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A new salicylic acid-derivatized kojic acid vanadyl complex: Synthesis, characterization and anti-diabetic therapeutic potential

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ABSTRACT

The molecular mechanisms of vanadium toxicity suggest that incorporation of antioxidant groups in the structure of vanadium complexes could be a preferable strategy in designing novel hypoglycemic vanadium complexes with proper efficacy and safety. By conjugating a pyrone skeleton to provide a coordination group and antioxidative motifs, we synthesized a novel complex of bis ((5-hydroxy-4-oxo-4 H-pyran-2-yl) methyl 2-hydroxy- benzoatato) oxovanadium (IV) (BSOV). Evaluation of the anti-diabetic effects of BSOV using streptozotocin (STZ)-induced diabetic rats with bis (maltolato) oxovanadium (BMOV) as a positive control showed that BSOV effectively lowered blood glucose level, ameliorated damages of hepatic and renal function in diabetic rats and improved lipid metabolism. The signs of potential alteration of renal function caused by BSOV and BMOV were observed and are discussed. Overall, the experimental results suggest BSOV as a potent hypoglycemic agent and further studies using this strategy for anti-diabetic agents.

1. Introduction

Vanadium compounds have shown substantial promise as hypoglycemic agents for the pharmacotherapy of diabetes [1,2] and cancer chemoprevention agents [3,4]. Potential metal toxicity and poor pharmacokinetic behavior have limited the development of vanadium drugs. To overcome these problems, a number of vanadium compounds with organic ligands have been synthesized and evaluated for their insulin mimetic activity, e.g., bis(ethylmaltolato)oxovanadium(IV) (BEOV) by Orvig et al. [5,6], bis(allixinato)- oxovanadium(IV) and bis (picolinato)oxovanadium(IV) by Sakurai et al. [7–10] and vanadium(III, IV,V)-dipicolinate by Crans et al. [11,12]. Recently, BEOV was in the phase II clinical trial, however, a potential side effect, i.e., kidney changes, prevented further applications [13].

Previously, great efforts have been put in elucidating the mechanisms of vanadium toxicity [14–16]. Although the toxicity of vanadium compounds involves many aspects, e.g., damage of cell tight junction, cell apoptosis, and immunosuppression, vanadium-induced reactive oxygen species (ROS) and mitochondrial damages have been suggested to be the major adverse reaction mechanisms especially for normal cells [17]. Treatment with antioxidants has significantly reduced vanadium toxicity both in vitro and in vivo [18]. Co-administration of vanadate in an herbal decoction was shown to prevent observable vanadium toxicity without compromising its anti-diabetic potential [19].

Based on the molecular mechanism of vanadium toxicity, incorporation of antioxidant groups in the structure of vanadium complexes could be a preferable strategy in designing novel anti-diabetic vanadium complexes. Previously, direct coordination of vanadyl ions with natural antioxidants produced novel complexes such as bis (quercetinato)oxovanadium(IV) (BQOV) [20,21] and bis[curcumino] oxovanadium (BCOV) [22]. BQOV was shown to improve carbohydrate metabolism and to reduce overall oxidative stress [20,21]; BCOV was shown to improve the cardiovascular complications associated with diabetes [22].

To develop anti-diabetic vanadium agents with desirable efficacy and safety, we designed a novel type of ligands containing a pyrone skeleton as coordination motif and an antioxidative group derived from natural antioxidants. In the present work, we report the synthesis and evaluation of bis((5-hydroxy-4-oxo-4 H-pyran-2-yl) methyl 2-hydroxybenzo- atato) oxovanadium, BSOV. The hypoglycemic effects were tested on streptozotocin-induced diabetic rats. The salicylate group is expected to provide a balanced lipo/hydrophilicity of the complex and a property of antioxidative activity.

2. Experimental

2.1. Chemicals and instrumentation

Vanadyl sulfate ($VOSO_4 \cdot xH_2O$, $x = 3$ to 5) was purchased from Aldrich, streptozotocin (STZ) from Sigma and L-Ascorbic acid from Beijing Chemical Reagent Company (Beijing, China). All other

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chemicals and solvents were reagent grade and used without further purification unless otherwise specified. The positive control, bis (maltolato)oxovanadium (BMOV), was prepared and purified according to the published method [23].

