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Vanadium-enriched chickpea sprout ameliorated hyperglycemia and impaired memory in streptozotocin-induced diabetes rats

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Abstract Vanadium compounds have been recognized for their hypoglycemic effects; however, potential short and long-term vanadium toxicity has slowed the acceptance for therapeutic use. In the present work, three batches of vanadium-enriched chickpea sprout (VCS) were prepared by incubating chickpea seeds in presence of 200, 100, and 50 μg/ml of sodium orthovanadate (SOV). The effects of oral administration of chickpea sprout (CS) and VCS food for 8 weeks on streptozotocin-induced (STZ) diabetic rats were investigated. Both CS and VCS food was found to ameliorate some hyperglycemic symptoms of the diabetic rats, i.e. improve lipid metabolism, decrease blood glucose level, prevent body weight loss, and reduce impairment of diabetic related spatial learning and memory. Serum insulin was substantially elevated in treated diabetic rats, which is probably one important reason for the hypoglycemic effect. Compared with CS alone, VCS100 food exhibited remarkably enhanced effectiveness in alleviating diabetes induced hyperglycemia and memory loss. Moreover, vanadium-enriched chickpeas appeared to abolish the vanadium induced toxicity associated with administration of this metal for diabetes during the 8-week study period. This study suggested further work of the vanadium speciation in CS and novel hypoglycemic mechanism for the antidiabetic activity of vanadium agents. Vanadium containing (VCS) food could be a dietary supplement for the diabetic status.

Keywords Chickpea sprout · Enriching · Streptozotocin-induced diabetes · Rats · SOV

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Abbreviations

SOV	Sodium orthovanadate
STZ	Streptozotocin
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
TC	Total cholesterol
TG	Triglyeride
OGTT	Oral glucose tolerance test
VCS	Vanadium-enriched chickpea sprout
CS	Chickpea sprout

Introduction

Diabetes mellitus (DM) is a metabolic disorder increasing tremendously all over the world (King et al. 1998) and remains as a major health concern for humans (Wan et al. 2007; Santoso 2006; Girach and Vignati 2006). Although insulin and many oral hypoglycemic drugs have been used as therapeutic agents (Srivastava and Mehdi 2005), more efficient therapeutic approaches are in significant need (Girach and Vignati 2006; Gandorfer 2006).

Vanadium compounds have demonstrated insulin-mimetic effects with in vitro and in vivo studies (Shechter 1990; Srivastava 2000; Cam et al. 2000; Brichard 2003). Vanadium compounds improve glucose homeostasis in animal models of both Type 1 and Type 2 DM, but the acceptance of vanadium compounds as antidiabetic agents has been slowed due to the concern for short-term gastrointestinal stress and potential long-term toxicity with vanadium accumulation (Domingo 2002). Recent vanadium permeability and toxicity studies (Shukla et al. 2006; Zhang et al. 2006; Yang et al. 2004) suggested that co-administration with botanic antioxidants might helpful for reducing vanadium toxicity. Vanadate salts ingested with a lichee black tea decoction (Clark et al. 2004a, b) or a green tea (Soussi et al. 2006) produced a significant reduction in vanadate toxicity and accumulation in diabetic rats.

In the present work, the hypoglycemic effects of chickpea sprout (CS) and vanadium-enriched chickpea sprout (VCS) were tested using an STZ-induced diabetic rat model as a novel vanadium delivery system. Chickpea (*Cicer arietinum*), an Asian traditional health food has been investigated its potential hypoglycemic effects in diabetic animal models by several groups (Shuiwei et al. 2005; Guixiang and Jinbao 2005; Siddiqui and Siddiqi 1976; Zulet and Martinez 1995). Chickpea seeds and the sprout contain variety of antioxidants such as, isoflavone, biochanin A, B, and C, and others entities (Jinfu et al. 2004). However, our previous studies revealed that the hypoglycemic effects of chickpea seeds and sprout were very limited. However, the VCS food was shown to effectively ameliorated hyperglycemia and impaired memory without observable short-term vanadium toxicity.

Materials and methods

Materials

Chickpea seeds were collected in November 2005 from Xinjiang, China. All chemicals and reagents, unless specifically mentioned, were obtained from Sigma Chemicals Co. (St. Louis, MO, USA) and Roche Inc. (Germany).

