ORIGINAL ARTICLE

EVALUATION OF PROTECTIVE EFFECTS OF QUERCETIN AND VANADYL SULPHATE IN ALLOXAN INDUCED DIABETES MODEL

BRUNO ŞTEFAN VELESCU[1#], VALENTINA ANUŢA[2#]*, ANTON ALDEA[3#], MARIANA JINGA[4,7#], PAVEL CĂLIN COBELESCHI[5#], CRISTINA ELENA ZBÂRCEA[1#], VALENTINA UIVAROSI[6#]

[1] *"Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy, Department of Pharmacology and Clinical Pharmacy, 6 Traian Vuia Str., 020956, Bucharest, Romania*
[2] *"Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy, Department of Physical and Colloidal Chemistry, 6 Traian Vuia Str., 020956, Bucharest, Romania*
[3] *BLD Pharma SRL, 96A Intrarea Sulfinei, 077125, Măgurele, Jud Ilfov, Romania*
[4] *"Dr. Carol Davila" Central Military Emergency Hospital, Internal Medicine and Gastroenterology Clinic, 88 Mircea Vulcănescu St., 10816, Bucharest, Romania*
[5] *Transilvania University, Faculty of Medicine, Department of Internal Medicine, 56th Nicolae Bălcescu St., Braşov, Romania*
[6] *"Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy, Department of Inorganic Chemistry, 6 Traian Vuia Str., 020956, Bucharest, Romania*
[7] *"Carol Davila" University of Medicine and Pharmacy, Faculty of Medicine, Eroilor Sanitari Blvd, Bucharest.*

**corresponding author:* vali_anuta@yahoo.com
[#] *All authors have equal contribution*

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Abstract

Various vanadium compounds demonstrated decreasing blood sugar levels in different diabetes animal models, and are considered to be promising antidiabetic agents. Oxovanadium(IV) complexes with flavonoid ligands, with beneficial effects in diabetic pathology, like the complex di-μ-hydroxobis(quercetinatooxovanadium)(IV), proved to decrease the elevated blood sugar levels and the total cholesterol, without impact on the insulin levels in alloxan induced diabetic rats. The aim of the study was to evaluate the effect of quercetin and vanadyl sulphate on rats exposed to alloxan, as diabetogenic agent. The rats' treatment consisted in daily oral administered doses of 0.01 mmol/kg b.w. of vanadyl sulphate, 0.02 mmol/kg b.w. of quercetin and respectively 0.01/0.02 mmol/kg b.w. of vanadyl sulphate - quercetin mixture, over a 4 weeks period. The experimental results pointed out protective effects of quercetin and vanadyl sulphate in alloxan exposed rats on the induced diabetic onset, rats' glycaemia and impaired lipid metabolism.

Rezumat

Diferiţi compuşi ai vanadiului au demonstrat efecte de scădere a valorilor glicemiei animalelor, în diferite modele experimentale de diabet şi prezintă potenţial terapeutic ca agenţi antidiabetici. Complecşi ai oxovanadiului(IV) cu liganzi din clasa flavonelor, care au efecte benefice în patologia diabetică, precum di-μ-hydroxobis(quercetinatooxovanadium)(IV), au scăzut, la şobolani cu diabet aloxanic, valorile glicemiei şi colesterolului total, fără să modifice valorile insulinemiei. Scopul acestui studiu a fost evaluarea efectelor tratamentului cu quercetină şi sulfat de vanadil la şobolani cărora li s-a administrat aloxan, ca agent diabetogen. Tratamentul animalelor a constat în administrarea orală zilnică a 0,01 mmoli/kg corp de sulfat de vanadil, 0,02 mmoli/kg corp de quercetină şi respectiv 0,01/0,02 mmoli/kg corp de amestec sulfat de vanadil - quercetină, timp de 4 săptămâni. Rezultatele experimentale au evidenţiat efecte protectoare ale quercetinei şi sulfatului de vanadil în modelul experimental ales, atât asupra instalării diabetului indus, cât şi asupra profilului glucidic şi lipidic.

