

***Campanumoea javanica* Bl. activates the PI3K/AKT/mTOR signaling pathway and reduces sarcopenia in a T2DM rat model**

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Abstract

Objective: Sarcopenia causes loss of skeletal muscle and function, thus seriously affecting the physical function and quality of life in the elderly. This article discusses the specific molecular mechanism and ameliorating effects of Tudangshen (TDS) on sarcopenia in elderly rats with type 2 diabetes mellitus (T2DM).

Methods: Elderly Sprague-Dawley (SD) rats were randomly selected and fed with a high-fat diet combined with an intraperitoneal injection of streptozotocin to establish T2DM model. The model rats were stratified and randomly divided into the model group, metformin group, TDS high-dose group, TDS medium-dose group, and TDS low-dose group according to blood glucose combined with body weight, and the same batch of old SD rats was set as the normal control group. The effects of TDS in an elderly T2DM sarcopenia rat model were evaluated by observing the body positions of the rats, analyzing blood biochemistry, testing exercise capacity, and pathologically staining sectioned gastrocnemius muscle tissues. The molecular mechanisms of the effects were analyzed using quantitative real-time polymerase chain reaction and western blotting.

Results: TDS has no statistically significant effect on blood glucose, insulin and glycosylated serum protein in aged rats with T2DM, but it can reduce levels of glycosylated serum protein, total cholesterol, triglycerides, and low-density lipoprotein; it improves pathological changes in rat gastrocnemius muscle tissues, and increases muscle cell activity in elderly rats with T2DM and sarcopenia. TDS also promoted the upregulation of the expression of mammalian target of rapamycin (mTOR)/protein kinase B (PKB/AKT)/phosphatidylinositol 3-kinase (PI3K)/ribosomal protein S6 kinase/eukaryotic initiation factor 4E binding protein 1 mRNA in rats and triggered an increase in corresponding protein levels.

Conclusions: TDS alleviated muscle decline in elderly rats with T2DM by activating the PI3K/AKT/mTOR signaling pathway and regulating the synthesis of corresponding proteins.

Keywords: *Campanumoea javanica* Bl., Sarcopenia, Tudangshen, Type 2 diabetes mellitus, PI3K/Akt/mTOR

Graphical abstract: <http://links.lww.com/AHM/A11>

Introduction

A recent report published by the World Health Organization has shown that diabetes is a growing global health challenge that affects 8% of the global adult population^[1]. The International Diabetes Federation estimates that 578 and 700 million adults will have diabetes by 2030 and 2040, respectively^[2], and the estimated annual healthcare cost will be in the order of 850 billion US dollars^[3]. Type 2 diabetes mellitus (T2DM) is a complex chronic metabolic disease characterized by insufficient insulin secretion from the pancreatic islet β cells, leading to impaired insulin sensitivity

(insulin resistance), high blood sugar levels, and other life-threatening complications^[3–4].

Sarcopenia is a painful syndrome caused by persistent skeletal muscle loss and decreased strength and function that increases morbidity and mortality among the elderly^[5]. It is characterized by a progressive and systemic loss of muscle quality and strength, leading to disability, reduced quality of life (QOL), and even death^[6]. Therefore, patients with sarcopenia are more likely to be hospitalized for long periods^[7]. It seriously affects the QOL and independence of elderly persons and increases medical and social burdens. The global prevalence of present sarcopenia is 10%^[8], and the rapid, worldwide increase in the elderly population will significantly increase this ratio, and consequently, the costs of medical care.

Sarcopenia is associated with poor glucose metabolism. Low muscle mass is an early factor in the induction of diabetes and predicting its occurrence^[9–10]. Diabetes and sarcopenia are age-related diseases. From the age of 30 years, humans lose muscle at a rate of 1% per year, and the muscle mass in patients over 60 drops on average 5% to 13%^[11]. Compared with the age-matched normal controls, elderly patients with T2DM suffer more obvious muscle mass, strength, and function decline^[12]. Sarcopenia is a chronic complication of T2DM in elderly persons^[13].

Tudangshen (TDS; root of *Campanumoea javanica* Bl.) is a popular Chinese traditional herbal medicine that invigorates the spleen and *qi*, replenishes the lungs, and

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relieves coughs. Indications are *qi* deficiency and fatigue, spleen deficiency, diarrhea, and hyperhidrosis. *Campanumoea javanica* Bl. belongs to the Campanumoea family, which is mainly distributed in southwest and south China^[14]. TDS has been used for therapeutic purposes in China^[15]. However, most current research on TDS has focused on the medical effects of *Campanumoea javanica* Bl. polysaccharide. There are no reports of TDS for treating sarcopenia with T2DM.

Elderly people with T2DM are prone to sarcopenia^[16]. In clinical practice, maintaining the balance of skeletal muscle protein metabolism is essential for treating sarcopenia in elderly patients with T2DM. Mammalian target of rapamycin (mTOR) is the center of protein synthesis and metabolism regulation. The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB/AKT)/mTOR pathway is a key signal transduction pathway in the mTOR signaling network that regulates protein synthesis and metabolism^[17]. In addition, the PI3K/AKT/mTOR pathway plays a key role in glucose homeostasis^[18]. Considering the crucial role of the PI3K/AKT/mTOR pathway in maintaining normal cell function, genetic variations in this pathway might affect the development of T2DM. Therefore, we assessed changes in the muscle cell morphology and exercise capacity of model mice on TDS treatment. We also evaluated the effects of TDS on the PI3K/AKT/mTOR signaling pathway and analyzed the corresponding protein expression to gain insight into the specific mechanism of action. Graphical abstract: <http://links.lww.com/AHM/A11>.