Animal model and treatment

Adult male albino Wistar rats (155–190 g) were from the Institute of Experimental Animals, Tongji Medical School, Huazhong Technology University (SCXK2004-0007), and housed in air-conditioned room (temperature: 23 ± 10°C, relative humidity: 55 ± 5%, 12-h/day photoperiod) in the animal facility of Taishan Medical University. The animal experiments were conducted in accordance with the guidelines for the Care and Use of Laboratory Animals.

The rats assigned to the test groups received a single intravenous tail vein injection of STZ (45 mg/kg) freshly prepared in 0.1 M citrate buffer (pH 4.5). The seven control rats (C) received the same volume of citrate buffer each. On day 7 following STZ administration, animals with a plasma glucose level of 15 mmol/l or higher were considered diabetic. On day 10, diabetic animals were randomly divided into five groups of seven animals. The control rats (C) and diabetic rats (D) receiving only standard pellet food and given tap water ad libitum. Four treated groups (one D + CS and three of D + VCS50–200) were given pellet food containing 5% of CS and VCS powder respectively, for 8 weeks before sacrificed.

Preparation of chickpea sprout and vanadium-enriched chickpea sprout powder

The CS was prepared according to the method described previously (Placido et al. 1977; Gallardo et al. 1991). Briefly, the seeds of *Cicer arietinum* (chickpea) were washed in sterile, double-distilled water. Then three batches chickpea seeds were incubated in water (1.3 ml/g) first at 50°C for a minimum of 30 min and then at 38°C for ~12 h in water containing 50, 100, and 200 μg/ml sodium orthovanadate (SOV), respectively. Subsequently, the

germinated seeds were further incubated at 28°C to CS while watering three times a day in an earthenware tray for another 5 days. Then the CSs were washed thoroughly with running tap water, dried at 60°C and ground to fine powder. The rats' food containing CS or VCS were prepared by adding 5% (w/w) the CS powder in the standard rat food pellets.

The vanadium concentrations of VCS powder were determined using a biological specimen analysis method (GB/T5009-2003) by use of a plasma emission spectroscopy (IRIS Intrepid II).

Blood glucose, plasma insulin, lipid profile, body weight and oral glucose tolerance tests (OGTT) measurement

Body weight and blood glucose were monitored daily. Blood samples were collected by tail nick. The blood for glucose analysis was centrifuged at 3,500 × *g* for 20 min at 4°C. The plasma glucose levels were determined immediately using a Glucometer Elite system (Roche Diagnostic, Germany).

Plasma insulin concentrations were determined using a radioimmunoassay kit (Boster Biologicals Company, Wuhan, China) using the manufacturer protocol. Plasma total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were determined using the diagnostic kits (Ortho-Clinical Diagnostic, Inc., USA) with manufacturer protocol.

The rats were anesthetized with an intraperitoneal (i.p.) injection of 4 ml/kg body weight, 1% sodium pentobarbital (40 mg/kg body weight). The left femoral artery was exposed and isolated through a middle inguina incision. A thin silastic catheter was introduced and secured with a silk ligature. The free end of the catheter was attached to short segments of steel tubing and tunneled subcutaneously through the back to the top of the skull and through a skin incision to the exterior. After patency had been checked, the catheter was flushed and filled with 0.5 ml 0.9% NaCl containing Heparin (20 IU/ml), and then capped.

The OGTT studies were then carried out in the morning after an overnight fasting. Throughout the study, rats were awake and allowed to move freely in a large cage. The 0-h (baseline) blood samples were drawn from the arterial cannula and analyzed for plasma glucose, insulin and lipid profile as described above. Then a glucose solution (1 g/ml) was immediately given to the animal at a dose of 2 g/kg body weight via gavage, and then the glucose levels were checked using the method mentioned above at the intervals of 30, 60, 90, and 120 min, respectively.