Keywords: quercetin, vanadyl sulphate, alloxan induced diabetic rats, hypoglycaemic activity, hypolipidemic activity

Introduction

Various vanadium compounds demonstrated decreasing the blood sugar levels in different diabetes animal models [1], and are considered to be promising antidiabetic agents [2, 3]. The oxivanadium compounds inhibit the dephosphorylation of the insulin receptor, through the inhibition of protein tyrosine phosphatase PTP1B [4], where the PTP-ase is the main negative regulatory factor in insulin signalling pathways [5]. Vanadium compounds act as insulinotropic agents, at pancreatic level, and reduce β-cells death, and enhance the proliferation of viable β-cells [6]. The pancreatic β-cells are more sensitive to oxidative stress due to their low content of antioxidants when compared with other tissues [7]. Bioactive compounds that improved hyperglycaemia mediated oxidative stress have been assessed to be potent antidiabetic agents. Quercetin, a main representative of natural flavonoid

class, has been reported to possess hypoglycaemic activity by improving the glucose and oxidative metabolisms which are affected during diabetes. After the treatment with intraperitoneal injection of quercetin (10 and 15mg/kg b.w./day for 10 days) in streptozocin (STZ)-induced diabetic and normal rats it was observed that the plasma glucose level of diabetic rats decreased in a dose dependent manner, with a significant reduction of plasma cholesterol and triglycerides. In normal rats, after the quercetin administration, the plasma glucose level of normal animals remained unaffected with an increase of plasma cholesterol and triglycerides [8]. Decrease in plasma glucose, alleviation of diabetic symptoms and liver injury, were observed in male STZ induced diabetes mice that received 0.1 and 0.5% quercetin respectively, supplemented diet for 2 weeks [9]. Diabetic status in rats with STZ-induced diabetes was found to be ameliorated in the quercetin-fed diabetic group compared to a starch-fed diabetic group, at a dose of 1 g per kilogram of diet for 6 weeks [10]. Beneficial effects of quercetin on plasma glucose were explained by mechanisms such as: increase in hepatic glucokinase activity, increase in the number of pancreatic islets [8], inhibition of cyclin dependent kinase inhibitor 1A (CDKN1A) expression in pancreas [9], inhibition of insulindependent activation of phosphoinositide 3-kinase (PI-3K) [11].

Apart of the beneficial effects on diabetes status, it was evidenced a protective effect of quercetin on hyperglycemia induced by STZ in rats. In a study with quercetin administered for 3 days (15 mg/kg b.w. day, intraperitoneal injection) prior to STZ induction of diabetes, the quercetin administration being continued to the end of the study (for 25 days), it was observed that quercetin had no effect on the plasma glucose level of normal animals, but its pre- treatment was able to prevent the induction of diabetes by single intraperitoneal injection of STZ exposed rats [12].

The effectiveness of vanadium compounds appears to be correlated to the oxidation state of the metal [13], the chemical nature of the compound (inorganic salt or complex combination) [14] and nevertheless the used ligand [15, 16]. The lower toxicity of oxovanadium(IV) salts [17] lead to the development of different series of oxovanadium(IV) complexes with different ligands. Oxovanadium(IV) complexes with flavonoid ligands, with beneficial effects in diabetic pathology [18, 19], like the complex di-μ-hydroxobis(quercetinatooxovanadium(IV) [20], proved to decrease the elevated blood sugar levels and the total cholesterol, without impact on the insulin levels in alloxan diabetic rats [21].

