



Protective Effects of an Oxovanadium(IV) Complex with N₂O₂ Chelating Thiosemicarbazone on Small Intestine Injury of STZ-Diabetic Rats

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Abstract

Vanadium compounds are being investigated as potential therapeutic agents in the treatment of many health problems, primarily diabetes. We aimed to provide the effect of N(1)-4-hydroxysalicylidene-N(4)-salicylidene-S-methyl-isothiosemicarbazidato-oxovanadium(IV) (VOL) on small intestinal injury in experimental male diabetic rats. Four groups were created of 3.0–3.5-month-old rats. The rats were made diabetic by a single dose of streptozotocin (STZ) at 65 mg/kg and grouped as follows: control animals, VOL-given control animals, STZ-induced diabetic animals and STZ-induced diabetic animals given VOL. A daily dose of 0.2 mM/kg vanadium complex was administered orally for 12 days after the inducement of diabetes. On the 12th day, small intestine tissue samples were taken. According to the data obtained from the biochemical analysis, reduced glutathione (GSH) level, catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD), Na⁺/K⁺-ATPase and paraoxanase (PON) activities were increased, whereas sialic acid (SA), xanthine oxidase (XO) and disaccharidases (maltase and saccharidase) activities were decreased in the small intestine tissue of VOL-treated diabetic rats. Microscopic examinations revealed a remarkable decrease in the mucosal necrotic areas, discontinuity in the brush border, deterioration of the villi integrity and oedema inside the villi, but with a mild decrease in the inflammatory cells, deterioration and loss of integrity of the gland in the small intestine of VOL-treated diabetic rats. Moreover, VOL treatment markedly decreased the proliferation of villus cells and especially inflammatory cells in the small intestine of diabetic rats. According to the obtained data, the administration of VOL is a potentially convenient strategy to reducing small intestine injury in diabetic rats.

Keywords Diabetes mellitus · Vanadium complex · Small intestine · Oxidative stress

Introduction

Diabetes mellitus (DM) is a type of metabolic disorder, characterized by impairments in protein, lipid and carbohydrate metabolism as a result of insulin resistance, chronic hyperglycaemia and insufficient insulin secretion [1–3]. According to the World Health Organization (WHO), the

incidence of adult diabetic patients in the world will increase from 177 to 370 million by 2030 [3]. The nature of metabolic disturbances in DM has a significant adverse effect on the organism resulting in cardiovascular, kidney and neurological diseases [4–6], all contributing to a decreased quality of life [7]. Dysfunction caused by the destruction of β cells in the pancreas is common in both type 1 diabetes (T1DM) and type 2 diabetes (T2DM). The extremely increase in diabetes worldwide reveals the need for insulin and various problems associated with insulin injection. Oral insulin mimic compounds would be ideal to overcome the problems caused by diabetes [8].

The small intestine tissue plays an essential role in the regulation of glucose metabolism and the pathogenesis of DM. The gastrointestinal symptoms in diabetes are associated with manifestations such as diarrhoea, faecal incontinence, constipation, dyspepsia, nausea and vomiting [9]. Therefore, improvement of hypoglycaemic condition with glycaemic control is a target of diabetic small intestine therapy. The permanent

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hyperglycaemic state in DM causes the formation of oxidative stress and reactive oxygen species (ROS) [10, 11]. In order to combat excess of ROS production, the activity of the body's natural protective mechanisms is increased. Eventually, antioxidant depletion occurs against natural defences in small intestinal enterocytes, resulting in uncontrolled ROS formation [12, 13]. In addition, in a typical hyperglycaemic state, disruption of antioxidant balance can lead to a decrease in both circulating repair factors and levels of inhibition factors involved in mucosal healing. This can cause damage to healing mechanisms as in diabetic enteropathy [13]. Increased oxidative stress excessively increases cytokine production through numerous mechanisms, with various derivatives of oxygen molecules acting as secondary messengers that cause transcription of immune-related cytokines in the small intestine tissue [14].

