Preparation and Spectroscopic of Vanadyl(Ⅱ) Vitamin D_3 Amino Acid Mixed Complexes as Insulin Mimetic Drug

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Abstract A new six intraperitoneal injection insulin-mimetic vanadyl(Ⅱ) compounds $[(VD_3^{-1})(VO^{+2})(AA_n^{-1})]$ (where (n=1～6); AA_1=isoleucine, AA_2=threonine, AA_3=proline, AA_4=phenylalanine, AA_5=lysine and AA_6=glutamine) were synthesized by the chemical reactions between vitamin D_3 (VD_3), $VOSO_4$ and amino acids (AA_n) with equal molar ratio 1∶1∶1 in neutralized media. The structures of these complexes were elucidated by spectroscopic methods like, infrared and solid reflectance spectroscopes. Magnetic moments and electronic spectra reveal square pyramid geometrical structure of the complexes. The infrared spectra assignments of these complexes revealed that the chelation towards vanadyl(Ⅳ) ions existed *via* deprotonation of the hydroxyl group of VD_3 drug ligand and so amino acids act as bidentate ligand *via* N-amino and O-carboxylate groups. The anti-diabetic efficiency of these complexes were evaluated against streptozotocin induced diabetic male albino rats.

Keywords Insulin alternative; Diabetes; Drug; VO^{2+} ion; Vitamin D_3; Amino acid; Spectroscopic
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Introduction

The expanding knowledge of the role of vanadium in biological systems and of the potential of vanadium compounds as therapeutic agents has led to a continuously increasing interest in the coordination chemistry and solution chemistry of this element[1]. Within the spectrum of vanadium complexes that have been synthesized as model compounds for the understanding of vanadium-controlled biological systems or as potential therapeutic agents with insulin-mimetic properties, one finds a substantial number of oxovanadium (Ⅴ) chelate complexes with a variety of donor set[2]. Moreover, the discoveries of several medicinal properties of vanadium complexes viz., insulin-mimetic, anticancer, antitumour and antifungal/antibacterial activities[1-2] have stimulated further research in this area. Diabetes mellitus was a serious metabolic disease that tendency to diseases and multiple-organ impairment[3]. The lacks of β-cells in the pancreas were the main cause of pathophysiological markers in the progress of both two types of diabetes either 1 or 2[4]. Therefore, the great therapeutic goal is to achieve the remarkable production and generation of pancreatic islets that would consequently ameliorate diabetes and reduced its complications[5]. Vitamin D_3 (Fig. 1) is synthesized in human skin on exposure to ultraviolet (UV) radiation from sunlight, or it can be obtained from food. Vitamin D has a well-known role as regulator of calcium homeostasis and is critical for bone mineralization[6]. In addition, vitamin D has been associated with various regulatory effects on the immune system[7]. Vitamin D deficiency in animals and hu-

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mans produces defects in bone mineralization, such as rickets and osteomalacia, diseases characterized by an increase in osteoid and impaired calcium phosphate deposition[8]. An increased prevalence of diabetes has been described in Vitamin D deficient individuals[9]. This work aimed to synthesis and spectroscopic characterizations of new vanadyl(Ⅳ) vitamin D_3 complex, for the purpose of use as a treatment for diabetes, that induced by streptozotocin (STZ) in male albino rats.

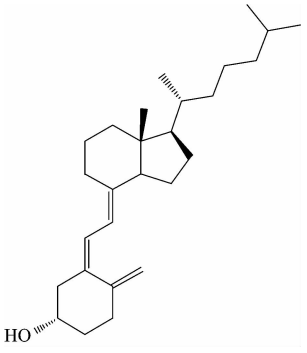

Fig. 1　Chemical structure of vitamin D_3 (VD_3)

1　Experimental

1.1　Chemical and reagents

All chemicals and solvents used in this investigation were of highest purity grade (Aldrich) and employed without further purifications. Vitamin D_3 pure drug, vanadyl(Ⅱ) sulfate and amino acids (isoleucine, threonine, proline, phenylalanine, lysine and glutamine as represented in were bought from Aldrich Company.

1.2　Synthesis of vanadyl(Ⅳ)-vitamin D_3-amino acid complexes

The solid vanadyl (Ⅳ)-vitamin D_3-amino acid complexes with genaral formula $[(VD_3^{-1})(VO^{+2})(AA_n^{-1})(H_2O)]$ (Ⅰ—Ⅵ) (where AA_1 = isoleucine, AA_2 = threonine, AA_3 = proline, AA_4 = phenylalanine, AA_5 = lysine and AA_6 = glutamine) were prepared by employing equal molar ratios of (VD_3 : $VOSO_4$: AA_n). These complexes were isolated by directly mixing 1.0 mmol of isoleucine (0.131 gm), threonine (0.119 gm), proline (0.115 gm), phenylalanine (0.165 gm), lysine (0.146 gm) and glutamine (0.146 gm) with (40 mL) of CH_3OH/H_2O solution of vitamin D_3 (0.384 gm, 1.0 mmol) and then added of $VOSO_4 \cdot H_2O$ salt (0.181 gm, 1.0 mmol). All these mixtures were neutralized using diluted ammonia solution (conc. 5%) at pH (8.0～9.0) and refluxed at ～60 ℃ for 30 min till the precipitates settled down then left to evaporate slowly at room temperature overnight. The solid colored precipitates were filtered off, washed several times by minimum amount of hot methanol and dried at 60 ℃, then stored in a vacuum desiccator over anhydrous $CaCl_2$.