The synthesized ligands and their complexes were characterized by infrared (IR) spectroscopy, mass spectrometry, elemental analysis, and NMR spectroscopy. Where appropriate, ^{1}H NMR was use for further characterization. Infrared spectra were recorded as KBr disks in the range 4000–400 cm^{-1} on a Nexus-470 FTIR spectrophotometer (Nicolet Instruments, USA). Mass spectra (positive ion mode) were obtained with a QSTAR (Applied Biosystems, USA) quadrupole time-of-flight (TOF) hybrid mass spectrometer.

2.2. Synthesis

The synthesis route is illustrated in Scheme 1:

2-(chloromethyl)-5-hydroxy-4 H-pyran-4-one (Chlorokojic, 2). Compound **2** was prepared according to a previously reported method [24].

(5-hydroxy-4-oxo-4 H-pyran-2-yl)methyl 2-hydroxybenzoate (3). Compound **3** was prepared using a modified literature procedure [25]. Briefly, sodium salicylate (1 g, 6.3 mmol) and chlorokojic acid (0.9 g, 5.6 mmol) were dissolved into 80 ml of N,N- dimethylformamide. The resulting solution was heated with stirring for 2 h in an oil bath at 110 °C. After cooled to room temperature, the solution was poured into water. The product (1.3 g, 80%) was obtained by filtration and purified by the recrystallization from methanol. IR (cm^{-1}, KBr disk) data: 3268 cm^{-1}(ν_{O-H}); 3500–3000 cm^{-1}(ν_{C-H}); 1686, 1651 cm^{-1}(salicylic acid $\nu_{C=O}$); 1619, 1594, 1484 cm^{-1}(pyrone $\nu_{C=O}$, ring $\nu_{C=C}$); ^{1}H NMR (DMSO-d_6, 400 Hz, s = singlet, m = multiplet) data: δ 5.22 (s, 2 H), 6.85 (s, 1 H), 6.94–7.83 (m, 4 H), 8.13 (s, 1 H), 9.26 (s, 1 H), 10.31 (s, 1 H); *m/z* 263.05 (calc. 263.21) $[M+1]^+$.

Bis ((5-hydroxy-4-oxo-4 H-pyran-2-yl)methyl 2-hydroxybenzoatato) oxovanadium (BSOV). (5-Hydroxy-4-oxo-4 H-pyran-2-yl) methyl 2-hydroxybenzoate (2.7 g, 10.3 mmol) and sodium hydroxide (0.4 g, 10.0 mmol) were dissolved into 100 ml water and 1.9 g of vanadyl sulfate was added. The resulting mixture was heated with stirring for 12 h in an oil bath at 90 °C. After the mixture was cooled to room temperature, the precipitate was filtered. The yield of the complex was 65% on the basis of the ligand. The prepared complex was characterized by elemental analysis and IR absorption, and Mass spectrometry. IR (cm^{-1}, KBr disk) data: 3500–300 cm^{-1}(ν_{O-H}, ν_{C-H}); 1679 cm^{-1}(salicylic acid $\nu_{C=O}$); 1610, 1565, 1512, 1473 cm^{-1}(pyrone $\nu_{C=O}$, ring $\nu_{C=C}$); 984 cm^{-1}($\nu_{V=O}$). ESI-MS data: *m/z* 590.01 (calc. 590.35) $[M+1]^+$. Anal. Calcd. for $C_{26}H_{18}O_{13}V$:C, 52.99; H, 3.08. Found: C 52.77, H 3.12.