The aim of the current study was to evaluate the effect of a quercetin and vanadyl sulphate treatment on rats exposed to alloxan, as diabetogenic agent. The rats' treatment consisted in daily oral administrated doses of 0.01 mmol/kg b.w. vanadyl sulphate, 0.02 mmol/kg b.w. of quercetin and respectively 0.01/0.02 mmol/kg b.w. of vanadyl sulphate - quercetin mixture, over a 4 weeks period. The experimental results pointed out protective effects of quercetin and vanadyl sulphate treatment in alloxan exposed rats, on the induced diabetic onset, rats' glycaemia and impaired lipid metabolism.

Materials and Methods

Materials

Alloxan monohydrate, vanadyl sulphate pentahydrate and quercetin where purchased from Sigma-Aldrich, Germany. All chemicals were of analytical grade and used without further purification.

Animals

Male Wistar rats weighing 202 ± 13 g obtained from the "Cantacuzino" Institute, Bucharest, Romania were used. The rats were housed in plastic cages in an air-conditioned animal room and fed on granulated food with free access to water. The temperature and relative humidity were continuously monitored using a thermohygrometer. The temperature was generally maintained between 20ºC and 22ºC and the relative humidity also, between 35 - 45%.

All procedures were carried out in accordance with the Directive 86/609/EEC of 24th November 1986, on the protection of animals used for experimental and other scientific purposes.

Experimental design

The animals were randomly divided into 5 groups of 8 rats each: (1) (N) rats treated with distilled water 1 mL/100 g b.w. for 28 days, the control group, including healthy rats, not subjected to diabetes induction; (2) (D) rats treated with distilled water 1 mL/100 g b.w. for 28 days subjected to diabetes induction; (3) (V) rats treated with vanadyl sulphate 0.01 mmol/kg b.w., aqueous solution 0.1 mmol % for 28 days; (4) (Q) rats treated with quercetin 0.02 mmol/kg b.w., aqueous suspension 0.2 mmol % for 28 days; (5) (VQ) rats treated with vanadyl sulphate - quercetin mixture 0.01/0.02 mmol/kg b.w., aqueous suspension of vanadyl sulphate/quercetin 0.1/0.2 mmol% for 28 days.

All substances were daily oral administered, in a corresponding volume of 1 mL/100 g b.w., for 28 days.

In the 28th day of the experiment, 2 hours after the treatments administration, the animals were ex-sanguinated and serum was collected for biochemical analyses.

Diabetes induction

In the 21st day of the experiment, after an 18 hours fasting period, and 2 hours after the treatments administration, animals from groups D, V, Q and respectively VQ were exposed to 130 mg/kg b.w.

alloxan as 13% saline solution, intraperitoneally. Diabetes onset was determined in the 25th day of the experiment. Animals with blood sugar levels over 200 mg/dL were considered diabetic [22, 23].

Venous glucose evaluation

Venous glucose measurements were performed on blood collected (by puncture) from the tail vein, with a commercial kit for glucose monitoring BioLand G-423 glucometer, BioLand Technology LTD, in days 1, 8, 15, 22, of the experiment.

Biochemical analysis

At the end of the experiment, biochemical parameters (glucose, total cholesterol, HDL-cholesterol, tri-glycerides, alanine aminotransferase (ALT), aspartate aminotransferase (ALT)) were assessed using commercially available diagnostic kits (Liquick Cor®) from Cormay.

Statistical analyses

Statistical analyses were performed with GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. The experimental data was tested for the normal distribution applying D'Agostino & Pearson test (data set ≥ 8) or Kolmogorov-Smirnov (data set < 8). If the results distribution did not pass the normality distribution tests, outliers were identified and excluded by applying the exclusion criteria described by Romanian Pharmacopoeia 10th edition [24]. Results are expressed as mean ± standard deviation. The applied t test has a 90% confidence interval (CI90%). Statistical significance was considered for $p < 0.05$.

Results and Discussion

Venous glycaemia dynamics prior to diabetes induction

The mean venous glycaemia values, prior to diabetes induction and statistical significance are presented in Table I and depicted in Figure 1.