1.3　Instruments

The elemental analyses of %C, %H and %N contents were performed by the microanalysis unit using a Perkin Elmer CHN 2400 (USA). The vanadium metal content was determined gravimetrically by the direct ignition of the respected complexes at 800 ℃ for 3 hrs till constant weight. The residue was then weighted in the form of vanadium oxide. The molar conductivity of freshly prepared 1.0×10^{-3} mol·cm^{-3} dimethylsulfoxide (DMSO) solution was measured for the dissolved vanadyl (Ⅳ) vitamin D_3 amino acid complexes using Jenway 4010 conductivity meter. The Solid reflectance spectra were measured on UV-3101 PC, Shimadzu, UV-Vis Spectrophotometer. The infrared spectra with KBr discs were recorded on a Bruker FT-IR Spectrophotometer (4 000 ～ 400 cm^{-1}). Magnetic data were calculated using Magnetic Susceptibility Balance, Sherwood Scientific, Cambridge Science Park Cambridge, England, at Temp 25 ℃ in Cairo University. The electron spin resonance (ESR) spectra for vanadyl (Ⅳ) vitamin D_3 amino acid complexes were performed on Jeol, JES-FE2XG, ESR-spectrometer, Frequency 9.44 GHz with Jeol Microwave unit. The thermal study TG/DTG-50H was carried out on a Shimadzu thermogravimetric analyzer under nitrogen atmosphere till 800 ℃. Scanning electron microscopy (SEM) images were taken in Quanta FEG 250 equipment. The transmission electron microscopy images were performed using JEOL 100s microscopy. The X-ray diffraction patterns for the vanadyl (Ⅳ) vitamin D_3 amino acid complexes were recorded on X'Pert PRO PANanalytical X-ray powder diffraction, target copper with secondary monochromate.

1.4　Experimental animals design

Male albino rats (weighing 100～120 g) were purchased from National Research Center in Cairo (Egypt). Animals were allowed free access to diet and water in good air conditioned room and were allowed free access and tap water for two weeks before starting the experiment. We have followed the European community Directive (86/609/EEC) and national rules on animal care. Animals were divided into four groups with 10 animals in each group as following:

Group (Ⅰ): This served as normal control. Group (Ⅱ): This untreated diabetic group (positive control) that was injected intraperitoneally (i.p) by a single dose of STZ (50 mg·kg^{-1} body weight)[10]. Group (Ⅲ): This was injected i.p. with STZ (50 mg·kg^{-1} body weight) then injected each alternative day i.p. by vanadyl sulfate (Ⅳ) alone at a dose of (40 mg·kg^{-1} body weight) for 30 days. Group (Ⅳ): This was injected with STZ (50 mg·kg^{-1} body weight) and then injected each alternative day i.p. by vanadyl (Ⅳ) vitamin D_3 amino acid complexes (Ⅰ—Ⅵ) at a dose of (40 mg·kg^{-1} body weight)[11] for 30 days.

1.5　Induction of experimental diabetes

Experimental diabetes was induced in 18 hrs fasted rats

by single i. P. injection of STZ in a dose of 50 mg • kg^{-1}[11] freshly prepared in cold 0.1 mol • L^{-1} citrate buffer (pH 4.5). STZ injected rats were provided with a 5% glucose drinking solution for the first 24 hrs to ensure survival[12]. Animals were considered diabetic when their blood glucose level exceeded 220 mg • dL^{-1}[13] and were included in the study after 72 hrs of STZ injection. Blood samples of the fasted rats were collected from the medial retro-orbital venous plexus immediately with capillary tubes (Micro Haematocrit Capillaries, Mucaps) under ether anesthesia[14]. Hemoglobin (Hb) measurements were determined using cell counter (Sysmex, model KX21N) in grams per deciliter (g • dL^{-1}) of blood. Insulin was assayed using insulin-I125 kit according to Woodhead et al.[15] using Radioimmunoassay kit obtained from Radioassay System Laboratories Inc (England). Triglycerides, cholesterol, high density lipoprotein-cholesterol (HDL-c) and Low density lipoprotein-cholesterol (LDL-c) levels were determined using fully auto-chemistry analyzer (Roch Integra 400 plus analyzer). The level of LDH, ALT, creatinine and uric acid were determined using fully auto-chemistry analyzer (Roch Integra 400 plus analyzer). The activities of G6PDH (glucose-6-phosophate dehydrogenase) were determined using the commercial kits. SOD was determined according to using biodiagnostic kit. Firstly, 0.5 mL of EDTA blood washed four times with normal saline to obtain washed erythrocytes. The washed erythrocyte made up to 2.0 mL with cold redistilled water and mixed then stand at 4 ℃ for 15 min. 100 mL of this lysate was mixed with working reagent and the absorbance was measured at 560 nm for 5 min for both control and for sample. Small pieces of liver and pancreas tissues were freshly collected directly and were done according official method[16]. Data were collected, arranged and reported as mean ± standard error of mean (S. E. M) for all groups were summarized and then analyzed using the computer program SPSS/version 15.0)[17].

2 Results and discussion

2.1 Interpretations of the chemical structure

The new six vanadyl(Ⅳ) complexes which are synthesized in situ mixed ligands of vitamin D_3 and amino acid chelates have higher melting point >260 ℃, with yield 80%～85%. The microanalytical, physical and chemical formulas of these complexes are summarized in Table 1. This resulted data is in a good agreement with general formula of $[(VD_3^{-1})(VO^{+2})(AA_n^{-1})(H_2O)]$ complexes. The absence of SO_4^- ions was confirmed by using 10% stock solution of $BaCl_2 \cdot 2H_2O$ reagent. The molar conductance data for the vanadyl(Ⅳ) vitamin D_3 amino acid complexes in DMSO solvent (1.0×10^{-3} mol • L^{-1}) were found to be within limit of 28～36 $\Omega^{-1} \cdot cm^2 \cdot mol^{-1}$ at room temperature. These values confirm that to be a non-electrolytic statement[18], hence the molar conductance values indicated that absence of SO_4^- ions inside the coordination sphere. The experimental results were agreement with check out of SO_4^- ions using $BaCl_2 \cdot 2H_2O$ reagent after the dissociation of vanadyl(Ⅱ) complexes in concentrated nitric acid.