Materials and methods

Preparation of experimental drugs

Ground TDS was soaked in distilled water for 2 h and extracted by refluxing twice for 2 h each in 10 volumes of water. The supernatant was concentrated, stored at 4°C, and diluted with distilled water immediately before use.

The rat equivalent dose of metformin hydrochloride tablets (China-US Shanghai Squibb Pharmaceutical Co., Ltd., Shanghai, China) was calculated based on the ratio of the surface area of humans to rats; 0.5% carboxymethylcellulose-Na suspension of metformin was prepared.

Experimental groups and establishment of senile T2DM sarcopenia model

This study complied with the animal ethics guidelines of the Biological and Medical Ethics Committee of Minzu University of China (ECMUC2019005AA). Seventy male, 13-month-old Sprague-Dawley (SD) rats (quality certification no: SCXK [jing] 2018-0010) from the Beijing Keyu Animal Breeding Center (Beijing, China) were reared for 3 months to ensure they reached an advanced age. The rats were randomly assigned to control ($n=10$) and T2DM ($n=60$) groups. The control group was fed regular chow, while the T2DM group was fed a high-fat diet for 8 weeks and intraperitoneally injected 25 mg/kg of streptozotocin to induce T2DM. Blood was collected from the tail to measure random blood glucose (RBG). In the T2DM group, 50 SD rats with RBG >11.1 mmol/L were grouped using a random number table. The T2DM group was divided into model, metformin, and TDS groups (TDS-L, TDS-M, TDS-H; $n=10$ each). The normal control group was fed a standard diet and

intraperitoneally injected with the same volume of normal saline. The administration regimen was as follows: TDS-H, 6.3 g/d/kg; TDS-M, 3.15 g/d/kg; TDS-L, 1.58 g/d/kg. The TDS stock solution (2 g/mL) was diluted to 3 mL/kg for intragastric administration, and metformin was administered at a dose that is common for rats (105 g/kg/d). Body weight and blood glucose levels were measured weekly. All rats were sacrificed 4 weeks after the intervention.

Rat body shape test

The rats were weighed and length was measured three times before sacrifice^[19]. The average weight, body length, and corresponding Lee's index were calculated for each group^[20], and the effects of TDS on elderly rats with T2DM sarcopenia were evaluated.

Gastrocnemius tissue wet weight

The gastrocnemius muscle tissue was removed, and rinsed with PBS, weighed (wet weight), and stored at -80°C ^[21]. Average (means $\pm$ SEM) tissue weights were calculated to evaluate specific changes in tissue quality in all six study groups.

Biochemical analysis

Blood glucose levels were measured using an animal blood glucose meter (Shanghai Yuyan Scientific Instrument Co., Ltd., Shanghai, China). Insulin was measured using ELISA kits (Cat. No.: SEKM-0141; Solarbio Co., Ltd., Beijing, China), and glycated serum protein level (GSP; Cat. No. BC4945) was measured. The rats fasted overnight for 10 h, and venous blood samples were collected in the morning. Total cholesterol (TC), triglycerides (TGs), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels were determined by colorimetry.

Rat behavior

Rat grip strength was assessed using a model ZS-ZL grip tester (Article No.: 1056049; Beijing Zhongshi Dichuang Technology Development Co., Ltd., Beijing, China). The tester was placed on a level surface, and the end of a tension gauge was fixed. The rat was placed on the wire mesh, and the tail was gently pulled along the longitudinal axis of the wire mesh, causing the paws to grasp the wire mesh. The pulling force slowly increased until it exceeded the grasping power of the rat, and the rat was pulled away from the wire. During the test, the tension meter automatically recorded the maximum grip strength. Each measurement was repeated three times, and the average value was recorded. The exercise capacity of the rats was assessed using a ZS-PT-III animal experimental treadmill (Beijing Medcona Biotechnology Co., Ltd., Beijing, China). The taret parameters were 2 min in duration and a constant speed of 12 m/min. Two rats were tested simultaneously by placing them in different tracks of the treadmill stimulated with a 2.0 mA current. The maximum amount of time that the rats ran was recorded.

Histological analysis

Gastrocnemius muscle samples were fixed in 10% formaldehyde, washed with sterile PBS, embedded in

paraffin blocks, and cut into 5- μ m-thick sections using a cryostat. The sections were loaded onto cationic slides and dried at 60°C for 1 h, deparaffinized, rehydrated, and stained with hematoxylin-eosin (HE)^[22]. The stained sections were stored in darkness, incubated for 30 min in 36% triethyl phosphate with 3 mg/mL of Oil Red O^[23], rinsed three times with ddH₂O, and covered with resin-bonded glass coverslips. All experiments were repeated at least three times to confirm the equivalence of staining between batches.

The activity of ATPase (pH 10.5) in gastrocnemius muscle sections was determined by staining as described^[24], and relative ATPase expression was estimated. Rehydrated frozen sections were incubated at pH 10.8 for 15 min, incubated in 20 mM sodium barbiturate containing 9 mM CaCl₂, and at pH 9.5 in 2.7 mM ATP for 45 min. The sections were rinsed three times in 1% CaCl₂ for 1 min each, soaked in 2% CaCl₂ for 2 min, and then rinsed with tap water. The sections were stained with 1% ammonium sulfide, rinsed with tap water, dehydrated in 95% absolute ethanol, and rendered transparent with xylene. The sections were sealed with resin, and then pathological changes were assessed using a fluorescence inverted microscope (magnification: 100 $\times$ or 400 $\times$).

Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

Total RNA was extracted using RNA Fast kits (Solarbio Co., Ltd.). Total RNA extracted from BRL cells using high-purity RNA kits was reversely transcribed using PrimeScriptTM RT-PCR kits (Takara Bio Inc., Kusatsu, Japan) then DNA regions of interest were amplified and the Ct value of each template was detected by qRT-PCR using an ABI StepOnePlusTM RT-PCR system (Thermo Fisher Scientific Inc., Waltham, MA, USA). The relative RNA concentration was calculated using the 2^{- $\Delta\Delta$ Ct} method, where Ct is the average threshold cycle value and values were normalized to those of β -actin. Table 1 shows the primer sequences obtained from NCBI (<https://www.ncbi.nlm.nih.gov/>).

Western blotting

Tissues in each experimental group were lysed in ice-cold RIPA buffer (1% PMSF) and homogenized to extract total protein, and concentrations of which were determined using the BCA method. The same amounts of protein from

samples were resolved by 10% SDS polyacrylamide gel electrophoresis at 80 V for 20 min. When the bromophenol blue indicator penetrated the separation gel, electrophoresis proceeded at 110 V until the indicator reached 0.5 cm from the bottom of the gel. Thereafter, separated proteins on the gel were blotted onto PVDF membranes. After blocking non-specific protein binding with QuickBlockTM Blocking Buffer (Beyotime, Shanghai, China) for 30 min, the membranes were incubated with primary antibodies against the following proteins at 4°C overnight: mammalian target of rapamycin complex 1 (mTORC1; 1:1,000), PI3K (1:4,000), protein kinase B (AKT; 1:1,000), ribosomal protein S6 kinase 1 (S6K1; 1:1,000), and eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1; 1:1,000). The membranes were incubated with a secondary antibody at 37°C for 1 h and washed three times with TBST for 5 min each; proteins were detected using enhanced chemiluminescence and exposed to X-ray film. Images were acquired and optical density was measured using a ClineX ChemiScope fluorescence and chemiluminescence imaging system (ClineX Science Instruments Co., Ltd., Shanghai, China). All experiments were conducted in triplicate.

Statistical analysis

Unless otherwise specified, all experiments were repeated in triplicate. Significant differences between means were assessed using analysis of variance and values with $P < 0.05$ were considered significantly different. All data were statistically analyzed using SPSS Statistics 25.0 (IBM Corp., Armonk, NY, USA).

Results

Construction and evaluation of elderly T2DM sarcopenia rat model

The rats were continuously gavaged with TDS for 4 weeks. We evaluated whether our elderly rat model of T2DM-induced sarcopenia was appropriate based on physiological, biochemical findings, and organ coefficients.

Physiological and biochemical changes in model rats

Figure 1D shows that the gastrocnemius muscle content was reduced to 66.8% in the model rats compared with the controls. The blood sugar level significantly increased to 3.7 fold compared with that of normal rats (Figure 2A).

Table 1
Primer sequences for RT-PCR.

Genes		Primer sequences	Size (bp)	Primer bank
<i>mTOR</i>	Forward	GGGCGGTAGCGATAATGGAG	104	NM_026270
	Reverse	TGCCGTCATCTGTGCTTTCC		
<i>AKT</i>	Forward	ATCGTGTGGCAAGATGTGTATGAG	197	NM_001165894
	Reverse	GCTGAGTAGGAGAACTGGGGAAA		
<i>PI3K</i>	Forward	CGGGATGCCAACTCTATGC	109	NM_020272
	Reverse	CACGATGGATGACAATGAAATG		
<i>S6K1</i>	Forward	GGGCATTACATCAAAGGGG	213	NM_003161
	Reverse	CCACCAATCCACAGCACGG		
<i>4EBP1</i>	Forward	AGTGTGCGAACTCGCCTGTG	146	NM_007918
	Reverse	ACCACCTGCCCGCTTATCTT		

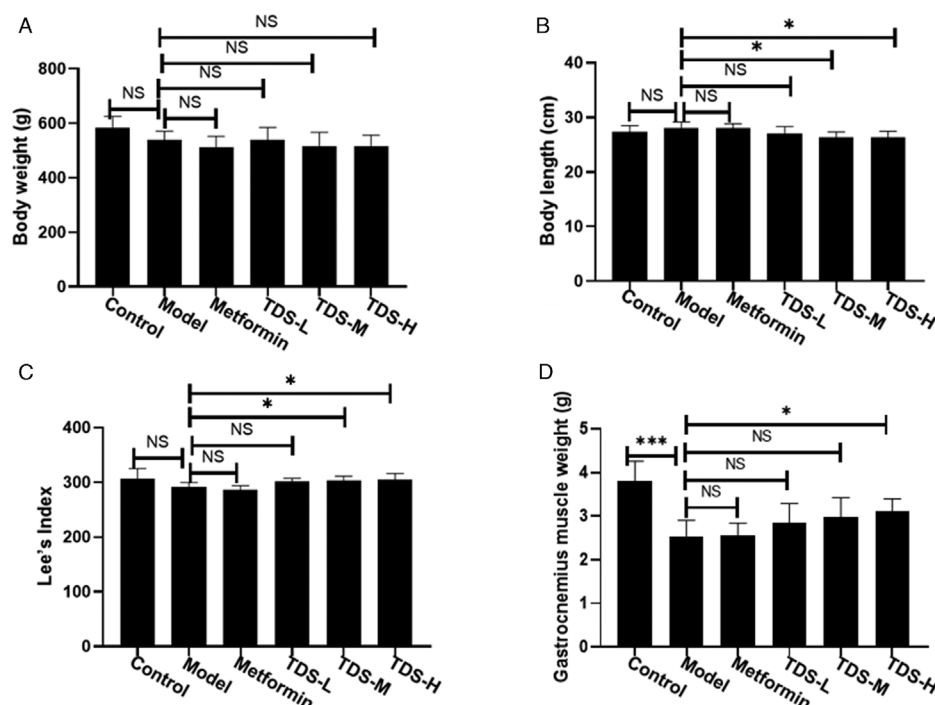


Figure 1. Physiological indicators of rats treated with TDS. Rat body weight (A) and length (B). (C) Lee index of mice. (D) Weight of rat gastrocnemius muscle. Effects of D-gal on muscle fiber morphology. Results are expressed as means $\pm$ SEM and the difference “*” between the control and TDS groups was significant ($P < 0.05$). In addition, ** $P < 0.01$, *** $P < 0.001$, and NS indicates not significant. TDS: Tudangshen.

