levels of Keap1, Nrf2 and NQO1 in the UI group were not significantly different from those in the IM group ($p > 0.05$). This demonstrated that GARSE can alleviate oxidative stress by acting on the Keap1/Nrf2/HO-1 pathway, and the treatment effects were best after 5 kGy irradiation.

Discussion

In this study, different doses of $^{60}\text{Co-}\gamma$ rays affected the contents of the total saponins in GAR, and then affected the scavenging rates of DPPH, ABTS and PTIO free radicals, because the main characteristic active ingredient of GAR is ginsenoside that has the antioxidant effects (Xu et al., 2021). Interestingly, when the irradiation dose was >5 kGy, the content of the total saponins decreased, and the content was inversely proportional to the irradiation dose. KC Le et al. proposed that low-dose γ -rays can increase the saponin of GAR by 4.2 times due to the changes in the activities of the main enzymes that were involved in the biosynthesis (Le et al., 2019). However, long-term exposure to high doses of γ -rays had negative effects, which may be related to the restriction of cell cycle in G2/M phase and the increase of genetic instability. The negative effects of irradiation were also seen in the study conducted by Alijaniha et al. A dose of 5 kGy reduced the total phenolic and flavonoid contents of *Cuscuta chinensis* extracts by 12% and 18%,

respectively (Ernawati et al., 2021). In combination with this experiment, different irradiation doses can lead to differences in the results: The activity of ginsenosides was attenuated by >5 kGy of $^{60}\text{Co-}\gamma$ radiation and activated by low dose of irradiation. It was speculated that irradiation might have caused a conversion or synergistic effect between ginsenoside monomers. The changes of specific ginsenoside monomers need to be explored through in-depth component analysis, and we will focus on the investigation in future studies. For different active substances, the applicable irradiation doses are different. Improper application of irradiation techniques can be counterproductive. A potential threat arises for future applications in functional foods and pharmaceuticals. Besides, the possibility of sterilization with the package is a major advantage of irradiation technology. However, Caire-maurisier et al. put forward that the irradiation of polyethylene terephthalate with 25 kGy electron beam produced polymers, including benzoic acid, terephthalic acid, etc (Caire-Maurisier et al., 2019). Kremser et al. discovered that 45 kGy γ -rays irradiation decreased the elongation at the break of polypropylene by 46% (Kremser et al., 2020). Therefore, the use of irradiation technology for food processing should also be concerned about the risk to food safety caused by its impacts on packaging materials.

The cell experiments showed similar results. When the irradiation dose was >5 kGy, the protective effects on HMCs decreased, which was manifested in oxidative stress-related indexes, indicating that $^{60}\text{Co-}\gamma$ ray irradiation affected the contents of oxidative stress-related saponin monomer or other active components with the effects. In fact, oxidative stress is a key factor in the occurrence and development of diabetes and its related complications. This process could not only promote the onset of diabetes, but also aggravate the disease and its related complications (Morandi et al., 2021). Acute hyperglycemia can lead to the increased ROS synthesis and nitric oxide consumption in mitochondria, thereby advanced glycation end products (AGEs) were produced, NADPH oxidase was induced. And the inflammatory cascade and sorbitol pathway were upregulated. Then, oxidative stress was exacerbated. In the case of chronic hyperglycemia, ROS production persists, so antioxidant enzymes and non-enzymatic antioxidants in various tissues are severely inhibited, which further exacerbates the oxidative stress. In order to combat the production of reactive oxygen species, the body has an antioxidant defense system, including CAT, SOD and other antioxidant enzymes (Pisoschi et al., 2021).

In vivo experiments revealed that the protective effects of oxidative stress related to the indexes of GARSE irradiated by 5 kGy $^{60}\text{Co-}\gamma$ ray were better than that of non-irradiated GARSE, and the effects became better with the increase of dose, which suggested that the protective effects of the drug on diabetes can be targeted at the aspects of antioxidant stress. In addition with STZ to beta cells in mice led to the serious lack of insulin, glucose in the body can't be used, which makes the body for energy cannot satisfy the consumption, thus producing hungry feed more symptoms. However, the intake of glucose metabolic utilization cannot be led to a rise in blood sugar levels, and substantial accumulation of glucose could cause a huge burden to the kidney (Tekula et al., 2018). With a lot of water with the urine, excess glucose would get together, thus causing increased urine output, and the loss of body water led to the rise of blood concentration, which could result in the symptoms of thirst, because the power from glucose metabolism can only consume large molecules, such as proteins and fat nutrients in the body, thus leading to weight loss for the typical symptoms of diabetes (Jamali-Raeufy et al., 2019). The present experiment produced results that are consistent with the above. Combined with the cellular experiments, it was found that the low dosing concentration in the animal experiments in this experiment was 100 times higher than the cellular experiments, but the therapeutic effects were not significant. The difference may be due to the difference in the bioavailability of the drug.