2.3. In vitro tests of antioxidant activity

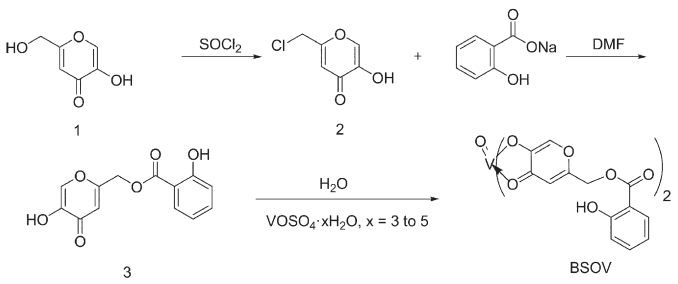
The antioxidant activity of vanadium complexes and free ligand were tested by hydroxyl radical scavenging capacity (HRSC) as previously described [26]. Briefly, antioxidants at various concentrations were added to Fenton reaction media containing 0.010 mM Rhodamine B, 1.0 mM $FeSO_4$, 5 mM of cetyl trimethyl ammonium bromide (CTAB), and 20 mM acetic acid (pH 2.8). After addition of 2.0 mM (final concentration) of H_2O_2, the reactions were left for 10 min at room temperature. Then absorbance A_s at 550 nm was measured. The antioxidant recovery capacity R was calculated as:

$$R = (A_s - A_b) / (A_0 - A_b)$$

Where A_0 is the control ($c_{antioxidant} = 0$) and A_b is the reagent blank. Then the data was further fitted to the following equation:

$$\frac{1}{R} = \frac{1}{c_{antioxidant}} \frac{K}{k \cdot R_{max}} + \frac{1}{R_{max}}.$$

Then R_{max} and k/K were obtained using an Origin 7.0 program. HRSC index was calculated by setting the k/K value of ascorbic acid as 1.0.



Scheme. 1. The pathway for synthesis of BSOV.

2.4. Animal treatments

Male Sprague–Dawley rats, weighing about 220–240 g, were obtained from Experimental Animal Center of Peking University, and maintained on a light/dark cycle. All animals were allowed free access to standard, solid rat chow and water. Temperature and relative humidity were maintained at 24 °C and 50%, respectively. Rats were acclimatized for 7 days prior to induction of diabetes. All care and handling of animals were performed with the approval of Institutional Authority for Laboratory Animal Care.

Diabetes was induced by a single intravenous injection of freshly prepared STZ (55 mg/kg body weight) in 0.1 mol/L citrate buffer (pH 4.5) [27]. The control rats were injected with an equal volume of vehicle. Rats were supplied with 5% glucose solution for 48 h after STZ injection in order to prevent hypoglycemia. Seven days after the streptozotocin administration, blood was collected from the tail vein and serum samples were analyzed for blood glucose. Animals showing fasting (12 h) blood glucose higher than 13.3 mmol/L were considered to be diabetic and used for the study. All drug candidates were administered as suspensions in 0.5% methyl-cellulose (MC) by oral gavage in a volume of 5.0 ml/kg body weight at a dose of 0.4 mmol/kg body weight for 4 weeks. The control groups received an equivalent volume of 0.5% methyl-cellulose alone.

A total of 56 rats (32 diabetic rats and 24 control rats) were randomly divided into seven groups: Group I, Control rats ($n = 8$); Group II, Ligand-treated non-diabetic rats ($n = 8$); Group III, BSOV-treated non-diabetic rats ($n = 8$); Group IV, Diabetic rats ($n = 8$); Group V, Ligand-treated diabetic rats ($n = 8$); Group VI, BSOV-treated diabetic rats ($n = 8$); and Group VII, BMOV-treated diabetic rats ($n = 8$). The BMOV-treated group was considered as the positive control group.

Throughout the experimental period, the body weight and drinking water intake were monitored daily. In the meantime, the food consumption was recorded. Blood samples were obtained from the tail vein of the rats and blood glucose levels were determined with an Accu-Chek blood glucose monitor (Roche Diagnostics GmbH, Mannheim, Germany).

2.5. Glucose tolerance tests (OGTT)

After the administration of BMOV or BSOV for 4 weeks, OGTT was carried out. The rats were fasted for 12 h, and glucose (200 mg/mL) was given by oral gavage at a dose of 2 g/kg body weight. Blood samples were obtained from the tail vein at 0, 30, 60, 120 and 180 min after glucose loading, respectively. Blood glucose levels were measured with an Accu-Chek blood glucose monitor.