Figure 2C and E show that GSP and TGs levels increased 1.36 and 3.95 fold, respectively, in the model group. The rats exhibited muscle degeneration and atrophy, leading to decreased muscle mass and increased blood sugar and lipid values.

Pathological changes in model rats

Figure 3 shows pathological changes in the gastrocnemius muscle tissues of the model rats assessed using inverted fluorescence microscopy. The gastrocnemius tissues were arranged regularly, and atrophy, edema, rupture, or necrosis of muscle cells were not evident in the control group. In contrast, muscle tissue arrangement was disordered, muscle cells were atrophied, edema and inflammatory cell infiltration were obvious, some of the muscle fibers had undergone autolysis, and the fiber membrane was slightly ruptured in the model rats (Figure 3A). Oil Red O staining (Figure 3B) revealed increased fat content in tissues of the model compared with that in normal rats. Activities of ATPase were lower in the gastrocnemius muscle tissues of the model than in normal rats (Figure 3C).

Effects of TDS on the physiological indexes of rats

Figure 1A shows that body weight did not significantly reduce of the TDS, metformin, and model groups compared with the controls. Figure 1B shows that TDS (TDS-M, TDS-H) reduced the length of the rats. Figure 1C shows that TDS (TDS-M, TDS-H) significantly increased the Lee index of rats ($P < 0.05$). Compared with the model group, TDS-H increased the weight of the gastrocnemius muscle ($P < 0.05$), while TDS-M and TDS-L slightly relieving symptoms, but not significantly

(Figure 1D). Compared with the control group, the weight of the gastrocnemius muscle was reduced by 33.2% in the model ($P < 0.001$).

TDS reduced GSP and blood lipids in elderly rats with T2DM

Figure 2A shows increased and decreased blood glucose levels in the model and metformin groups, respectively ($P < 0.05$), and TDS did not affect blood glucose levels. Metformin can increase insulin levels in model rats ($P < 0.05$). TDS showed no significant difference in increasing insulin levels (Figure 2B). Metformin can decrease GSP level in model rats ($P < 0.01$). TDS showed no significant difference in decreasing insulin levels (Figure 2C). Compared with the control group, the levels of TC, TG and LDL in the model group increased, while the HDL level decreased. Metformin can reduce TC, TG and LDL levels and increase HDL levels in model rats ($P < 0.05$). TDS can reduce TG and LDL levels ($P < 0.05$) to a certain extent. TDS reduces TC level and increases HDL level (Figure 2D–G).

TDS improved pathological changes of gastrocnemius muscle

The HE staining results (Figure 3) show regularly arranged tissues in the control group have no obvious muscle cell atrophy, edema, fracture, or necrosis. Compared with the normal group, the number of gastrocnemius muscle fibers and cell nuclei in the model group decreased, but the cell diameter increased, and cells were more edematous. Metformin resulted in a relatively regular cell arrangement, and the numbers of cell nuclei were somewhat restored. Cell edema was not evident in the TDS-H group, and the numbers of nuclei and