Vanadium is an abundant element on earth which distribution takes a large place in all organisms [15–17]. Some previous studies indicated that some synthesized vanadium complexes have exhaustive antidiabetic activities and a wide variety of other pharmacological properties in vitro and in vivo [18–21]. Many researches are widespread on the development and creation of new metalopharmaceuticals, and these types of pharmaceuticals are extremely desirable for the treatment of DM. Based on our previous works, we noticed that vanadium might be very useful in the treatment of DM [22, 23].

Thiosemicarbazones are an important class of ligands for metal complexes because of biological and therapeutic properties such as antioxidant [24, 25], cytotoxic [24, 26–29], antidiabetic [30], antimalarial [31, 32], antiviral [33], antibacterial [34], anticancer [35, 36] and antiprotozoal [29, 37]. In the present study, we aimed to demonstrate biochemical and pathological effects of N(1)-4-hydroxysalicylidene-N(4)-salicylidene-S-methyl-isothiosemicarbazidato-oxovanadium(IV) (VOL), which is a vanadium compound bearing a thiosemicarbazone, on small intestinal injury in male streptozotocin (STZ)-induced diabetic rats.

Materials and Methods

Preparation of VOL

VOL complex was obtained through 4-hydroxysalicylidene-S-methylisothiosemicarbazone and salicylaldehyde in the presence of vanadyl sulphate by template reaction (Fig. 1).

Starting Material 4-Hydroxysalicylidene-S-methylisothiosemicarbazide was prepared by refluxing from the reaction of 4-hydroxysalicylaldehyde (1 g, 1 mmol) with S-methylisothiosemicarbazide (0.776 g, 1 mmol) in ethanol (25 ml). After 4 h, the precipitated powder with pinkish cream colour was filtered and washed with ethanol [30]. The yield was 91% (1.48 g) and m.p is 180–181 °C.

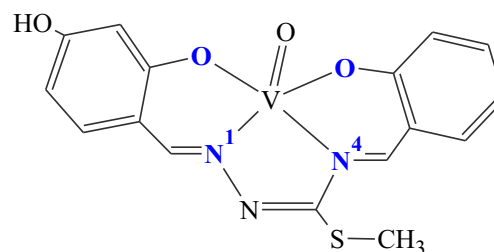


Fig. 1 The oxovanadium(IV) complex with dibasic N_2O_2 -chelating thiosemicarbazidato ligand (symbolized by VOL)

VOL The complex was obtained from 4-Hydroxysalicylidene-S-methyl-isothiosemicarbazone and salicylaldehyde in the presence of $VOSO_4 \cdot 5H_2O$ in 1:1:1 molar ratio by the previously described method [30]. The physical properties of amorphous brown powder were controlled: yield, 72%; m.p, > 380 °C.

Animals Approval

Twenty one clinically healthy Swiss Albino male rats (of between 3.0 and 3.5 months old) were used. Experiments were reviewed and approved according to the Animal Care and Use Institute's Committee of Istanbul University.

Induction of Diabetes

STZ was freshly prepared by dissolving in cold 0.01-M sodium buffer of citrate–hydrochloric acid (pH = 4.5) [38] and used for induction of diabetes by a single intraperitoneal injection (i.p) of 65 mg/kg body weight.

Experimental Design

The animals were randomly allocated according to the order, nondiabetic group ($n = 5$), VOL-treated nondiabetic group ($n = 5$), STZ-diabetic group ($n = 6$) and STZ-diabetic animals given with VOL group ($n = 5$). VOL was given to the rats by gavage technique (upon dissolution in gum Arabic solution) at a dose of 0.2 mM/kg/day for 12 days after rats became diabetic. Body weights of all animals were taken on 0, 1 and 12 experimental days and tail vein blood samples were measured for the same days.

Biochemical Assays

On day 12 of the experiment, animals were fasted overnight and were subsequently sacrificed under ether anaesthesia. The small intestine of all animals was removed and processed for study. The ortho-toluidine method was used for the determination of 18-h fasting blood glucose for all animals [39]. The data of body weights and fasting blood glucose levels have been published in a previous article describing the effects of VOL on the pancreas tissue [30].