2.6. Serum biochemical parameters test

The rats were deprived of food overnight, and blood samples were collected for determination of biochemical parameters. Biochemical parameters in serum, including total cholesterol (TCHO), triglycerides (TG), alanine amino transferase (GPT), aspartate amino transferase (GOT), creatinine (CRE), blood urea nitrogen (BUN), and albumin (ALB) were determined using assay kits from Randox (Antrim, UK) on an OLYMPUS AU400 chemistry analyzer (Olympus, Tokyo, Japan).

2.7. Statistical analysis

All data are presented as means ± SD. The statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests using a SPSS 13.0 software. Statistical significance was accepted at the level of $p<0.05$.

3. Results

3.1. In vitro antioxidative activity

The antioxidant activity of BSOV, BMOV and ligand were tested as hydroxyl radical scavenging capacity (HRSC), Table 1. The salicylic acid-derivatized ligand gave better HRSC index than the parental compounds. Interestingly, both vanadyl complexes, especially BSOV, exhibited excellent antioxidant activity.

3.2. Blood glucose level

Fig. 1 illustrates the changes of fasting blood glucose level in control and diabetic rats treated with BSOV and BMOV each at a dose of 0.4 mmol/kg body weight/day. Compared with the blood glucose level of STZ-diabetic rats, the blood glucose level of diabetic rats treated with BSOV and BMOV reverted back close to normal levels; while the salicylic acid-derivatized ligand did not show any hypoglycemic effects.

3.3. Glucose tolerance test

To evaluate the improvement in the glucose tolerance ability in diabetic rats, an OGTT was performed after the 4 weeks treatment (Fig. 2). After oral administration of glucose, the blood glucose concentration in diabetic rats reached a maximal concentration in 1 h and returned to the baseline in the next 2 h. Although both BSOV and BMOV-treated diabetic rats still showed significant fluctuation in blood glucose level, the peak values were much lower than that of the diabetic rats ($p<0.01$) and returned close to the normal blood glucose level at 2–3 h after glucose administration, indicating comparable extent of improvement in glucose tolerance in the two vanadium compound-treated rats. The ligand treatment did not affect glucose tolerance with significance in diabetic or none diabetic rats.

Table 1
Hydroxyl radical scavenging capacity of vanadium compounds and the references.

Compounds	Fitting results			HRSC index
	R_{max}	k/K	r^2	
L-ascorbic acid	1.32	0.31	0.9673	1.0
Salicylic acid	1.12	0.72	0.9677	2.3
Kojic acid	1.36	2.01	0.9606	6.5
Salicylic-derivatized ligand	0.95	2.53	0.8284	8.1
BSOV	0.73	14.34	0.9399	46.3
BMOV	1.23	2.45	0.9293	7.9

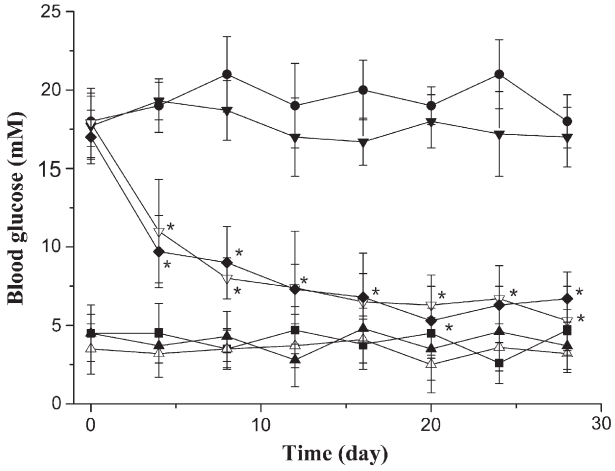


Fig. 1. Changes in blood glucose levels upon vanadium treatment. Control rats (■), Ligand-treated normal rats (▲), BSOV-treated normal rats (△), Diabetic rats (●), Ligand-treated diabetic rats (▼), BSOV-treated diabetic rats (▽), BMOV-treated diabetic rats (◆). Values are expressed as means ± SD. $^{*}p<0.01$ *vs.* diabetic rats.