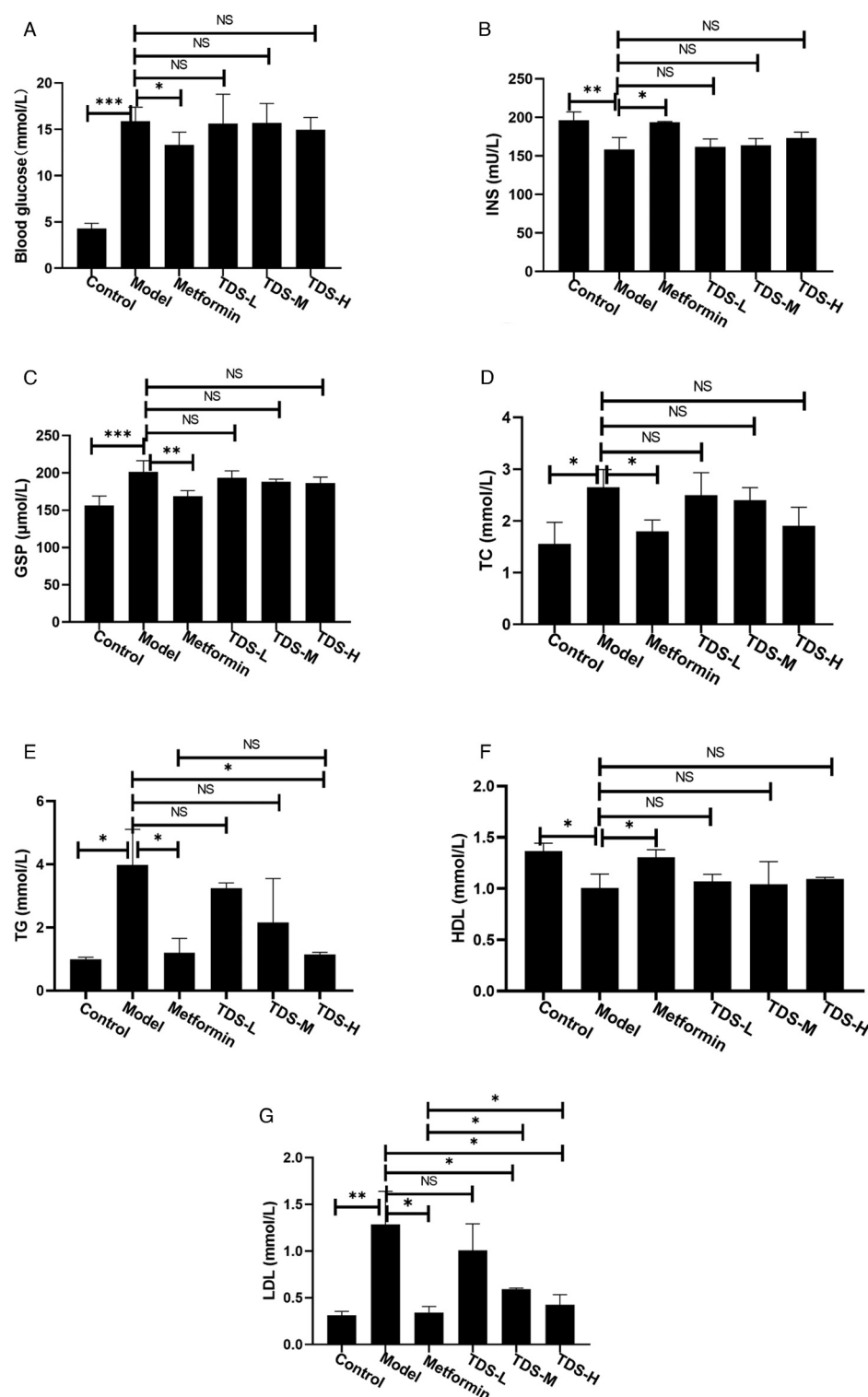


Figure 2. Blood biochemical indexes of rats. Changes in (A) blood glucose, (B) insulin, (C) glycated serum proteins, (D) total cholesterol (TC), (E) triglycerides (TG), (F) high-density lipoprotein (HDL), and (G) low-density lipoprotein (LDL). NS: Not significant. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

gastrocnemius muscle fibers were improved. The control group was stained faintly reddish with Oil Red O, indicating a lower fat content in the gastrocnemius muscles of normal rats (Figure 4). Compared with the normal group, the numbers of gastrocnemius muscle fibers, nuclei, and cell diameter were reduced, and cell rupture was more serious in the model rats. Metformin resulted in a more regular cell arrangement, and the

numbers of cell nuclei were recovered. However, mechanical tears that occurred perhaps during slicing were more serious. In addition, significant cell edema was not evident in the TDS-H group, and the number of nuclei and gastrocnemius muscle fibers was improved. The ATPase content determined as the relative intensity of the stain in the muscle cells was lower in the model, than in the normal rats (Figure 5). Metformin promoted more

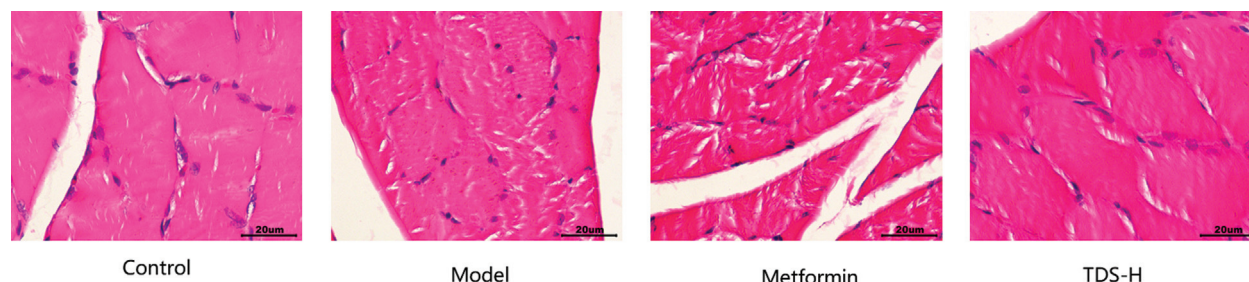


Figure 3. Results of HE staining. Only cells in TDS group are intensely stained ($\times 40$). HE: Hematoxylin-eosin; TDS: Tudangshen.

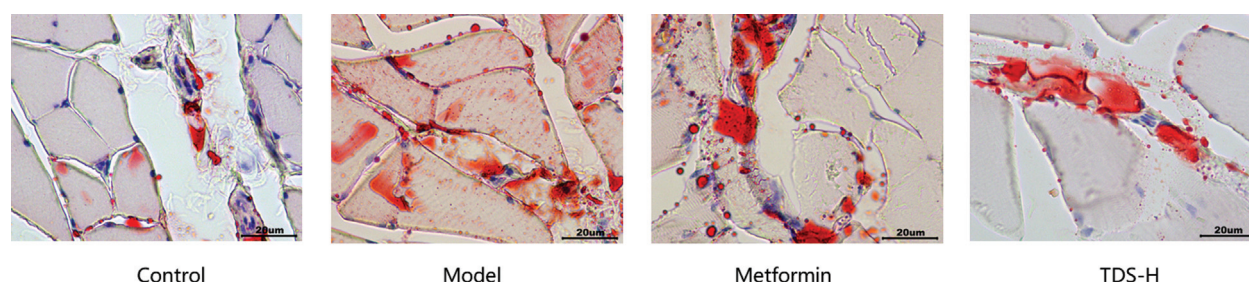


Figure 4. Results of Oil Red O staining ($\times 40$). Only TDS-H group is intensely stained. TDS-H: Tudangshen high-dose.