Table 1 Elemental analysis and physical data of $[(VD_3^{-1})(VO^{+2})(AA_n^{-1})(H_2O)]$ complexes

Complex	Color	M. wt. (g • $mole^{-1}$)	(calcd.)/found % C	% H	% N	% V	μ_{eff} BM	$\Lambda m/(\Omega^{-1} \cdot cm^2 \cdot mol^{-1})$
$[(VD_3)(VO)(AA_1)(H_2O)]$ $(C_{33}H_{57}NO_5V)$	Brown	598	(66.22) 66.17	(9.53) 9.40	(2.34) 2.25	(8.52) 8.50	2.1	29
$[(VD_3)(VO)(AA_2)(H_2O)]$ $(C_{31}H_{53}NO_6V)$	Green	586	(63.48) 63.29	(9.04) 8.45	(2.38) 2.26	(8.70) 8.66	2.2	36
$[(VD_3)(VO)(AA_3)(H_2O)] \cdot 6H_2O$ $(C_{32}H_{65}NO_{11}V)$	Green	690	(55.65) 55.54	(9.42) 9.15	(2.02) 2.00	(7.39) 7.30	2.0	31
$[(VD_3)(VO)(AA_4)(H_2O)] \cdot 5H_2O$ $(C_{36}H_{65}NO_{10}V)$	Yellowish green	722	(59.83) 59.83	(9.00) 8.98	(1.93) 1.92	(7.06) 7.03	2.2	28
$[(VD_3)(VO)(AA_5)(H_2O)] \cdot 2H_2O$ $(C_{33}H_{62}N_2O_7V)$	Green	649	(61.01) 60.90	(9.55) 9.48	(4.31) 4.27	(7.85) 7.82	2.3	30
$[(VD_3)(VO)(AA_6)(H_2O)]$ $(C_{32}H_{54}N_2O_6V)$	Yellowish green	613	(62.64) 62.18	(8.80) 8.72	(4.56) 4.49	(8.31) 8.27	2.1	36

The electronic diffuse reflectance spectra of $[(VD_3^{-1})(VO^{+2})(AA_n^{-1})(H_2O)]$ complexes show the characteristic bands of VO(Ⅳ) in a square pyramidal configuration. The absorption bands at around 760～775 nm and the bands at about 600～630 nm are assigned to the spin allowed $^2B_2 \rightarrow {}^2E$ and $^2B_2 \rightarrow {}^2B_1$ transitions, respectively[19]. The weak bands at about 505～570 and 410～440 nm can be assigned to the ligand-to-metal charge transfer (L-M_{CT}) band. Magnetic measurement was carried out according to the Gauy method. Magnetic moment of vanadyl (Ⅳ) vitamin D_3 amino acid complexes were measured at room temperature and effective magnetic moments μ_{eff} value inserted within the 2.0～2.3 BM range

that assigned to a one electron of the $3d^1$ system of square pyramidal oxovanadium(Ⅳ) complexes[19].

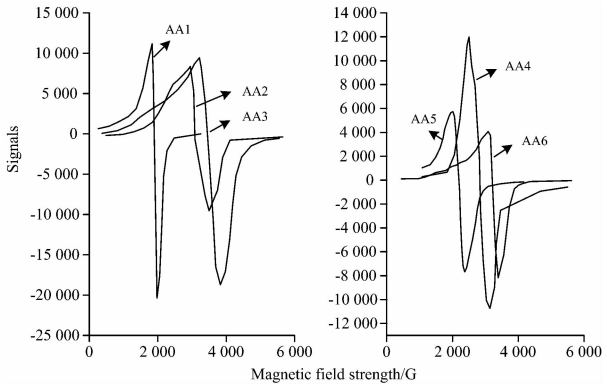

Fig. 2　ESR spectra of $[(VD_3^{-1})(VO^{+2})(AA_n^{-1})(H_2O)]$ complexes (where AA_1 = isoleucine, AA_2 = threonine, AA_3 = proline, AA_4 = phenylalanine, AA_5 = lysine and AA_6 = glutamine)

The ESR spectrum of the vanadyl(Ⅳ)/VD_3/amino acid complexes were scanned in DMSO at room temperature and shown in Fig. 2. The values of $g_{\parallel}$ and $g_{\perp}$ factors are matched with square pyramidal vanadyl complexes[20]. The $g_{\parallel}$, $g_{\perp}$, $A_{\parallel}$ and $A_{\perp}$ values were calculated from the spectrum and the trend of g values calculated from the spectrum ($g_{\parallel} < g_{\perp} < 2$), which are in good agreement for a square pyramidal structure.