It is well known that diabetes has major impacts on heart and kidney. Our study also implied the significant complications of liver and kidney, which was caused by the excessive production of AGEs and ROS in

kidney for the chronic and long-term hyperglycemia, thus leading to oxidative damage (Rosas-Martínez et al., 2021). Renal injury was mainly characterized by the thickening of glomerular basement membrane, mesangial dilation, glomerulosclerosis, and tubulointerstitial fibrosis. The results of kidney staining in this experiment showed mild lesions, which may have only reached the initial stage of diabetes, rather than the stage of the complications of nephropathy due to the lack of experimental induction time. However, there has been a trend of kidney disease, which provided a basic reference for diabetic kidney complications.

Due to T1DM, the main factors of β -cell damage include oxidative stress-mediated mitochondrial damage, which causes the imbalance in redox homeostasis. However, the Nrf2/Keap1/HO-1 pathway serves as a key cellular defense mechanism to further alleviate T1DM by regulating second-stage detoxification and antioxidant genes to counteract oxidative stress (Bhakkialakshmi et al., 2015). Fig. 4b displays that Keap1 and Nrf2 bind in the cytoplasm and promote ubiquitination under normal physiological conditions. However, when being stimulated by oxidative stress, Nrf2 translocates to the nucleus, forms a dimer with Maf therein, and binds to the antioxidant response element ARE, which initiates the transcription and translation of downstream antioxidant enzyme-related genes, including HO-1, NQO1, SOD, CAT, etc (Adelusi et al., 2020). Keap1 and Nrf2, as receivers and initiators of the Nrf2/Keap1/HO-1 pathway defense mechanism after stimulation by oxidative stress, were first dissociated and translocated. Then antioxidant enzymes such as HO-1 and NQO1 downstream of the pathway were produced in a controlled manner and the defense mechanism was activated. The state of the Nrf2/Keap1/HO-1 pathway was directly responded to by changes in the content of these four proteins. The results of this experiment indicated that hyperglycemia caused the decrease of Nrf2 in the cytoplasm, and oxidative stress caused Nrf2 to undergo nuclear transfer. However, the levels of HO-1 and NQO1 did not increase as a result, which may be due to body's own activation of Keap1/Nrf2. The HO-1 and NQO1 produced by the Nrf2/Keap1/HO-1 pathway are excessively consumed by the oxidative stress response, which in turn leads to the increase of oxidative stress (Ding et al., 2019). Some studies have confirmed that Nrf2 depletion can increase blood sugar levels, aggravate glucose intolerance and impair insulin signaling (Zhang et al., 2020). The treatment of GAESR alleviated the depletion of Nrf2, HO-1 and NQO1, which suggested that GAESR can down-regulate the expression of Keap1, and activate Nrf2 to up-regulate the expression of HO-1 and NQO1 to combat oxidative stress damage under hyperglycemia. Keap1/Nrf2/ARE signaling pathway has been regarded as an important therapeutic target for the prevention of oxidative stress disease, diabetes and end-stage diabetic nephropathy.

The subsequent experiments can be further advanced from the direction of kidney complications. In addition, on the basis of exploring the hypoglycemic effects of irradiated GARSE, the signal pathway level can be extended. In conclusion, our experiment confirmed that $^{60}\text{Co-}\gamma$ ray irradiation could affect the antioxidant effects of GARSE in vivo and in vitro, and the difference in the treatment effects of irradiated and non-irradiated GARSE could be reflected in the effects of alleviating oxidative stress.

Conclusion

This work reports the antioxidant and antidiabetic properties of GARSE under different $^{60}\text{Co-}\gamma$ irradiation on HMCs with high glucose damage and STZ-induced T1DM mice. The results showed that the total saponin content and the antioxidant radical scavenging ability of GARSE were significantly enhanced after being treated with $^{60}\text{Co-}\gamma$ irradiation at 5 kGy. At the same time, the antioxidant capacity of HMCs damaged by high glucose was significantly enhanced. Over 5 kGy showed a weakening effect. On the other hand, at a dose of 500 mg/kg-BW, the blood glucose values and lipid levels of T1DM mice were significantly reduced by the GARSE which irradiated with 5 kGy. The food utilization

and antioxidant capacity of T1DM mice were improved. The liver and kidney damage caused by STZ was alleviated. The Keap1/Nrf2/HO-1 pathway was activated. Good antioxidant effect and ability to alleviate diabetic complications of GARSE irradiated by 5 kGy were shown.

Ethics approval

Animal care and experiments were in accordance with the guidelines established by Regulations on the Control of Experimental Animals (Ministry of Science and Technology, China, 2017) and the study was approved by the Medical Ethics Committee, Affiliated Hospital of Yanbian University (Yanbian Hospital).

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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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