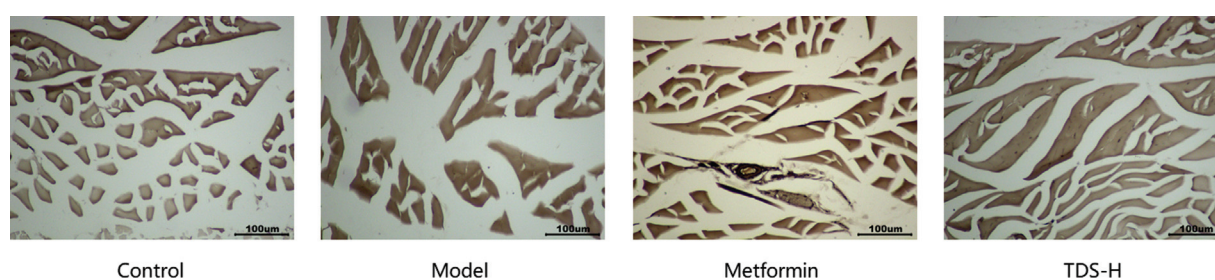


Figure 5. ATPase staining result ($\times 10$). Only TDS-H group is intensely stained. TDS-H: Tudangshen high-dose.

cellular ATPase production compared with the controls. However, TDS had a better therapeutic effect and elevated ATPase levels.

Behavioral changes in rats

The treadmill experiment revealed obvious dyskinesias in the model rats (Figure 6C). However, TDS-H increased the duration that the rats spent running and relieved dyskinesia in the model rats when compared with the relatively insignificant relief provided by metformin (Figure 6A and B). Figure 6D shows that the significantly reduced grip strength in the model rats was restored by TDS. The recovery effects of TDS-M and TDS-H were better than those of metformin.

Effects of TDS on mTOR/AKT/PI3K pathway

We determined mRNA levels of mTOR, AKT, PI3K, S6K1, and 4EBP1 using RT-PCR (Figure 7). The expression of mTOR, AKT, PI3K, S6K1, and 4EBP1 was downregulated in the model rats. Metformin did not significantly alter the expression of mTOR, S6K1, and 4EBP1 (Figure 7A, D, E) but downregulated AKT by 29.5% and upregulated PI3K by 35.3%. The difference

was that TDS-M and TDS-H promoted mTOR, PI3K, S6K1, and 4EBP1 expression. Although the difference was not significant, TDS-M and TDS-H upregulated AKT by 40.7% and 23.9%, respectively.

Effects of TDS on mTOR/AKT/PI3K/S6K1/4EBP1 protein expression

The expression of mTOR, AKT, PI3K, S6K1, and 4EBP1 significantly decreased in the model, compared with the control group (Figure 8). Compared with the model group, metformin slightly (1.2%–4.4%) increased the expression of related proteins, whereas TDS-H significantly altered the expression of AKT, PI3K, and S6K1 ($P < 0.05$) and upregulated mTORC1 and 4EBP1 26.7% and 19.6%, respectively, although the difference was not statistically significant. Therefore, the effects of TDS-L and TDS-M were weak.

Discussion

Sarcopenia is a chronic complication of T2DM, and currently no drugs are particularly effective. The present physiological and biochemical findings showed damaged gastrocnemius muscles and a significantly increased blood

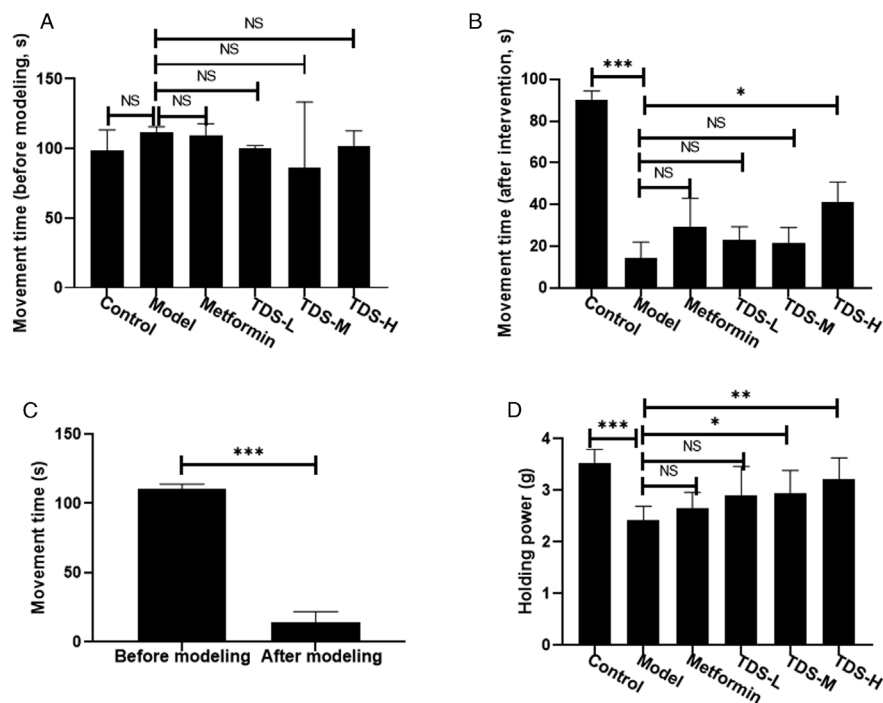


Figure 6. Behavioral changes in rats. (A–C) Effects of TDS on rat movement over time. (D) Effects of TDS on rat grip power. Data are expressed as means $\pm$ SEM; marks between groups indicate differences (NS indicates not significant, $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$). TDS: Tudangshen.

sugar level in our model rats with age-related T2DM and sarcopenia^[22]. We investigated the effects of different doses of TDS on basic physiological indicators of the rats, namely body weight, body length, and final gastrocnemius muscle weight. We found that TDS improved the Lee index and gastrocnemius muscle weight of model rats, indicating that TDS can slightly relieve the symptoms of sarcopenia.