The infrared spectra of VD_3, amino acids and their mixed vanadyl(Ⅳ) complexes are shown in Figs 3. The IR spectrum of vitamin D_3 shows a very strong broad band at 3 418 cm^{-1} which assigned to ν(O—H) stretching vibration, ionization of the OH group with subsequent ligation through oxygen atom seems a plausible explanation. It is difficult to distinguish between the ν(OH) of free vitamin D_3 and the stretching vibrational bands of water molecules of the complexes due to the overlapping between them values, and the appearance in one place[21]. The involvement of ν(OH) group of vitamin D_3 in the coordination process can be approved by following the stretching vibration band of ν(C—O) in the complexes where the ν(C—O) is shifted to lower wavenumber from 1 049 cm^{-1} in case of free ligand to about 1 005～1 031 cm^{-1} in the prepared complexes with noticeable decrease in its intensity and this result indicates that the OH group is participated in the complexation[22] and vitamin D_3 acts as monodentate ligand. Vitamin D_3 shows a strong band at 1 451 cm^{-1} for the phenyl skeletal, this band shifted to lower frequency in the complexes to 1 414～1 418 cm^{-1} and this supporting the participation of OH group in the formation of the complexes. Vitamin D_3 also shows two bands at 2 928 and 2 862 cm^{-1} due to the asymmetric and symmetric stretching vibrations of the CH_2 and CH_3[22], these bands not changed in the spectra of the complexes indicating that these groups are not participate in the formation of the complexes. The ν(V═O) stretching vibration in the prepared complexes is observed as expected band at about 929～943 cm^{-1}, which is a good agreement with those known for many vanadyl complexes[21]. The presence of water molecules in the above mentioned complexes is assisted by the presence of broad bands of strong intensity in the 3 201～3 431 cm^{-1} region which may be assigned to the OH stretching vibration for the coordinated water molecules in the vitamin D_3 complexes[22], and supported by bands in the range ～(844～848) cm^{-1} due to the rocking vibration motion δ_r(H_2O) of coordinated water molecules[21]. The formation of new complexes is also confirmed by the appearance of new bands that observed at 501～505 and 420～442 cm^{-1} which may be assigned for the ν(M—O) and ν(M—N), respectively[21-22]. As regards chelation through amino acids, the amino acid was found to be bidentate ligands and bound to the metal ion through the carboxylic OH and the amino group; NH_2. The δNH_3^+ band, which is characteristic of the zwitter ion, disappears in the spectra of the complexes. This fact indicates that the NH_2 group must be involved in coordination. This is supported by the appearance of broad and split bands in the spectra of the complexes, in the regions ～(3 201～3 431) cm^{-1}(stretching vibration; νNH_2) and sharp bands in the region 1 635～1 646 cm^{-1}(in plane deformation; δNH_2), assigned for the coordinated amino group[22]. The bands in the regions 1 633～1 582 and 1 463～1 412 cm^{-1}, due to ν_{asym}(COO—) and ν_{sym}(COO—) of the amino acids, appear in the complexes at 1 414～1 418 and 1 086～11 116) cm^{-1}. The shift of these two bands suggests the involvement of the carboxylic groups of the amino acids in complex formation[22-25]. The microanalytical and spectroscopic discussions of the vanadyl(Ⅳ) vitamin D_3 amino acid complexes confirm to the proposed stoichiometric formulations (Fig. 4).

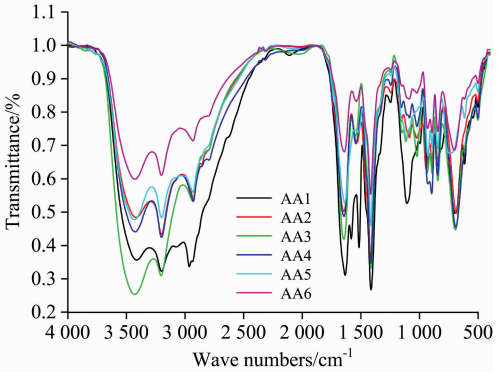

Fig. 3　Infrared spectra of $[(VD_3^{-1})(VO^{+2})(AA_n^{-1})(H_2O)]$ complexes (where AA_1 = isoleucine, AA_2 = threonine, AA_3 = proline, AA_4 = phenylalanine, AA_5 = lysine and AA_6 = glutamine)

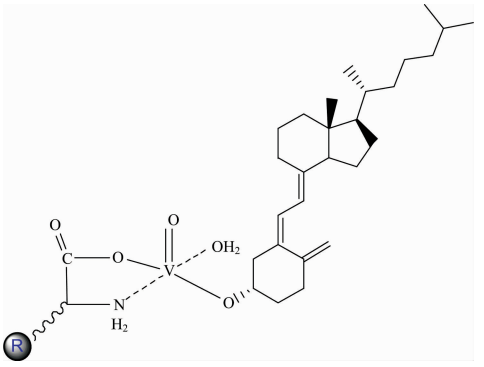

Fig. 4 Suggested structures of vanadyl(Ⅳ) complexes (R = complementary of amino acids)