Table I

Venous glycaemia evolution prior to diabetes induction

Venous glycaemia (mg/dL)				
Group	**Day 1**	**Day 8**	**Day 15**	**Day 22**
D	132.00 ± 9.17	121.13 ± 8.79	102.75 ± 6.98	89.50 ± 4.84
V	127.75 ± 11.54	103.50 ± 8.25	95.63 ± 17.58	76.38 ± 5.40
Q	128.13 ± 10.71	105.88 ± 10.37	89.25 ± 9.42	85.25 ± 6.09
VQ	139.63 ± 9.15	107.50 ± 15.43	97.00 ± 12.63	81.75 ± 5.31
Statistical analyses of the data (t test, CI90%, p)				
D *vs.* V	n/s	< 0.05	n/s	< 0.05
D *vs.* Q	n/s	< 0.05	< 0.05	< 0.05
D *vs.* VQ	n/s	< 0.05	n/s	< 0.05
V *vs.* Q	n/s	n/s	n/s	< 0.05
V *vs.* VQ	n/s	n/s	n/s	n/s
Q *vs.* VQ	n/s	n/s	n/s	n/s

CI90% – 90% confidence interval; n/s – not significant

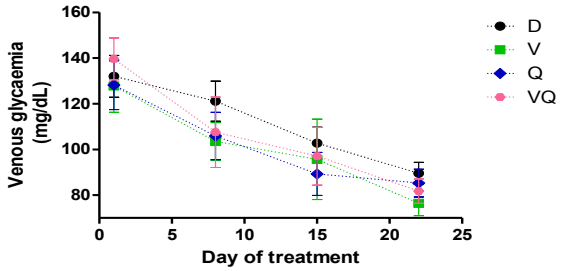

Figure 1.

Mean venous glycaemia dynamics prior to diabetes induction

The animals treated with vanadyl sulphate, quercetin and the mixture vanadyl sulphate-quercetin showed a decrease of the relative elevated initial venous glycaemia levels with statistical significance when compared within the groups ($p < 0.05$) or with the untreated animals (group D).

After 3 weeks of treatment with vanadyl sulphate, quercetin and the mixture vanadyl sulphate-quercetin, the most important decrease in venous glycaemia levels was found for the vanadyl sulphate group when compared to the D group.

Diabetes induction

In the 22nd day of the experiment animals from the groups D, V, Q, VQ were exposed to alloxan in order to induce diabetes. 72 hours after the alloxan administration, 70.83% animals were found diabetic, with a glycaemic increase of 446.07% compared to the baseline (Mann-Whitney test, $p < 0.05$).

Table II highlights are presented data regarding the treatment protection against diabetes onset incidence ratio (IR), the treatment protection against diabetes onset incidence ratio report (IRR), and diabetes onset % risk (RR%) reported to D group.

Table II

The treatments protection against diabetes onset incidence, reported to group D

	D	**V**	**Q**	**VQ**
IR	0.125	0.25	0.5	0.25
IRR *vs.* D		2	4	2
RR (%)		85.71	57.14	85.71

The non-diabetic animals IR was higher for the animals treated with quercetin when compared to the vanadyl sulphate (V) and the vanadyl sulphate - quercetin (VQ) groups. Apparently, in the experimental model used, nor vanadyl sulphate, neither vanadyl sulphate - quercetin mixture enhanced diet did not delay the diabetes onset after the alloxan administration.

Biochemical analysis

Glucose serum levels and the glycaemia lowering effect are presented in Table III. This was calculated as % of reduction in glycaemia level reported to diabetic group using the formula: (D group mean value - Treated group value)/D group mean value * 100.