Spatial learning and memory tests

One week before the end of the experiment, animals were trained in a water maze to test spatial learning and memory as described (Morris 1984). Briefly, the maze consisted of a circular pool (φ1.2 m × 50 cm) made of stainless steel. The pool was filled with tap water to a height of 27 cm, and a black escape platform was placed 2 cm beneath the water surface. The water temperature was kept at 25 ± 2°C during the test period. Maze performance was recorded using a video camera mounted in the ceiling and connected to a computerized video tracking system (Taimeng Technology Company Limited, Chengdu, China). In order to shield the platform, a whitening agent, titanium dioxide, was dissolved in water and made the water oyster white. The pool was conceptually divided in four quadrants according to the cardinal points (N, E, S, and W). In the first 4 days, the rats were trained daily using a single four-trial session. The rats were trained to swim to the escape platform from each quadrant, where they stayed for 30 s. Between each round of training, they were placed in a black plastic bucket and rested for 20 s. When rats failed to reach the platform within 120 s, they were gently guided to the platform. The test was carried out in the fifth day, escape latencies and swimming paths were recorded using the video tracking system for each rat.

Statistics

One-way analysis of variance (ANOVA) was used to compare the means ± SEM. Group comparisons were made using Duncan's post hoc test. The significance levels were tested at $P < 0.05$.

Results

The vanadium contents in vanadium-enriched chickpea sprout powder

Based on the vanadium content in VCS powder and daily food consumption of ~30 g pellet food/rat, the

vanadium dosage for the diabetic rats in the three groups was estimated to be 191.1, 217.3, and 989.2 μg/day/kg body weight, respectively (Table 1).

Effects of CS and VCS food on body weight, blood glucose level, and insulin level in STZ-diabetic rats

The rats took CS and VCS food as ordinary pellet food. No signs of gastrointestinal stress were observed during the experimental period in VCS groups. The changes in body weight, blood glucose level, and insulin level are summarized in Figs. 1 and 2; Table 2. The body weight of the diabetic rats (D) not treated decreased continually in the 8-week period; while the non-diabetic control rats (C) grew steadily. All groups of rats being treated with CS-food and VCS-food slowly gained weight. The VCS100 fed rats had the highest rate of body weight gain.

The D rats showed initial high blood glucose levels (~22 mM) that continually increased during the 8-week period. The blood glucose levels of four treated groups gradually decreased. The VCS100 treated rats had the best hypoglycemic effect among the four groups during the 8-week period. At the end of experiment, the blood glucose level reduced from 22 to 8.6 mM.

The diabetic rats (D) exhibited much lower insulin levels compared with the control rats (C). Treatment of diabetic rats with CS and VCS food effectively increased insulin levels. The insulin secretion of diabetic rats fed VCS100 group (34.1μIU/ml) was higher than the other three treated groups, and very close to the normal level of 39.5μIU/ml.

Effect of CS and VCS on glucose tolerance of diabetic rats

The effect of CS and VCS on glucose tolerance after 8-week treatment with CS and VCS food was shown in

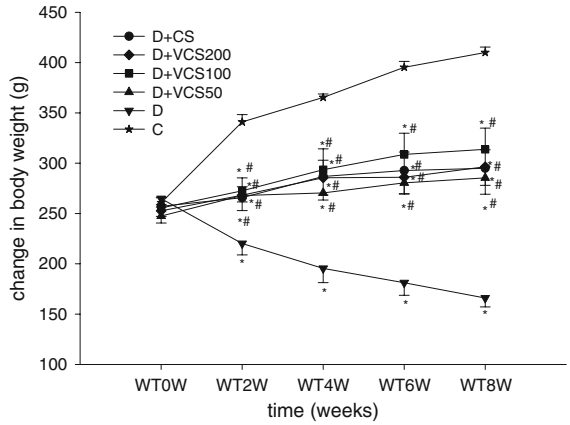

Fig. 1 Changes of body weight of rats in control (C) rats, diabetic (D) rats, diabetic rats treated with chickpea sprout food (CS), and three groups of diabetic rats treated with VCS food (D + VCS50, D + VCS100, and D + VCS200). There were seven rats in each group. Significant differences are shown at $P < 0.01$ by "*" (vs. the control group), and "#" (vs. the diabetic group)