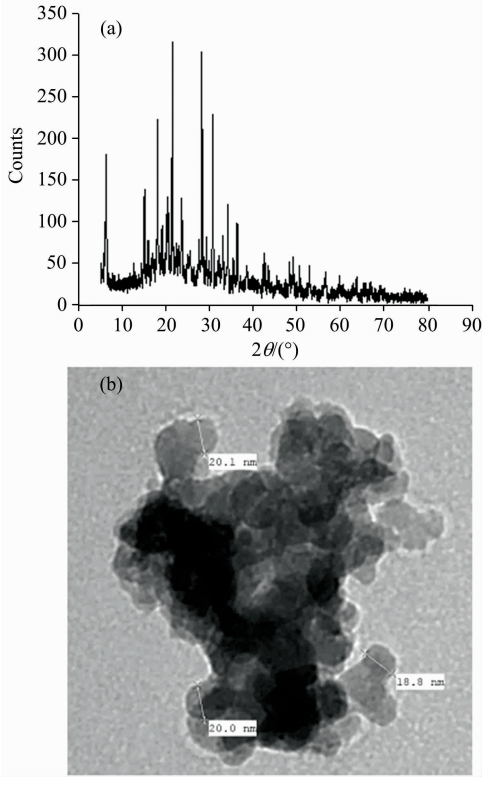

Fig. 5 XRD and TEM of the solid [(VD$_3$)(VO)(AA$_1$)(H$_2$O)] complex

The XRD diffraction patterns of the [(VD$_3$)(VO)(AA$_1$)(H$_2$O)] complex (Ⅰ) (Fig. 5) shows the presence of the characteristic peaks for vanadium (42.535°, 59.968° and 69.147° in accordance with JCPDS File 22-1058[26], vitamin D$_3$ (6.377°, 20.427°, 21.355°, 23.616°, 25.491°, 28.099°, 30.727°, 49.281°, 50.981°, 53.223°, 56.605°, 63.910°, 65.591° and 66.905°)[27] and isoleucine (14.997°, 17.992°, 18.919°, 32.235°, and 32.989°, 34.109°, 36.544°). The crystallite size of the vanadyl(Ⅳ) VD$_3$ complex can be calculated using Sherrer formula[28]. The calculation of particle size of the [(VD$_3$)(VO)(AA$_1$)(H$_2$O)] complex from the highest line diffraction peak at 21.355°. XRD pattern of vanadyl(Ⅳ) VD$_3$ complex (Ⅰ) has a nanocrystalline statement with ~20 nm size.

The distribution of the grain size is homogeneous complex, where medium to large particles size. According to TEM image (Fig. 5), the particles of complex (Ⅰ) exhibit regular spherical forms and their size is widely distributed ~ 20 nm.

2.2 Biological investigation of vanadyl(Ⅳ) complexes on diabetic rats

It was clear from Table (2) that the intraperitoneally injection of vanadyl(Ⅳ) sulfate alone and vitamin D$_3$ complexes (Ⅰ—Ⅵ) at a dose (40 mg · kg^{-1} body weight) each alternative day for 30 day significantly decreased the level of blood glucose of treated diabetic groups (positive control) specially group (Ⅳ$_1$) which injected by [(VD$_3$)(VO)(AA$_1$)(H$_2$O)] complex that contain isoleucine amino acid where the blood glucose decreased by 54.74% compared to positive control group, so that this complex considered the more effective one. Our results demonstrate that the administration of vanadyl(Ⅳ) sulfate and vitamin D$_3$ complexes to diabetic rats at the same dose elicited a significant increase in insulin hormone level with respect to untreated diabetic (STZ) group where the injection retrieve the normal level of insulin hormone specially group (Ⅳ$_1$) which injected by [(VD$_3$)(VO)(AA$_1$)(H$_2$O)] complex that contain isoleucine amino acid where the insulin increased by 51.89%[29].

Our results demonstrated that GPT activity increase in case of diabetic group and this increasing indicates that diabetes mellitus may induce hepatic dysfunction in streptozotocin (STZ) induced diabetic rats; hence restoration of normal level of this enzyme indicates normal functioning of liver under effect of treatment[30]. These results corroborate those of other authors who also reported increased GPT in induced diabetic rats by a single dose of STZ given intraperitoneally. The injection of vanadyl(Ⅳ) sulfate alone at dose (40 mg · kg^{-1} body weight) slightly increase the activity of GPT enzyme by 10.30% with respect to diabetic group, while the injection of vitamin D$_3$ complexes at the same dose slightly decrease the serum GPT activity specially [(VD$_3$)(VO)(AA$_2$)(H$_2$O)] complex that contain threonine amino acid where the GPT activity decreased by 13.59% compared to diabetic group and 22.50% compared to vanadyl(Ⅳ) sulfate alone at the same dose. These results indicate that the treatment with vitamin D$_3$ vanadyl(Ⅳ) amino acids complexes have low side effect and toxicity on liver cells of diabetic rats compared with vanadyl(Ⅳ) sulfate alone[30].

Our results as shown in Table 2, the treatment of diabetic rats with only vanadyl(Ⅳ) sulfate and vitamin D$_3$ complexes have a good effect in decreasing the creatinine level specially [(VD$_3$)(VO)(AA$_5$)(H$_2$O)] · 2H$_2$O complex that con-

tain lysine amino acid where the creatinine level decreased by 46.49% compared to diabetic (STZ) rats and thus indicating that vitamin D_3 complexes have no side effect on kidneys tissue and greatly improve the kidney function. The increased value of uric acid that was observed in diabetic rats is coinciding with Edwards[31-32] who found that the uric acid increased in diabetic mice and this may be due to breaking down of uric acid in diabetics to substances that called purines. According to our study the value of uric acid was decreased in treatment of diabetic rats with vitamin D_3 complexes specially $[(VD_3)(VO)(AA_5)(H_2O)]\cdot 2H_2O$ complex that contain lysine amino acid where the uric acid decreased by 25.88% compared with diabetic positive control group and this reduction in uric acid can explain due to the inhibited oxidative phosphorylation processes which leading to a decrease of protein synthesis.