Diabetes is one of the biggest health challenges to humanity, so optimal and continuous blood sugar control is essential for minimizing long-term complications. Type 1 diabetes (T1DM) requires lifelong insulin treatment, supplemented by education and psychological support. For T2DM, lifestyle intervention, drug combinations, and more frequent insulin administration are needed compared with T1DM^[25]. We found here that metformin

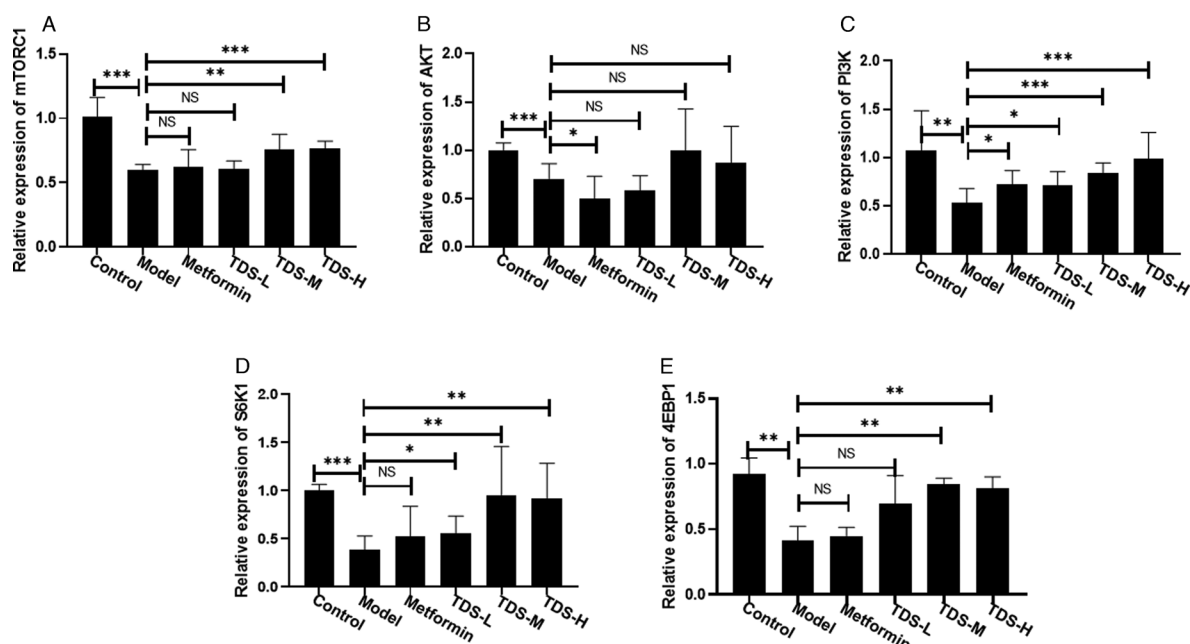


Figure 7. Effects of TDS on rat mTORC1, AKT, PI3K, S6K1, and 4EBP1 gene expression. Data are expressed as means $\pm$ SEM ($n = 3$ per group), and marks between groups indicate differences (NS indicates not significant, $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$). mTORC1: Mammalian target of rapamycin; TDS: Tudangshen.

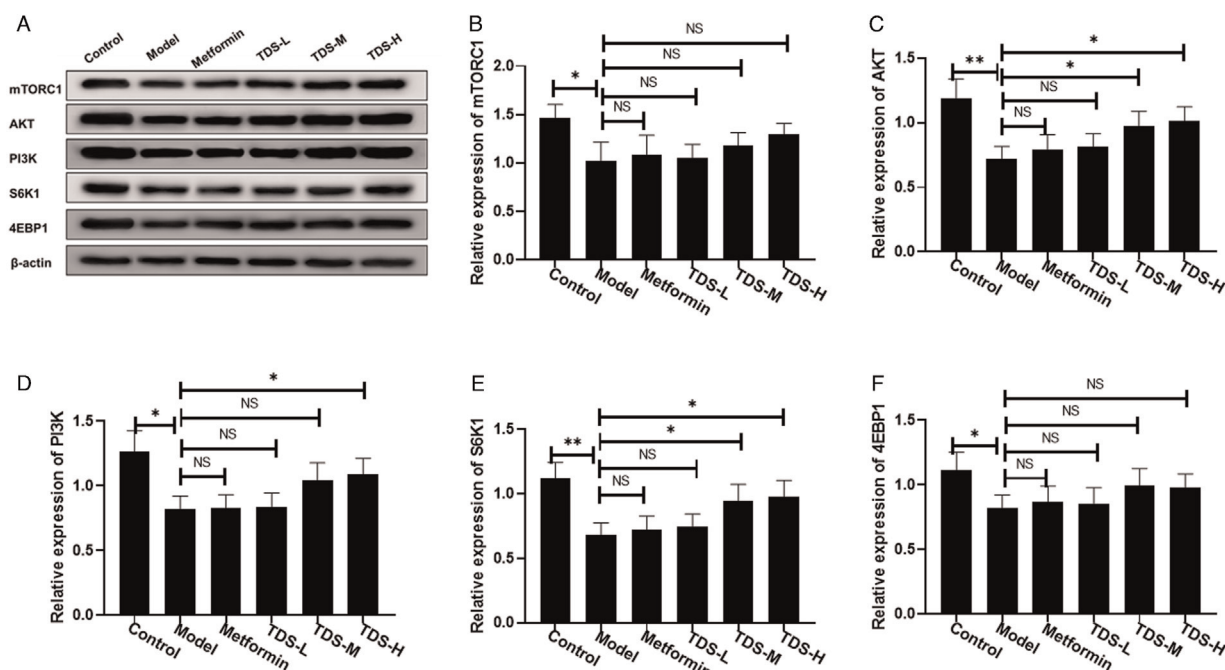


Figure 8. Effects of TDS on mTORC1, AKT, PI3K, S6K1, and 4EBP1 expression in rats. (A) Western blots. (B–F) Statistical results of western blots ($n=3$). Data are expressed as means $\pm$ SEM, and marks between groups indicate differences (NS indicates not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). TDS: Tudangshen.