3.4. Serum biochemical parameters test

The levels of TCHO, TG, GPT, GOT, BUN ALB, and CRE in serum have been summarized in Table 2. Compared to the control rats, results can be summarized as: (i) treatment with vanadium complexes ameliorated most of hyperglycemia-elevated serum biochemical parameters, i.e., BUN, TG, TCHO, GOT, and CRE. The effects of treatment with BSOV were similar to those of BMOV; (ii) significant decrease of TCHO and TG levels to even lower than those in normal control rats; (iii) no significant improvements of ALB levels of diabetic rats; (iv) with diabetic rats, the free ligand reduced the serum parameters similar to the complexes; and (v) both BSOV and free ligand did not significantly affect the serum parameters of normal rats.

3.5. Physiological parameters

The changes of body weights of control and diabetic rats are shown in Fig. 3. The none diabetic rats (control rats, ligand-treated, and BSOV-treated groups) steadily gained weight until the end of the study. While the diabetic rats (diabetic control and vanadium-treated groups) remain their body weight. At the end of the experiment,

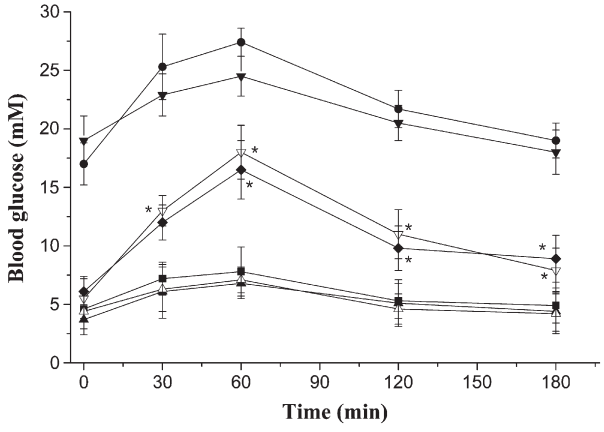


Fig. 2. Oral glucose tolerance test curves. Control rats (■), Ligand-treated normal rats (▲), BSOV-treated normal rats (△), Diabetic rats (●), Ligand-treated diabetic rats (▼), BSOV-treated diabetic rats (▽), BMOV-treated diabetic rats (◆). Values are expressed as means ± SD. $^{*}p<0.01$ *vs.* diabetic rats.

Table 2
Effects of BSOV on serum biochemical parameters in STZ-induced diabetic rats.

	BUN (mM)	ALB (g/L)	TG (mM)	TCHO (mM)	GPT (U/L)	GOT (U/L)	CRE (mg/dL)
Control	7.09 ± 0.60*	30.91 ± 1.87*	0.63 ± 0.19*	1.66 ± 0.32*	45 ± 9*	95 ± 18*	0.64 ± 0.07*
Normal + Ligand	6.02 ± 1.20*	31.63 ± 0.78*	0.55 ± 0.13*	1.38 ± 0.24*	52 ± 10*	103 ± 23*	0.71 ± 0.06
Normal + BSOV	7.14 ± 1.40*	30.66 ± 1.04*	0.74 ± 0.27*	1.56 ± 0.19*	57 ± 13*	116 ± 82*	0.67 ± 0.09*
Diabetic	32.53 ± 6.81#	26.16 ± 1.06#	0.94 ± 0.39#	2.26 ± 0.29#	245 ± 57#	711 ± 95#	0.77 ± 0.06#
Diabetic + Ligand	23.98 ± 3.98*,#	27.47 ± 0.94#	0.51 ± 0.48	1.03 ± 0.48*,#	206 ± 51#	218 ± 106*,#	0.57 ± 0.12
Diabetic + BSOV	22.79 ± 7.39*,#	28.28 ± 2.21#	0.48 ± 0.13*	0.82 ± 0.16*,#	209 ± 45#	255 ± 115*,#	0.58 ± 0.06*
Diabetic + BMOV	23.00 ± 5.89*,#	27.48 ± 1.56#	0.46 ± 0.29*	0.78 ± 0.25*,#	216 ± 27#	294 ± 91*,#	0.61 ± 0.03*