Table 2　Effect of vanadyl(Ⅳ) complexes (Ⅰ—Ⅵ) on insulin hormone, blood glucose level, serum GPT enzyme, creatinine, uric acid level, Lactate dehydrogenase, glucose-6-phosphate dehydrogenase activities, hemoglobin levels, superoxide dismutase enzyme (SOD) and lipid profile in normal and diabetic rats

Biological test	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group $Ⅳ_1$	Group $Ⅳ_2$	Group $Ⅳ_3$	Group $Ⅳ_4$	Group $Ⅳ_5$	Group $Ⅳ_6$
Insulin/(IU·mL^{-1})	57.64±1.76	23.78±2.50	41.44±1.23	49.43±2.66	46.33±2.32	45.91±1.98	47.74±2.27	44.65±1.74	48.32±1.99
Glucose/(mg·dL^{-1})	77.55±4.93	410.23±14.52	283.51±9.21	185.65±7.34	210.37±8.32	217.11±6.55	206.26±8.86	211.38±6.71	199.22±8.22
GPT/(U·L^{-1})	72.33±6.61	111.50±7.44	124.31±10.5	101.22±6.49	96.34±4.87	105.31±4.61	99.52±5.66	103.72±7.12	100.28±4.87
Creatinine/(mg·dL^{-1})	0.52±0.12	1.14±0.18	0.85±0.15	0.73±0.14	0.77±0.17	0.68±0.18	0.79±0.22	0.61±0.21	0.67±0.14
Uric acid/(mg·dL^{-1})	3.52±0.24	4.79±0.37	3.86±0.29	3.77±0.25	3.81±0.31	3.61±0.30	3.70±0.26	3.55±0.27	3.69±0.33
LDH/(U·L^{-1})	295.43±15.33	409.55±13.27	434.72±19.7	391.23±17.44	403.28±16.32	377.51±14.88	360.64±19.30	382.44±18.59	400.33±16.91
G6PD/(U·L^{-1})	12.13±0.64	7.92±0.47	9.25±0.41	10.52±0.42	10.34±0.54	9.74±0.46	10.81±0.53	9.93±0.63	11.37±0.44
hemoglobin/(g·dL^{-1})	12.82±0.44	9.83±0.37	10.85±0.51	11.42±0.31	11.22±0.44	12.31±0.47	12.02±0.51	11.63±0.62	11.01±0.57
SOD/(U·mL^{-1})	307.53±15.1	259.41±21.66	280.37±18.7	297.47±14.77	291.36±16.51	284.66±19.33	291.22±16.11	290.08±13.82	286.07±19.54
Cholesterol(mg·dl^{-1})	75.66±7.65	210.52±10.57	129.66±8.77	113.56±5.43	117.45±6.13	123.65±7.33	129.43±9.12	125.77±7.38	130.55±6.53
Triglyceride/(mg·dl^{-1})	139.67±9.45	197.46±11.86	156.77±10.5	129.38±8.38	135.71±10.63	144.66±8.44	131.65±7.34	137.21±7.66	140.44±8.51
HDL-c/(mg·dl^{-1})	42.33±3.12	21.44±1.77	32.32±2.11	37.11±2.12	35.32±2.77	36.49±2.43	36.28±2.22	35.78±1.87	36.74±1.93
LDL-c/(mg·dl^{-1})	31.33±4.22	52.57±5.32	42.88±4.71	36.58±4.49	39.43±4.89	38.45±5.11	37.07±3.76	38.12±4.18	38.08±4.75

The activity of LDH enzyme in different experimental groups is presented in Table 2. In general the activity of serum LDH in STZ diabetic rats was significantly increased compared to normal control group and this is mainly due to the leakage of LDH into the blood because of STZ toxicity in the liver. The administration of vanadyl sulfate alone at a dose (40 mg·kg^{-1} body weight) each alternative day increase the activity of LDH by 5.78% compared with the diabetic positive control group while the administration of vitamin D_3 complexes at the same dose elicited a significant decrease in the activities of LDH compared to diabetic positive control group specially group $Ⅳ_4$ that injected by $[(VD_3)(VO)(AA_4)(H_2O)]\cdot 5H_2O$ complex which contain phenylalanine amino acid where this complex afforded a significant decrease in LDH by 11.94%, and this reflects the ameliorative role of vanadyl(Ⅳ) complexation with vitamin D_3 and amino acids in reducing the tissue damage that caused by diabetes[33]. G6PD is the principal source of the major intracellular reductant, NADPH, which is required by many enzymes, including enzymes of the antioxidant pathway[34]. G6PD deficiency is a hereditary condition in which red blood cells breakdown when the body is exposed to certain drugs or the stress of infection. Our study demonstrate that the activity of G6PD was decreased in all STZ diabetic rats as compared to normal control (Table 2), the same observations have been reported previously[35]. The treatment of diabetic STZ rats with vitamin D_3 complexes significantly increased the G6PD activity as compared to positive diabetic group especially group $Ⅳ_6$ that injected by $[(VD_3)(VO)(AA_6)(H_2O)]$ complex which contain glutamine amino acid where the G6PD activity increased by 30.34%. This results concerning the significant effect of vanadyl(Ⅳ) complexation with vitamin D_3 and amino acids on G6PD level.

Our obtained results Table 2 revealed that the diabetic untreated group (STZ) afforded a highly considerable decline in Hb content with respect to the normal controller group and this reduction is mainly due to the reduction in insulin level, where insulin generally enhances the anabolic effect of protein which may be responsible for the decreasing level of Hb in diabetic animals[36]. The treatment of diabetic rats with vitamin D_3 complexes elicited a significant increasing in Hb content in comparison with untreated STZ diabetic rats at the end of the study specially $[(VD_3)(VO)(AA_3)(H_2O)]\cdot 6H_2O$ complex that contain proline amino acid where Hb increased by 20.14%, and this a sign on low toxicity effect of vitamin D_3 complexes on living system of experiment animals.