Oxidative Stress Markers and Antioxidant Enzyme Activities

The small intestinal tissue homogenates were made 10% (w/v) by using a glass equipment. After centrifugation, the clear supernatants were collected and used for the estimation of reduced glutathione (GSH), sialic acid (SA), protein levels, antioxidant levels and disaccharidase enzyme activities. The small intestine GSH, SA and protein levels were determined by the methods of Beutler [40], Warren [41] and Lowry et al. [42], respectively. The activities of enzymes were measured with following techniques: of catalase (CAT) according to Aebi [43], glutathione peroxidase (GPx) according to Paglia and Valentine [44] as modified by Wendel [45], glutathione-S-transferase (GST) according to the Habig and Jacoby [46], superoxide dismutase (SOD) according to Mylorie et al. [47], Na⁺/K⁺-ATPase according to Ridderstap and Bonting [48], paraoxanase (PON) according to Furlong et al. [49], xanthine oxidase (XO) according to Corte and Stirpe [50] and disaccharidase (maltase and saccharidase) according to the Dahlqvist [51] by diluting in appropriate rates.

Histopathology

The small intestine tissue pieces were kept in Bouin's fixative. The water in the tissue pieces was removed by passing through the rising alcohol series, and after xylene application, they were embedded in the paraffin blocks. After applying periodic acid-Schiff (PAS) dye to 5-μm-thick sections, they were analysed with an Olympus CX-45 microscope with DP71 digital camera attachment.

Immunohistochemistry

Proliferative cell nuclear antigen (PCNA) was labelled using the Streptavidin-Biotin-Peroxidase method. Three percent hydrogen peroxide was applied to suppress the activity of endogenous peroxidases. Following the block solution which is a component of the Histostain Plus Broad Spectrum Kit (Zymed 85-9043), the mouse polyclonal antibody against PCNA (NeoMarkers MS-106-P) was incubated at 1:50 dilution for 1 h at room temperature. After applying biotinylated secondary antibody and streptavidin peroxidase, which are the other components of the same kit, the reaction was developed with 3-amino-9-ethylcarbazole. Mayer's Haematoxylin was applied for counter staining. Sections were analysed with an Olympus CX-45 microscope with DP71 digital camera attachment. Ten fields (0.0506 mm²) were randomly counted per section from 5 animals of each group.

Statistical Evaluation of Data

The obtained all biochemical data were evaluated using an unpaired *t* test and analysis of variance (ANOVA) using the statistical computer package NCSS. The results were expressed as mean ± SD. GraphPad Prism Software, version 4.00 (San Diego, CA), was used for immunohistochemical analysis. Data were stated as mean ± S.E.M. One-way ANOVA and Tukey's test were used for statistical analysis. *p* < 0.05 was accepted as significant.

Results

VOL complex was obtained via template synthesis method as previously reported [30]. Firstly, S-methyl-isothiosemicarbazide was prepared through the reaction of a methyl halogenide and thiosemicarbazide. Thereafter, a 4-hydroxysalicylidene-S-methyl-isothiosemicarbazide as starting material was obtained from the reaction of 4-hydroxysalicylidene with the prepared S-methyl-isothiosemicarbazide. Then, VOL was synthesized by reacting to the starting material with salicylaldehyde in the presence of VOSO₄·5H₂O in 1:1:1 molar ratio. All processes were monitored via thin-layer chromatography. The reaction was tracked by IR spectroscopy. In the IR spectrum of the VOL complex, the disappearance of stretching and bending vibration bands of NH₂ group of the starting material at 3445, 3337 and 1624 cm⁻¹ and appearance of the characteristic (V=O) and (V-O) bands at 987 and 478-434 cm⁻¹ were proofs that VOL complex was formed [30].