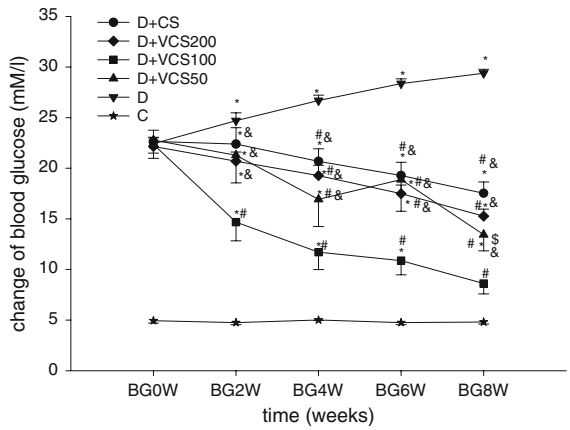

Fig. 2 Changes of blood glucose in control (C) rats, diabetic (D) rats, diabetic group treated with chickpea sprout food (CS), and three groups of diabetic rats treated with VCS food (D + VCS50, D + VCS100, and D + VCS200). There were seven rats in each group. Significant differences are shown at $P < 0.01$ by "*" (vs. the control group), "#" (vs. the diabetic group), "&" (vs. the VCS100 group) and "$"(vs. the CS group)

Table 1 Vanadium contents in chickpea sprout powder and the dosages for animals

Label	$Na_3VO_4 \cdot 12H_2O$ concentration in cultural water (μg/ml)	Vanadium content in chickpea sprout powder (μg/g dry weight)	Estimated vanadium dosage of animals (μg/day/kg body weight)
VCS50	50	8.6 ± 0.3	191.1 (3.7 μmol)
VCS100	100	10.5 ± 1.2	217.3 (4.1 μmol)
VCS200	200	45.8 ± 5.6	989.2 (19.4 μmol)

Table 2 The insulin levels of test rats at the end of the 8-week treatment

Group	
C	39.5 ± 19.3
D	16.7 ± 1.0*
D + CS	22.0 ± 3.1*·**·***
D + VCS50	23.3 ± 3.5*·**·***
D + VCS100	34.1 ± 12.6**
D + VCS200	21.4 ± 3.6*·**·***

Each value is a mean of seven rats ±S.E.M

* Significantly different from normal control at $P < 0.01$

** Significantly different at $P < 0.01$ from diabetic rats

*** Significantly different at $P < 0.05$ from the VCS100 group

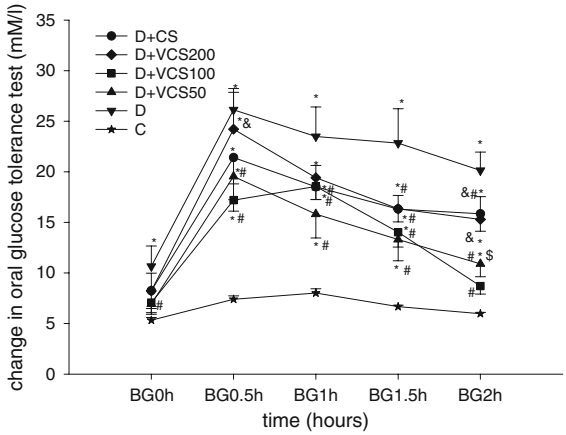

Fig. 3 OGTT curves for rats in control (C), diabetic (D), group treated with chickpea sprout food (CS), and three groups treated with VCS food (D + VCS50, D + VCS100, and D + VCS200). Data are average of seven rats in each group. Significant differences are shown at $P < 0.05$ by "*" (vs. the control group), "#" (vs. the diabetic group), "&" (versus the VCS100 group) and "$" (vs. the CS group)

Fig. 3 and the area under the curve (AUC) was calculated and listed in Table 3. During the 2 h following glucose ingestion, all the treated rats exhibited smaller area under curve than D rats with hypoglycemic potency of VCS100 > VCS50 > VCS200 > CS. The D + VCS100 group showed a similar curve shape for OGTT curve to the control group animals.

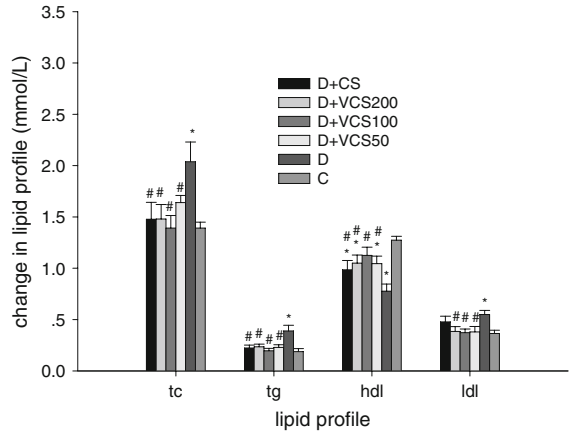

Fig. 4 Serum lipid profiles of diabetic rats treated with VCS. For each experimental group, the histograms are presented in the order of diabetic rats treated with chickpea sprout food (D + CS), three group of diabetic rats treated with VCS food (D + VCS200, D + VCS100, and D + VCS50, respectively), diabetic rats (D), and the control (C). Data are average of seven rats in each group. Significant differences are shown at $P < 0.05$ by "*" (vs. the control group) and "#" (vs. the diabetic group)