The efficiency of vitamin D_3 complexes on the treatment of diabetic STZ rats recorded a slight decreasing in SOD level

compared with the normal controller group. It was clear from Table 2, that the level of SOD in STZ diabetic rats treated with VO(Ⅳ) complexes specially group (Ⅳ$_1$) which injected by [(VD_3)(VO)(AA_1)(H_2O)] complex that contain isoleucine amino acid only decreased by 3.27% compared to normal group. The reduced activity of SOD could be due to its degradation or inhibition as a result of the increased production of free radicals in diabetes mellitus[37]. The treatment with vitamin D_3 complexes increased the activity of SOD and may help in controlling free radicals in diabetic rats.

It was apparent from Table 2 that there were a significant increase in the levels of serum triglycerides, total cholesterol, LDL-c in diabetic untreated STZ rats but there were marked reduction in HDL-c in STZ diabetic rats, the main cause for this marked increment of serum lipids in diabetic rats is mainly as a result of increasing the free fatty acids mobilization from the peripheral deposits, since insulin inhibits the hormone sensitive lipase[38]. Excess fatty acids in the serum of diabetic rats are converted into phospholipids and cholesterol in the liver. Liver, an insulin dependent tissue that plays a pivotal role in glucose and lipid homeostasis and it is severely affected during diabetes. Diabetes results in decrease in glucose utilization and an increase in glucose production in insulin-dependent tissues, such as liver. It was clear from the present study that the administration of vitamin D_3 complexes at a dose (40 mg · kg^{-1} body weight) led to significant improvement in the lipid parameters specially the administration of [(VD_3)(VO)(AA_1)(H_2O)] complex that contain isoleucine amino acid where total cholesterol (TC), triglycerides (TG) and LDL-c are significantly decreased by 46.05%, 34.47% and 30.41% respectively while HDL-c level is significantly increased by 42.22% in the serum of diabetic rats compared with positive control diabetic group. These results go in head to head with Toshio et al.[39] who reported that vanadium salts have great role in stimulating the release of lipoprotein lipase (LPL) especially in combination with adenosine and these findings are greatly agreed with our results. Lipoprotein lipase (LPL) catalyzes the hydrolysis of plasma triglycerides and thereby regulate the uptake of fatty acids by tissues as adipose tissues, skeletal muscles and cardiac muscles and this explain the significant increase of serum lipid parameters in diabetic untreated rats and the role of vitamin D_3 VO(Ⅳ) complex in ameliorating lipid profile picture and decreasing these parameters. Our results are also greatly agreed with [40] who clarified that daily oral administration of sodium metavanadate to STZ diabetic rats induced normalization of blood glucose and triglycerides levels, so all these findings suggested that vanadate salts can treat and reduce the hypertriglyceridemia that occurred during diabetes and this occurs through stimulating the secretion of LPL from adipose tissues. All these previous studies reinforced our results as the complexation between vitamin D_3 and vanadyl sulfate has greatly succeeded in reverting high values of triglycerides and cholesterol levels induced by STZ to nearly normal values.

The cells of the pancreas from normal control group were all present in their normal proportions and showing normal structure consisting of normal pancreatic tissue and normal sized islet of langerhans surrounded by normal pancreatic acini. The islets contain alpha cells secreting glucagon and beta cells secreting insulin as shown in Fig. 6. On the other hand, pancreatic tissues in STZ diabetic control rats showing dilated congested vascular spaces surrounded by aggregates of inflammatory cells and pancreatic acini. The islets are largely occupied by a uniform eosinophilic material and few atrophic cells with reduction in its size. Eosinophilic materials also surround the blood vessel as shown in Fig. 6. Pancreatic tissues in diabetic rat treated with only $VOSO_4$ showing mild improvement of the size of islet of langerhans with dilated congested vascular space surrounded by few aggregates of inflammatory cells as shown in Fig. 6. Pancreatic tissues in diabetic rat treated with vitamin D_3/vanadyl/isoleucine system complex [(VD_3)(VO)(AA_1)(H_2O)] showed showing a good response with return of islet of langerhans to its normal size and absence of inflammatory cell and no eosinophilic deposits were seen as shown in Fig. 6. In the present study the histopathological of pancreas of normal control rats did not show any notable changes in its histology throughout the 30 days study. In contrast, STZ administration elicited severe injury to pancreas, leading to decrease in the islet cell numbers and in the diameter of pancreatic islets where the islets were shrunken in diabetic rats when compared with normal rats. This destruction of the islets leads to an absolute lack of insulin that characterizes diabetes mellitus. The administration of $VOSO_4$ showing mild expansion of islets and significantly reduced the injuries to pancreas within 30 days of treatment and recover the damage of pancreatic tissue. The treatment of the diabetic rats with vanadyl complexes specially [(VD_3)(VO)(AA_1)(H_2O)] complex return the normal pancreas histological structure with rich vascular supply and this may be due to the role of the prepared complexes in recovering the damage of pancreatic tissue that caused by STZ-induced diabetes. In conclusion, this study investigated the effect of STZ on a β cells and threw light on the potential of vanadyl complexes in the prevention or treatment of diabetes.