Impact of VOL on Small Intestine GSH and SA Levels

The data showing the reduced GSH and SA levels in nondiabetic and treated animals are presented in Table 1. A insignificant decrease of GSH level was shown in the diabetic group

Table 1 The levels of reduced glutathione and SA in the control and experimental animals' small intestine tissues

Groups	GSH (nmol GSH/ mg protein)*	SA (mmol/mg protein)*
Control	9.11 ± 0.43	344.42 ± 32.35
Control + VOL	5.66 ± 0.08 ^a	302.48 ± 72.37
Diabetic	7.79 ± 0.87	469.34 ± 10.53 ^a
Diabetic + VOL	8.56 ± 0.33	209.40 ± 64.71 ^b
<i>P</i> _{ANOVA}	0.001	0.014

*Mean ± SD

^a *P* < 0.05 compared with control group

^b *P* < 0.05 compared with diabetic group

when compared with control rats. VOL administration resulted in a nonsignificant increase of GSH level in the diabetic group. Interestingly, there was a significant decrease in the GSH level in VOL-treated rats in comparison with non-treated control rats ($p < 0.05$). A profound increase of SA levels in diabetic animals in comparison with the control group ($p < 0.05$) was observed. Orally treated VOL significantly decreased the level of SA in diabetic group ($p < 0.05$).

Impact of VOL on Small Intestine Antioxidant Enzymes and Na^+/K^+ -ATPase Activities

The small intestinal CAT, GPx, GST, SOD and Na^+/K^+ -ATPase activities are given in Table 2. Small intestinal CAT, GPx, GST and SOD activities were significantly decreased in diabetic group compared with the control group ($p < 0.005$ for CAT, $p < 0.0001$ for GPx and GST, $p < 0.05$ for SOD). The vanadium complex significantly increased the activities of these enzymes to near normal in VOL-treated diabetic group ($p < 0.0001$ for CAT, GPx and GST; $p < 0.05$ for SOD). Another remarkable output is that the control animals given with VOL showed a significant decrease in GPx and GST activities compared with control rats ($p < 0.005$, $p < 0.0001$). A mean decrease in Na^+/K^+ -ATPase activity was noticed in diabetic rats as compared with the controls ($p < 0.05$). On the other hand, diabetic rats that received VOL had a significantly increased Na^+/K^+ -ATPase activity in comparison with non-treated diabetic rats ($p < 0.005$).

Impact of VOL on Small Intestine PON, XO and Disaccharidase Activities

The small intestinal PON, XO and disaccharidases (maltase and saccharidase) activities are given in Table 3. A significant decrease in PON activity of diabetic rats compared with

control rats ($p < 0.005$) was noticed. The administration of VOL significantly raised PON activity in the diabetic group ($p < 0.0001$). XO activity was significantly increased in the diabetic rats compared with the control ($p < 0.05$). However VOL treatment significantly decreased XO activity in diabetic group ($p < 0.05$). On the other hand, the small intestinal XO activity was increased significantly in the VOL-given control rats in comparison with non-treated control animals ($p < 0.0001$). In comparison with the control, the activities of intestinal maltase and saccharase in diabetic rats were significantly elevated ($p < 0.005$, $p < 0.0001$). The administration of VOL reduced the activity of these enzymes significantly ($p < 0.05$, $p < 0.0001$).

Impact of VOL on Small Intestine Histopathology

Microscopic examination reveals that VOL treatment restored intestinal morphological alterations observed in diabetic rats. A remarkable decrease in mucosal necrotic areas, discontinuity in the brush border, deterioration of the villi integrity and oedema inside the villi were observed. Moreover, a mild decrease in the inflammatory cells, as well as the deterioration and loss of integrity of glands in the small intestine by VOL treatment to diabetic rats (Fig. 2), was detected.

Impact of VOL on Small Intestinal Cell Proliferation

PCNA immunostaining was evaluated for three different types of cells that included crypt, villus and inflammatory cells in duodenum sections. There were no statistically significant changes in PCNA⁺ crypt cells between the groups. PCNA⁺ crypt cell numbers were as follows: C: 12.68 ± 3.84 , VOL: 24.95 ± 0.82 , D: 23.03 ± 4.06 and D + VOL:

Table 2 Small intestinal antioxidant enzymes and Na^+/K^+ -ATPase activities in control and experimental groups