Effects on blood lipids

The influence of 8-week administration with CS and VCS-food on lipid metabolism in diabetic rats was showed in Fig. 4. The results showed that the treatment recovered the blood levels of TC, TG, LDL, and HDL. The best effects were observed in the D + VCS100 group.

The effects of CS and VCS on spatial learning and memory of diabetic rats

Prevention or delaying of the progression of serious complications has always been desirable for diabetes

Table 3 The OGTT–AUC of test rats at the end of the 8-week treatment

Group	D + CS	D + VCS200	D + VCS100	D + VCS50	D	C
OGTT–AUC (mmol/l h)	34.2 ± 8.3*·**	35.9 ± 9.1*·**	28.7 ± 4.9*·**	28.9 ± 10.0*·**	43.9 ± 8.6*	13.9 ± 1.0

Each value is a mean of seven rats ±S.E.M

* Significantly different from normal control at $P < 0.01$

** Significantly different at $P < 0.05$ from diabetic rats

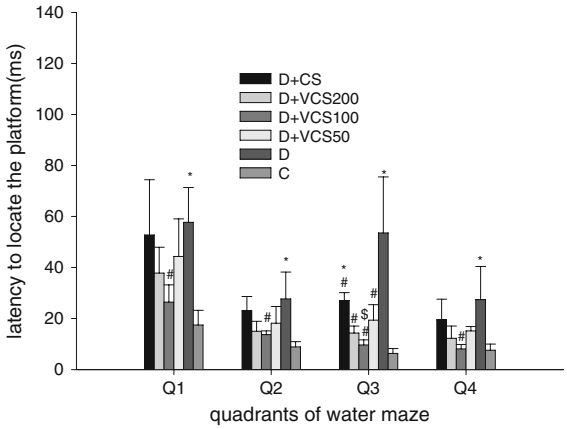

Fig. 5 Average latency to locate the platform in the water maze after 8-week treatment. For each experimental group, the histograms are presented in the order of diabetic rats treated with chickpea sprout food (D + CS), three group of diabetic rats treated with VCS food (D + VCS200, D + VCS100, and D + VCS50, respectively), diabetic rats (D), and the control (C). Data are average of seven rats in each group. Significant differences are shown at $P < 0.05$ by "*" (vs. the control group) "#" (vs. the diabetic group), and "$" (vs. the CS group)

(Hathan 1994). In diabetes, impaired cognition function possibly due to glucose insufficiency in brain and insulin deficiency has been recognized (Sima et al. 2004). In the present work, the effect of CS and VCS food on the neural function of diabetic rats, i.e. spatial learning and memory was measured using a water maze test and the results were shown in Fig. 5. Compared with the control rats (C), the diabetic rats (D) exhibit significant delayed latencies to locate the platform in all four quadrants ($P < 0.05$). After treatment, the time for locating the platform was shortened significantly. The best effect was again observed in the D + VCS100 group.

Discussions and conclusion

Vanadate has been shown to be a potent hypoglycemic agent, but with short-term effects such as diarrhea, decrease in weight gain, death due to dehydration; and potential long-term toxicities (metal accumulation in certain tissues such as kidney and liver, etc.) (Ramasarna 1996). To reduce the metal toxicity, we enriched vanadium in CS to provide a vanadium-content food together with certain botanic antioxidants. The hypoglycemic effects of the CS and VCS food were investigated.

During the 8-week experimental period, both CS and VCS food were found to improve hyperglycemic situation, including (i) lowering blood glucose levels (Fig. 2), (ii) improving glucose tolerance (Fig. 3 & Table 3), (iii) improving lipid metabolism (Fig. 4), (iv) improving function of spatial learning and memory (Fig.5), and (v) increase insulin secretion (Table 2). The overall effects of CS, although very limited, are similar to those of the antioxidants resveratrol (Baur et al. 2006).