Values are expressed as mean ± SD for ten rats in each group.
# $p<0.05$ or less vs. control rats (Dunnett's test).
* $p<0.05$ or less *vs.* diabetic rats (Dunnett's test).

the body weights of both vanadium- and ligand-treated groups were close to their control groups.

The daily food and water intake of diabetic rats were significantly higher than that of normal control rats (Fig. 4). Both BMOV and BSOV treatment significantly lowered food and water consumption 2 days after drug administration and remained constant throughout the experiment. During the experimental period, no signs of GI stress were observed for BMOV, BSOV and salicylic acid-derivatized ligand.

4. Discussion

The insulin-mimetic effect of vanadium compounds has been widely documented in diabetes animal models [7,27,28]. In this study, BSOV was designed to combine kojato and salicylic acid to provide a balanced lipo/hydrophilicity of the complex and a property of antioxidative activity. In testing the anti-diabetic effects of BSOV, BMOV, a well-known vanadium complex, was used as the positive control.

As shown in Figs. 1–4, BSOV was observed to (i) effectively lower blood glucose level to close to the normal in 4 weeks treatment (Fig. 1), (ii) significantly ameliorate the impaired glucose tolerance in STZ-diabetic rats (Fig. 2), and (iii) remarkably decrease food and water intake in group diabetic rats (Fig. 4). Compared to the positive control, the experimental results indicate that BSOV was as potent a hyperglycemic agent as the previously investigated BMOV.

In addition to the hypoglycemic effects, BSOV treatment ameliorated damages of hepatic and renal function in diabetic rats as revealed by decrease of GPT, GOT, and BUN levels (Table 2). It has been well documented that STZ-induced hyperglycemia causes profound alterations in the concentration and composition of lipids [29]. BSOV treatment was found to significantly reduce the elevated serum TG and TCHO concentrations (Table 2), suggesting enhancement of lipid metabolism. Overall, BSOV treatment improved hyperglycemia state in a similar extent to those of BMOV.

However, there were signs indicating potential toxicity of both BSOV and BMOV: the body weight of vanadium compound-treated diabetic rats did not grow as the normal rats, and the level of ALB was not improved. The reasons may be complex: (i) a malabsorption caused by vanadium complexes. However, since GI stress signs were not observed, further evidence needed to support this statement; (ii) the toxicity of the dissociated ligands. However, as shown in Table 2, the free ligand did not exhibit any significant toxicological effects on normal rats. The recovery of the altered organ function in the diabetic rats was probably due to the antioxidant activity; and (iii) the unexpected excretion of serum albumin (ALB) due to change of renal function. Previously, it was suggested that vanadium compounds can cause damage of cell tight junction [30] and the alteration of the tight junction of the podocytes in glomerulus was an important reason for urine protein elevation in diabetes [31–33].

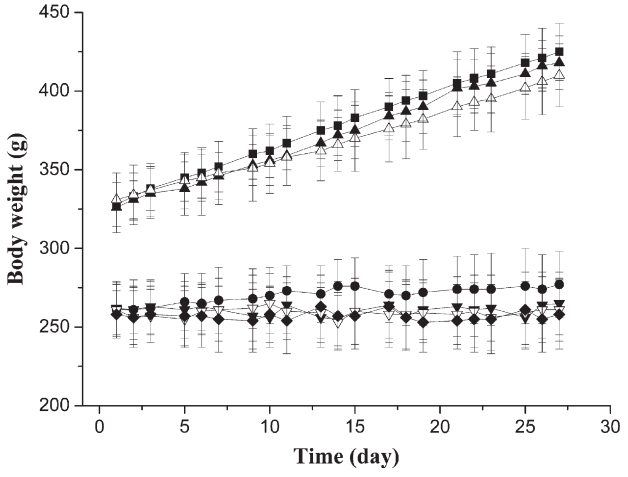


Fig. 3. Changes of body weight during vanadium treatment. Control rats (■), Ligand-treated normal rats (▲), BSOV-treated normal rats (△), Diabetic rats (●), Ligand-treated diabetic rats (▼), BSOV-treated diabetic rats (▽), BMOV-treated diabetic rats (◆). Values are expressed as means ± SD.