Groups	CAT (U/g protein)*	GPx (U/g protein)*	GST (U/g protein)*	SOD (U/g protein)*	$\text{Na}^+/\text{K}^+/\text{K}^+$ -ATPase ($\mu\text{mol Pi/g protein/h}$)*
Control	24.43 ± 2.64	25.91 ± 1.15	73.31 ± 2.97	6.31 ± 0.60	129.48 ± 4.11
Control + VOL	23.76 ± 3.22	21.36 ± 0.93^a	59.01 ± 2.39^c	6.43 ± 0.69	141.02 ± 1.86
Diabetic	13.06 ± 2.07^a	19.23 ± 1.04^c	50.36 ± 5.31^c	4.98 ± 0.31^d	113.69 ± 0.08^d
Diabetic + VOL	30.88 ± 1.94^b	29.25 ± 0.56^b	74.26 ± 3.70^b	6.65 ± 0.43^e	134.78 ± 1.70^f
P_{ANOVA}	0.0001	0.0001	0.0001	0.016	0.001

*Mean \pm SD

^a $P < 0.005$ compared with control group

^b $P < 0.0001$ compared with diabetic group

^c $P < 0.0001$ compared with control group

^d $P < 0.05$ compared with control group

^e $P < 0.05$ compared with diabetic group

^f $P < 0.005$ compared with diabetic group

Table 3 Small intestine PON, XO and disaccharidase activities in all groups

Groups	PON (U/mg protein)*	XO (U/g protein)*	Maltase (U/g protein)*	Saccharase (U/g protein)*
Control	8.51 ± 1.07	1.51 ± 0.09	5.09 ± 2.10	4.67 ± 2.45
Control + VOL	8.76 ± 0.68	3.59 ± 0.78 ^c	6.79 ± 3.74	7.43 ± 3.31
Diabetic	2.27 ± 0.70 ^a	2.79 ± 0.65 ^d	17.79 ± 0.59 ^a	24.68 ± 3.74 ^c
Diabetic + VOL	4.88 ± 0.62 ^b	0.46 ± 0.15 ^e	11.07 ± 0.63 ^e	10.54 ± 3.55 ^b
<i>P</i> _{ANOVA}	0.0001	0.002	0.003	0.002

*Mean ± SD

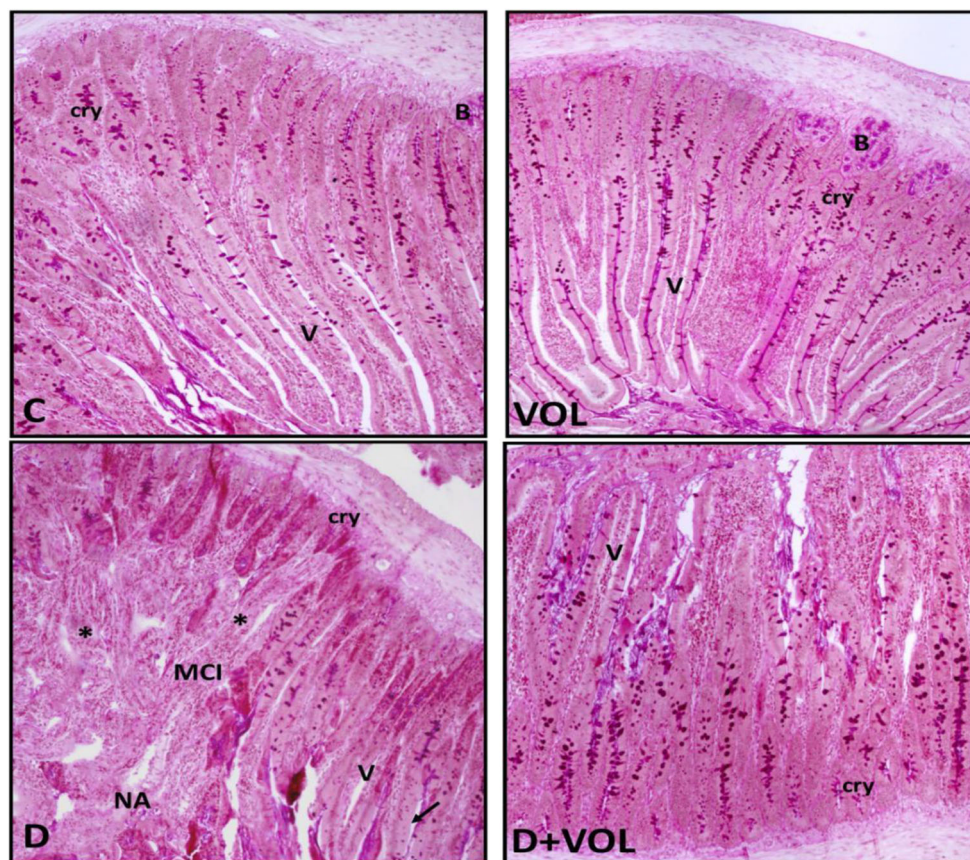
^a *P* < 0.005 compared with control group^b *P* < 0.0001 compared with diabetic group^c *P* < 0.0001 compared with control group^d *P* < 0.05 compared with control group^e *P* < 0.05 compared with diabetic group