affected blood sugar levels, whereas TDS did not obvious, metformin can reduce GSP in the model group, TDS has some effect on GSP. Our elderly T2DM sarcopenia rat models had high blood glucose levels, TDS was not as significant as metformin in insulin level changes. TDS did not alleviate blood glucose as effectively as metformin. In addition, TDS did not significantly change the insulin levels in rats^[26]. Glycosylated serum protein is a ketamine compound formed by a combination of sugar with serum protein, which reflects average blood glucose concentrations over the past 2 to 3 weeks and is of great significance to blood glucose monitoring in patients with diabetes^[27]. Herein, we found significantly increased GSP contents in model rats^[26]. In addition, TDS reduced TC, TG, LDL, and increased HDL levels. Although the homeostasis model assessment of insulin resistance is potentially important, it is not routinely assessed in general clinical practice. The ratio of TG to high-density lipoprotein cholesterol (TG/HDL) is a powerful predictor of insulin resistance and cardiovascular disease^[28–29] that is clinically applied as a simple and economical marker of insulin resistance^[30–31]. As a result, the TG/HDL ratio has become a more widely used measure of insulin resistance^[32]. Notably, the TG/HDL index was the lower in the rats treated with a high concentration of TDS (Figure 2E and F). This finding showed that concentrated TDS exerted good control in the T2DM rat model.

Sarcopenia is closely associated with many metabolic diseases, such as obesity, abnormal glucose metabolism, diabetes, high blood pressure, and dyslipidemia^[33–36]. The more prevalent type of diabetes, T2DM, seriously affects the health and QOL of elderly persons. Here, we explored the ability of TDS to alleviate sarcopenia in elderly rats with T2DM by staining tissue sections for pathological changes. We found that TDS alleviated pathological phenomena in gastrocnemius muscles and improved sarcopenia in elderly rat models of T2DM.

Exercise, especially resistance training, is recommended as the main treatment strategy for sarcopenia^[37], and functional activity and balance training seem to be the key to preventing falls. Although existing physical activity guidelines recommend strength training, they do not target strength recovery exercises^[38]. Therefore, targeted strength training is required to relieve sarcopenia. To this end, we tested the grip and exercise ability of rats to determine the degree to which TDS recovered muscle strength. Our results showed that TDS helped restore the grip strength of rats with sarcopenia. In terms of recovering the athletic ability to run, TDS significantly affected only the low-dose group. Exercise training and recovery can reduce risk and are the main treatment options for sarcopenia^[39]. The results of a cohort of elderly patients in Sweden showed that longer periods of moderate-to-vigorous physical activity can reduce the risk of sarcopenia^[38]. Therefore, we concluded that TDS can alleviate dyskinesia in model rats.

The PI3K/AKT/mTOR signaling pathway plays key roles in glucose homeostasis and in the response of cells to extracellular stimuli, such as insulin and insulin-like growth factor-1^[18]. In addition, mTORC1 regulates protein synthesis by phosphorylating S6K1 and 4EBP1^[40]. Therefore, phosphorylated S6K1 and phosphorylated 4EBP1 can be regarded as markers of activated mTOR signaling^[41–42]. The PI3K/AKT/mTOR signal transduction pathway is crucial in immune cells^[43], as through its action on activated downstream effector molecules, it can inhibit cell apoptosis and promote cell proliferation.

Therefore, the PI3K/AKT/mTOR pathway is closely associated with protein metabolism. We found here that medium and high doses of TDS promoted the expression of mTOR, AKT, PI3K, S6K1, and 4EBP1. Our western blotting results showed that TDS promoted the expression of related mRNAs and the synthesis of their corresponding proteins. This indicated that TDS can

alleviate sarcopenia by upregulating the PI3K/AKT/mTOR pathway.

Conclusions

This study is the first to explore how TDS improves T2DM-induced sarcopenia in elderly rats. TDS increases the Lee index and gastrocnemius muscle weight, down-regulates serum GSP, TC, and TG levels, upregulates HDL level, and improves fatty acid metabolism in rats with T2DM. TDS improved gastrocnemius striated muscle cell edema and waxy degeneration and reduced liver fat contents. TDS increased the amount of time that the rats spent running and improved their grip strength. TDS upregulated the mRNA and protein expression of mTOR, AKT, PI3K, S6K1, and 4EBP1. These findings provide new insights into the potential mechanism of TDS in reducing sarcopenia associated with T2DM and give new evidence for the scientific and rational use of TDS to improve sarcopenia in clinical practice.

Conflict of interest statement

The authors state that there are no conflicts of interests to declare.

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Author contributions

Xiangyu Zuo designed the study and experiments, searched the literature, and wrote the manuscript. Rongfei Yao and Linyi Zhao contributed to the discussion. Yinjiang Zhang participated in the study and contributed to the figures and tables. Zongran Pang and Binan Lu supervised the study and participated in data analysis.

Ethical approval of studies and informed consent

Not applicable.

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None.

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