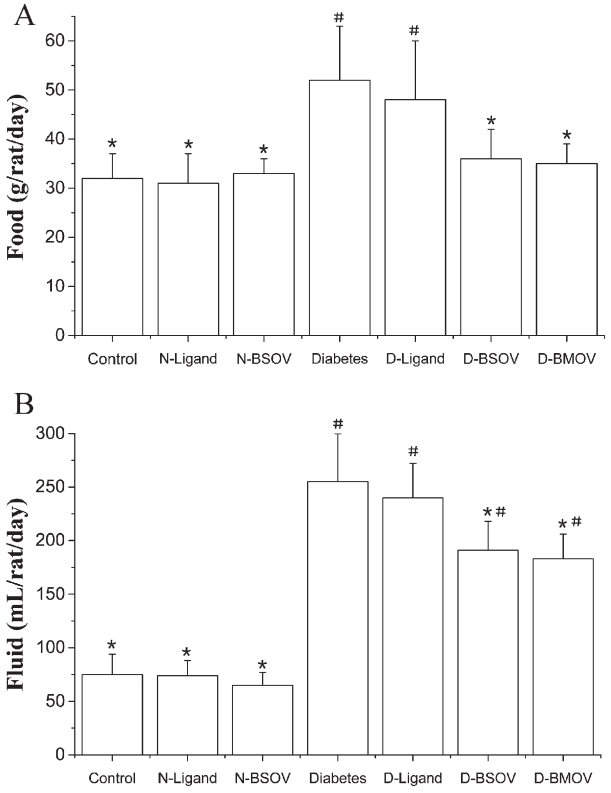


Fig. 4. Food (A) and water (B) intake of control and experiment groups of rats. Values are given as mean ± SD. #$P<0.05$ or less vs. control rats; *$p<0.05$ or less *vs.* diabetic rats.

Therefore, the vanadium toxicity on cell tight junction may increase leakage of kidney, thus ALB level did not increase with the overall improvement of the hyperglycemic status upon treatment with vanadium complexes.

BSOV was not substantially more effective and tolerated than BMOV as expected. Possible problems with BSOV include: (i) the salicylate group did not provide adequate antioxidant capacity; and (ii) the complexes degrade in blood. A preliminary test of plasma protein binding suggested that BSOV, similar to BMOV, may bind to protein and at least partially dissociate during incubation (data not shown). Improved antioxidant activity and *in vivo* stability of the vanadium complexes could then be expected.

In conclusion, a novel vanadium complex BSOV has been designed and synthesized based on the structural-ADMET properties relationship. Evaluation using STZ-induced diabetic rats indicated that BSOV exhibited anti-diabetic potency and the present results suggest that further studies are worthwhile using ligands derived from natural antioxidants.

Abbreviations

ALB	albumin
BCOV	bis[curcumino]oxovanadium
BEOV	bis(ethylmaltolato)oxovanadium(IV)
BMOV	bis(maltolato)oxovanadium
BQOV	bis(quercetinato)oxovanadium(IV)
BSOV	bis((5-hydroxy-4-oxo-4 H-pyran-2-yl) methyl2-hydroxybenzoatato)oxovanadium(IV)
BUN	blood urea nitrogen
CRE	creatinine
GOT	glutamine-oxaloacetic transaminase/aspartate aminotransferase
GPT	glutamic-pyruvic transaminase/alanine aminotransferase
TCHO	total cholesterol
TG	triglycerides
OGTT	glucose tolerance test
ROS	reactive oxygen species
STZ	Streptozotocin

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