Table III

Serum glycaemia dynamics in the studied groups of animals

Glycaemia (mg/dL)						
Group		**N**	**D**	**V**	**Q**	**VQ**
Mean ± S.D.		99.83 ± 11.93	869.36 ± 277.53	560.57 ± 275.09	325.70 ± 138.29	265.16 ± 144.26
t test, CI90%, p	**Group *vs.* N**	-	< 0.05	< 0.05	< 0.05	< 0.05
	Group *vs.* D	-	-	< 0.05	< 0.05	< 0.05
	Group *vs.* V	-	-	-	< 0.05	< 0.05
	VQ *vs.* Q	-	-	-	n/s	-
% reduction in rats' glycaemia		-	-	35.52 ± 31.64	62.54 ± 15.91	69.50 ± 16.59

CI90% – 90% confidence interval; n/s – not significant

At the end of the experiment, the animals treated with vanadyl sulphate, quercetin and the vanadyl sulphate-quercetin mixture demonstrated a decreasing effect of the elevated glycaemia levels when compared to the diabetic animals (group D) ($p < 0.05$). The most important glycaemia decrease was observed for the animals treated with the mixture vanadyl sulphate-quercetin, but the treatments did not normalise the glycaemia levels.

The lipidic profile was assessed as total cholesterol, HDL-cholesterol and triglycerides.

Total Cholesterol values and the lowering effect are presented in Table IV. This was calculated as % of reduction in total cholesterol level reported to diabetic group using the formula: (D group mean value - Treated group value)/D group mean value * 100.

Table IV

Serum total cholesterol dynamics in the studied groups of animals

Total Cholesterol (mg/dL)						
Group		**N**	**D**	**V**	**Q**	**VQ**
Mean ± S.D.		92.56 ± 3.71	178.90 ± 28.69	163.81 ± 28.57	125.75 ± 26.38	108.45 ± 18.64
t test, CI90%, p	**Group *vs.* N**	-	< 0.05	< 0.05	< 0.05	< 0.05
	Group *vs.* D	-	-	n/s	< 0.05	< 0.05
	Group *vs.* V	-	-	-	< 0.05	< 0.05
	VQ *vs.* Q	-	-	-	n/s	-
% reduction in rats' total cholesterol		-	-	8.43 ± 15.97	29.71 ± 14.74	39.38 ± 10.42

CI90% – 90% confidence interval; n/s – not significant

At the end of the experiment, the animals treated with quercetin and the vanadyl sulphate-quercetin decreased mixture the elevated total cholesterol levels when compared to the diabetic animals (group D) ($p < 0.05$). The vanadyl sulphate treatment did not influence the total cholesterol levels when compared to the diabetic animals ($p > 0.05$), but the treatments did not normalise the total cholesterol levels.

HDL-Cholesterol values and the variations, calculated as % of change in HDL-cholesterol level reported to diabetic group using the formula: (D group mean value - Treated group value)/D group mean value * 100, are presented in Table V.

Table V

Serum HDL-Cholesterol dynamics in the studied groups of animals

HDL-Cholesterol (mg/dL)						
Group		**N**	**D**	**V**	**Q**	**VQ**
Mean ± S.D.		35.03 ± 2.82	21.53 ± 7.63	20.19 ± 3.90	27.10 ± 8.21	28.59 ± 5.58
t test, CI90%, p	**Group vs. N**	-	< 0.05	< 0.05	< 0.05	< 0.05
	Group vs. D	-	-	n/s	n/s	n/s
	Group vs. V	-	-	-	< 0.05	< 0.05
	VQ vs. Q	-	-	-	n/s	-
% change in rats'HDL-cholesterol		-	-	-6.24 ± 18.10	25.84 ± 38.12	32.77 ± 25.92

CI90% – 90% confidence interval; n/s – not significant

At the end of the experiment, the animals treated with quercetin and the vanadyl sulphate-quercetin mixture increased the HDL-cholesterol levels when compared to the diabetic animals, without statistical significance ($p > 0.05$). The vanadyl sulphate treatment lead to a decrease in HDL-cholesterol levels when compared to the diabetic animals ($p > 0.05$), but the treatments did not normalise the HDL-cholesterol levels.