23.93 ± 5.84. Although the number of PCNA⁺- villus cell index were very low in the control group, it was observed that PCNA⁺- villus cell number increased in diabetic group (*p* < 0.01), while VOL administration to these rats resulted in a reduction in PCNA⁺- villus cell number (*p* < 0.01). PCNA⁺- villus cell numbers were as follows: C: 0.03 ± 0.03, VOL: 0.0 ± 0.0, D: 0.64 ± 0.07 and D + VOL: 0.19

± 0.06. The most prominent findings were in PCNA⁺ inflammatory cells. PCNA⁺ inflammatory cell number increased in diabetic group (*p* < 0.01), while VOL treatment to these rats resulted in a decrease in PCNA⁺ inflammatory cell number (*p* < 0.05). PCNA⁺ inflammatory cell numbers were as follows: C: 2.7 ± 0.29, VOL: 6.26 ± 0.26, D: 18.59 ± 2.13 and D + VOL: 10.11 ± 1.24 (Fig. 3).

Fig. 2 Histological appearance of duodenum from each group.

Normal histological appearance of duodenal mucosa of control or VOL-treated control rats can be seen in the photos. We observed a remarkable decrease in the mucosal necrotic areas (NA), discontinuity in the brush border (arrow), deterioration of integrity (*) of the villi (V), while mild decrease in the mononuclear inflammatory cells (MCI) and deterioration of crypt (cry) integrity in the small intestine by VOL treatment to diabetic rats. (B) Brunner's gland, (C) control rats, VOL: oxovanadium-treated rats, (D) diabetic rats, D + VOL: oxovanadium-treated diabetic rats. PAS, X135