Microscopically, liver from normal control group showed normal structure consisting of the central vein surrounded by rows and cords of healthy hepatocytes with central nucleus and blood sinusoids as shown in Fig. 7. On the other hand, Liver tissues in diabetic control rats showed a large area of hepatic necrosis infiltrated with inflammatory cells with markedly

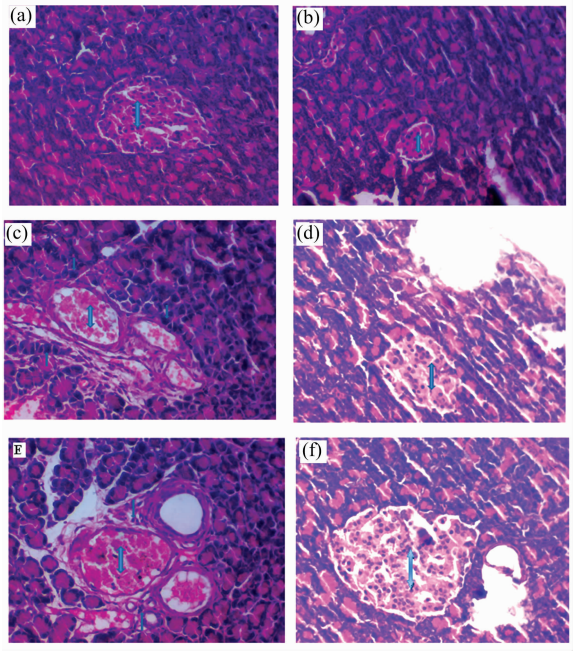

Fig. 6 （a）Normal control of pancreas（Phptomicrograph of normal pancreatic tissue showing normal sized islet of langerhans（↕）surrounded by normal pancreatic acini（H & E stain ×400））；（b）Positive D. M control of pancreas（Phptomicrograph of pancreatic tissue of diabetic rat showing atrophy of the islet of langerhans（↕）surrounded by normal pancreatic acini（H & E stain ×400））；（c）Positive D. M control of pancreas（Phptomicrograph of pancreatic tissue of diabetic rat showing dilated congested vascular spaces（↕）surrounded by aggregates of inflammatory cells（↟）and pancreatic acini（H & E stain ×400））；（d）Pancreas of treated group with（$VOSO_4$）（Phptomicrograph of pancreatic tissue of diabetic rat treated with $VOSO_4$ showing slight increase in the islet of langerhans（↕）（H & E stain ×400））；（e）Pancreas of treated group with（$VOSO_4$）Phptomicrograph of pancreatic tissue of diabetic rat treated with $VOSO_4$ showing still dilated congested vascular space（↕）surrounded by few aggregates of inflammatory cells（↟）.（H & E stain ×400）；（f）Pancreas of treated group with［（VD_3）（VO）（AA_1）（H_2O）］complex（Phptomicrograph of pancreatic tissue of diabetic rat treated with［（VD_3）（VO）（AA1）（H_2O）］complex showing a good response with return of islet of langerhans（↕）to its normal size（H & E stain ×400））

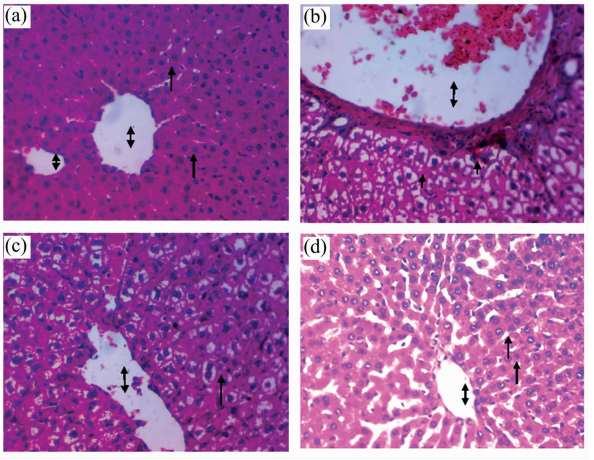

Fig. 7 （a）Normal Liver Control（Photometicrograph of normal liver tissue showing normal size central vein（↕）surrounded by rows and cords of normal hepatocytes（↟）with central nuclei and abundant eosinophilic cytom（H & E stain ×400）；（b）Positive control D. M Liver（Photometicrograph of liver tissue of diabetic rat showing markedly dilated congested central vein（↕）filled with red blood cells and surrounded by aggregates of inflammatory cells with rows and cords of hepatocytes showing sever fatty change（↟）（H & E stain ×400）；（c）Liver of treated group with（$VOSO_4$）（Photometicrograph of liver tissue from diabetic rat treated with only $VOSO_4$ showing moderately dilated congested central vein（↕）surrounded by rows and cords of hepatocytes showing moderate degree of fatty change（↟）（H & E stain ×400）and（d）Liver of treated group with［（FA）（VO）（AA_1）（NH_4）］complex（Photometicrograph of liver tissue of diabetic rat treated with［（FA）（VO）（AA_1）（NH_4）］complex showing return to the normal state with normal size of central vein（↕）surrounded by rows and cords of normal hepatocytes（↟）（H & E stain ×400）

dilated congested central vein filled with red blood cells and surrounded by aggregates of inflammatory cells with rows and cords of swelled and degenerated hepatocytes with sever fatty change as shown in Fig. 7. Liver tissues in diabetic rat treated with only $VOSO_4$ showed mild improvement of hepatocytes with moderately dilated congested central vein surrounded by rows and cords of hepatocytes showing moderate degree of fatty change with normal parenchymal histology as shown in Fig. 7. Liver tissues in diabetic rat treated with vitamin D_3/vanadyl/isoleucine system complex［（VD_3）（VO）（AA_1）（H_2O）］showed good improvement of the liver tissues and return to the normal state with normal size of central vein surrounded by rows and cords of normal hepatocytes and absence of inflammatory cells as shown in Fig. 7. In the current study, the results showed that, treatment of the diabetic rats with vanadyl complexes specially［（VD_3）（VO）（AA_1）（H_2O）］complex return the normal liver histological structure and this may be due to the role of the prepared complexes in diminish-

ing the oxidative stress on hepatic cells and diminishing hepatocellular damage and suppression of gluconeogenesis and consequently may alleviate liver damage caused by STZ-induced diabetes. These results are in agreement with those obtained by Subash et al.[41] as they studied the same effect of cinnamaldehyde on liver tissues.

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