Triglycerides values and the lowering effect, calculated as % of reduction in triglycerides level reported to diabetic group using the formula: (D group mean value - Treated group value)/D group mean value * 100, are presented in Table VI.

Table VI

Serum triglycerides dynamics in the studied groups of animals

Triglycerides (mg/dL)						
Group		**N**	**D**	**V**	**Q**	**VQ**
Mean ± S.D.		58.24 ± 4.23	165.63 ± 43.59	132.53 ± 85.14	63.38 ± 26.66	77.24 ± 22.50
t test, CI90%, p	**Group vs. N**	-	< 0.05	< 0.05	< 0.05	< 0.05
	Group vs. D	-	-	< 0.05	< 0.05	< 0.05
	Group vs. V	-	-	-	< 0.05	< 0.05
	VQ vs. Q	-	-	-	n/s	-
% reduction in rats' triglycerides		-	-	19.98 ± 51.40	61.73 ± 16.10	53.36 ± 13.59

CI90% – 90% confidence interval; n/s – not significant

At the end of the experiment, the animals treated with quercetin and the vanadyl sulphate-quercetin mixture decreased the elevated triglycerides levels when compared to the diabetic animals ($p < 0.05$), but did not normalise the triglycerides levels.

The hepatic function was assessed after performing the experiment as serum AST and ALT levels, and the results are presented in Tables VII and VIII.

Table VII

Serum AST dynamics in the studied groups of animals

AST (UI/L)						
Group		**N**	**D**	**V**	**Q**	**VQ**
Mean ± S.D.		2.56 ± 0.67	13.20 ± 6.00	2.92 ± 1.72	2.13 ± 0.63	3.26 ± 3.31
t test, CI90%, p	**Group *vs.* N**	-	< 0.05	n/s	n/s	n/s
	Group *vs.* D	-	-	< 0.05	< 0.05	< 0.05
	Group *vs.* V	-	-	-	n/s	n/s
	VQ *vs.* Q	-	-	-	n/s	-

CI90% – 90% confidence interval; n/s – not significant

At the end of the experiment, the animals treated with vanadyl sulphate, quercetin and the mixture vanadyl sulphate-quercetin decreased the elevated AST levels when compared to the diabetic animals ($p < 0.05$), succeeding to normalise the AST levels.

Table VIII

Serum ALT dynamics in the studied groups of animals

ALT (UI/L)						
Group		**N**	**D**	**V**	**Q**	**VQ**
Mean ± S.D.		24.326 ± 1.87	27.60 ± 3.22	27.06 ± 8.51	25.65 ± 7.75	25.76 ± 2.99
t test, CI 90%, p	**Group *vs.* N**		n/s	n/s	n/s	n/s
	Group *vs.* D			n/s	n/s	n/s
	Group *vs.* V				n/s	n/s
	VQ *vs.* Q				n/s	

CI90% – 90% confidence interval; n/s – not significant

After performing the experiment, the treatments did not influence ALT levels.

Conclusions

In the used experimental model, the administration of vanadyl sulphate, quercetin and the vanadyl sulphate-quercetin mixture generated a decrease in the venous blood glucose levels of the healthy animals, with a maximum, after 3 weeks, for the vanadyl sulphate group. The diet supplementation in vanadyl sulphate, quercetin and the vanadyl sulphate-quercetin mixture did not offered a statistical significant protection against alloxan induced diabetes onset, nevertheless quercetin showed the highest protection against diabetes onset incidence ratio (0.5).

After alloxan diabetes induction, the supplementation of the diet with vanadyl sulphate, quercetin and vanadyl sulphate-quercetin mixture proved protective

effects on hyperglycaemia and impaired lipid metabolism. The supplementation of diet decreased the elevated glycaemia, total cholesterol and triglycerides levels of the animals exposed to alloxan. It was observed a similar increase in the high-density lipoproteins, when compared to untreated diabetic animals, for the animals exposed to quercetin and to the vanadyl sulphate-quercetin mixture.

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