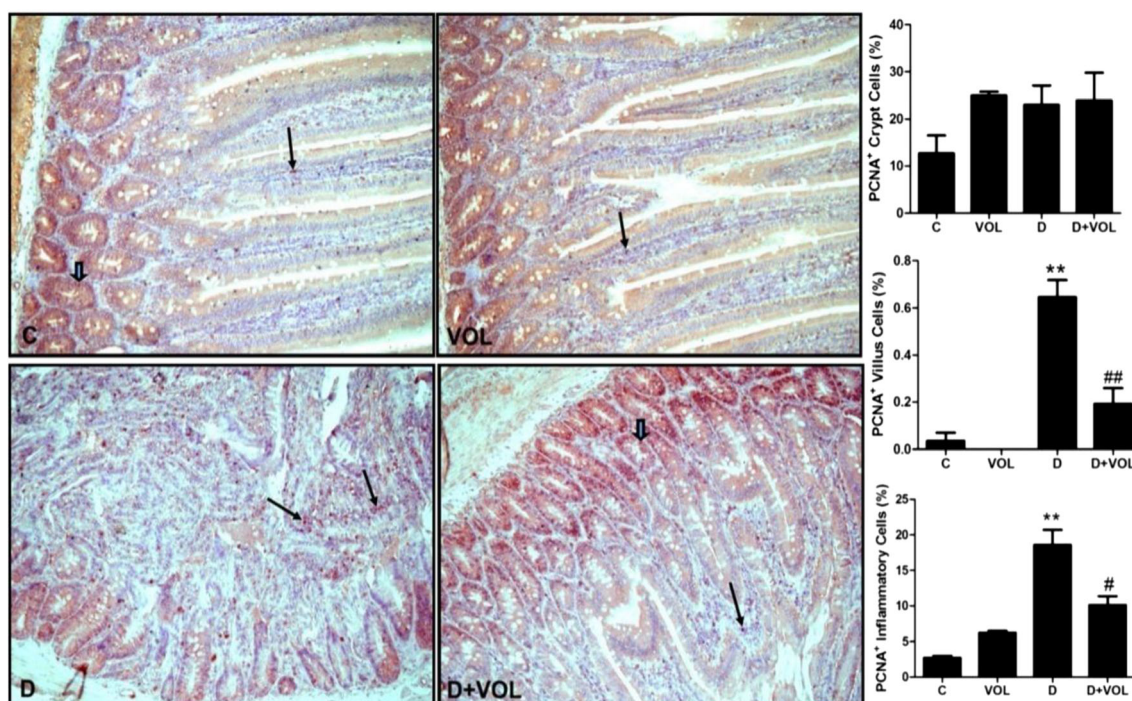


Fig. 3 PCNA⁺ crypt, villus and inflammatory cells in the duodenum. PCNA⁺ villus and inflammatory cell number increased in diabetic rats. VOL administration caused a significant decrease in the PCNA⁺ villus and inflammatory cell number in diabetic rats. PCNA⁺ inflammatory cells

(thin arrow), PCNA⁺ crypt cell (thick arrow). ** $p < 0.01$ vs control and # $p < 0.05$, ## $p < 0.01$ vs diabetic rats. Values are mean \pm S.E.M. $n = 5$ animals for each group. C, control rats; VOL, oxovanadium-treated rats; D, diabetic rats; D + VOL, oxovanadium-treated diabetic rats. X540

Discussion

Due to the increasing prevalence of DM in the world, studies are underway to investigate new antidiabetic agents [52]. Researches involving biochemistry, immunohistochemistry, histopathology and bioinorganic chemistry indicate that vanadium ligands and vanadium complexes are useful in the treatment of experimental diabetes [30, 53, 54]. In the present study, a vanadium complex was synthesized and tested for its hypoglycaemic effect in STZ-induced diabetic rats [30].

Increased ROS production or low ROS sweeping capacity causes oxidative stress to the pathogenesis of diabetic complications [55]. GSH is a cysteine-containing tripeptide whose sulfhydryl group (–SH) is involved in the reduction and conjugation reactions [56]. In our study, it was seen that GSH level was decreased in diabetic small intestinal tissue. The administration of VOL to diabetic rats improved GSH level, and this indicates the positive function of the VOL complex on improving the antioxidant status [30, 52].

An increase in glycoprotein components such as SA is another patho-biochemical modification that arises due to hyperglycaemia. It is proposed that under DM conditions, the increasing level of SA would cause insulin resistance and affect the functionality of the tissues [57]. In the present study, an elevated small intestine SA level of diabetic rats compared with the control group was observed. The treatment with VOL decreased the SA level of the diabetic group when

compared with non-treated diabetic animals. Regulation of glycoprotein component level under DM conditions further emphasizes the therapeutic effect of VOL in relieving DM-related complications.