It is conceivable that better hypoglycemic effects were observed in VCS food groups, especially theVCS100 group. The major improved differences between the CS alone and the VCSs are seen in hyperglycemia and memory loss. The diabetic rats fed with VCS100 pellet exhibited better glucose tolerance (Fig. 3 & Table 3) and their blood glucose level gradually reduced to be within normal range (below 10 mM) (Fig. 2). VCS100 food almost restored the function of spatial learning and memory in diabetic rats, especially in the later quadrants (Fig. 5). This protection of neural function was clearly a reasonable result of the effective control of hyperglycemic situation upon VCS100 food treatment. Since VCS food increased the basal insulin level close to the health C-group rats, restore/protect the function of pancreatic β cells upon VCS food treatment may account for at least part of the hypoglycemic effects.

Vanadium compounds have been shown to lower diabetic hyperlipidemia (Srivastava and Mehdi 2005; Gandorfer 2006; Shechter 1990; Srivastava 2000; Cam et al. 2000; Brichard 2003); Elevated plasma lipid levels are regarded as risk factors for the coronary heart disease. Herein, both CS and VCS food could effectively ameliorate the levels of TC, TG, LDL, and HDL (Fig. 4), but no significant difference was observed between the treatment groups. Similar results were observed in the studies of co-administration of vanadate with an antioxidant herb *Salvia miltiorrhiza Bunge* (data not shown). It is conceivable that the above fact is probably due to the strong therapeutic effect of the chickpea sprouts alone and suggests vanadium compounds might share a similar pathway with botanic antioxidants in regulating lipid metabolism.

It was noted that the order of the overall hypoglycemic effect is: VCS100 > VC50 ≥ CS ≥ VCS200, hereby, the highest dose of vanadium exhibit

marginal effect over the CS food base. In addition, the present effective dose (~4 μmol/kg body weight) of in term of vanadium element was significantly lower than the previously reported effective dose of $NaVO_3$ (~60 μmol/kg) and BMOV (80 μmol/kg) (McNeill et al. 1995; Meyerovitch et al. 1987). These results suggested further works on the possible reasons may be appropriate, which may lie on that: (i) the vanadium complexes formed in CS might be more potent or (ii) may have improved bioavailability. Although higher vanadate concentration in the sprout incubation solution could result in higher vanadium content in the sprout, but the amount effective vanadium specie might even less due to influence of high concentration of vanadate to the sprout growth. It is appropriate to elucidate the chemical speciation of vanadium-enriched in chickpea sprout and clarify whether the above low dosage of vanadium would result in vanadium accumulation and thus long-term vanadium toxicity; (iii) the botanic species e.g. isoflavone and biochanins might significantly enhance the hypoglycemic effects of vanadium complexes. Certain ratio of vanadium to antioxidant might be necessary for the enhancement effect. Nonetheless, novel mechanisms of the hypoglycemic effect of vanadium should be investigated, e.g. the role of vandyl ions in the signal transduction of 60 kDa heat shock protein (HSP60) proposed recently (Wan et al. 2007).

It is noted that during the period of treatment, no signs of short-term toxicity vanadium were observed and the body weight of the diabetic rats treated with VCS food increased steadily although slower than the healthy rats. The reasons for the low toxicity may include: (i) low vanadium dosage. The dosages of vanadium were estimated to be in the range of 190–990 μg/kg (Table 1); (ii) the botanic antioxidants such as flavonoids and biochanins effectively reduced vanadium toxicity as observed with lichee black tea decoction (Clark et al. 2004a, b) and green tea (Soussi et al. 2006).

In summary, vanadium was given in a low dosage to STZ-induced diabetic rats in a form of VCS food. The experimental results revealed that both CS and VCS food could ameliorate hyperglycemic situation and improved lipid metabolism, however, VCS100 food exhibited remarkably enhanced effectiveness compared with CS alone in alleviating diabetes induced hyperglycemia and memory loss, and importantly without observable short-term vanadium toxicity. The results suggested that the VCS food could be a novel dietary supplement beneficial for hyperglycemic situation and further works investigating its mechanism of hypoglycemic effects would be appropriate.

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