Antioxidant enzymes such as CAT, GPx, GST and SOD play an important role in scavenging the toxic intermediate of incomplete oxidation [52, 58]. The excessive production of H₂O₂ and O₂ from the auto oxidation of extremely high glucose concentration and non-enzymatic glycation of proteins may result from the decreased activities of SOD and CAT [59]. GPx and GST are GSH-related enzymes that have depressed or inactivated enzymatic activity in the small intestine tissue of DM. Higher protein glycation in DM is associated with inactivation of these enzymes. Indeed, glycation inactivates GPx due to the modification of arginine residues located in the glutathione binding site of the enzyme [60]. In this context, our data show decreased activity of these enzymes in the small intestine tissue of diabetic rats. Oral administration of VOL to diabetic rats resulted in improved activities of CAT, GPx, GST and SOD, which in turn indicates the antioxidant potential of the complex. Similarly, a decreased tissue Na⁺/K⁺-ATPase activity in experimental diabetes was reported [61]. In the present work, a 12-day treatment of diabetic rats with VOL significantly increased small intestinal Na⁺/K⁺-ATPase activity. It was suggested that the improvement of carbohydrate metabolism through vanadium's insulinomimetic and insulin enhancing effects in diabetic rats

reduced oxidative stress and increased the activity of Na⁺/K⁺-ATPase in the small intestine [62].

Previous studies show the association between lower PON1 and increased XO activities in DM [63]. According to Ibrahim et al., the reduced PON1 and elevated XO enzymatic activity is the reason for oxidative stress scenario in the cardiac tissue of diabetic rats [64]. Similarly to this study, we also noticed decreased PON and elevated XO activities in the small intestinal tissue of diabetic rats. According to the data of the present finding, VOL treatment restored the activities of both enzymes in the diabetic group.

Interestingly, the present study observed a decreased small intestinal GSH level as well as that of GSH-related enzyme activities (GPx and GST) in VOL-treated control rats. XO activity was also increased in the same group. The decreased GSH level in normal rats may be caused by the toxic effect of VOL in the small intestine. This is possibly a result of depleted GSH function, a compound that plays a role in the elimination of peroxides and many xenobiotic compounds. The declined thiol levels lead to GSH depletion and generation of ROS, thereby leading to a raised XO activity in the VOL-given control rats.

The inhibition of intestinal α -glycosidases (maltase and saccharase) delays the digestion of starch and causes reduced glucose absorption. Such effect would control/stabilize postprandial hyperglycaemia which is considered an important approach in the treatment of the DM [65]. In our research, the activities of intestinal maltase and saccharase due to hyperglycaemia were increased in diabetic rats. VOL administration inhibited the α -glycosidase activities in the diabetic group as to STZ-induced diabetic animals. It is reported that the expression of sugar carriers increases with duodenal saccharase and lactase activities in diabetic individuals, leading to hyperglycaemia due to excessive carbohydrate consumption/uptake [66]. Similar to these, an increase in maltase and saccharase activities of diabetic animals of our study suggests that carbohydrate digestion is accelerated. Our histopathological findings indicate the accumulation of inflammatory cell and necrotic areas, coupled with discontinuity in the brush border and oedema in the villi of the diabetic group. It may be possible to develop these histopathological changes in the villi area as a result of continuous rapid digestion and absorption. We observed a remarkable decrease in the mucosal necrotic areas, with mild decrease in the inflammatory cells, deterioration and loss of integrity of the small intestine glands upon VOL pre-treatment to diabetic rats. The low levels of maltase and saccharase activities in this group compared with diabetic animals suggest that carbohydrate digestion is maintained at normal levels. On the other hand, VOL pre-treatment markedly decreased proliferation of villus cells and especially inflammatory cells in the small intestine of diabetic rats. When all the findings are combined, it can be suggested that villus cells proliferate to meet the need for an increased glucose

absorption in diabetic rats, and these changes are suppressed by VOL pre-treatment. The most striking outcome is the possibility of VOL's preventive effect on necrosis and inflammation in the villi area through the inhibition of rapid carbohydrate digestion and absorption.

Conclusion

According to the obtained results, diabetes induces oxidative stress and disrupts the antioxidant defence system in the small intestine of rat tissue. The biochemical, immunohistochemical and histopathological results of the present study suggest that VOL is a potentially favourable complex for diminishing small intestine deterioration in diabetic rats. However, the same concentration of this vanadium complex has also been observed to cause irreversible damage in the small intestinal tissue when administered to the control animals.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that there is no any conflict of interest.

Ethics Approval Experiments were reviewed and approved according to the Animal Care and Use Institute's Committee of Istanbul University.

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