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Metabolic responses, PPAR-γ and TNF-α gene expression in type2 diabetic rats subsequent vanadyl sulfate treatment

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Abstract: Vanadyl sulfate (VS), is a component of some food supplements and experimental drugs. This study was carried out to present a novel method for induction of Type 2 diabetes in rats, then for the first time in literature, for evaluating the effect of VS on metabolic parameters and gene expression, simultaneously. 40 male wistar rats were distributed between the four groups, equally. High fat diet and fructose were used for diabetes induction. Diabetic rats treated by two different dose of VS for 12 weeks. Metabolic profiles were evaluated by commercial available kits and gene expression were assayed by real time-PCR. Compared to controls, in non-treated diabetic rats, weight, glucose, triglyceride, total cholesterol, insulin and insulin resistance were increased significantly (*p-value <0.05*) that indicated induction of type 2 diabetes. Further, the results showed that VS significantly reduced weight, insulin secretion, Tumor Necrosis Factor-alpha (TNF-α) genes expression, lipid profiles except HDL that we couldn't find any significant change and increased Peroxisome Proliferator-Activated Receptor- gamma (PPAR-γ) gene expression in VS-treated diabetic animals in comparison with the non-treated diabetics. Our study demonstrated that vanadyl supplementation in diabetic rats had advantageous effects on metabolic profiles and related gene expression.

Keywords: Vanadyl sulfate, metabolic profiles, PPAR-γ, TNF-α,

INTRODUCTION

Diabetes mellitus (DM) has been known as one of the main universal health difficulties. In the course of last decades, the prevalence of DM has been increased considerably, essentially due to rise in the incidence of type 2 DM (Lovic *et al*., 2020; Mirzaei *et al*., 2020). Trace elements display a central task in the suitable functioning of carbohydrate and lipid metabolism by various mechanism (Panchal *et al*., 2017; Mirhashemi *et al*.,2011; Akdas *et al*.,2020; Dubey *et al*., 2020). Vanadium is a group 3d transition metal that occurs in the humans as a modulator in the action of certain bone health conditions related endocrine markers and performances as an inorganic cofactor in many enzymatic reactions, having and assisting cellular bioactivities (Pal 2018; Treviño *et al*., 2019; Levina 2017). Primary attention to varied biological actions of vanadium concentrated on anti-diabetic activities but has moved to anti-cancer and anti-parasitic therapeutics (Levina 2017; Treviño *et al*., 2019; Tsave *et al*., 2016). Vanadyl sulfate has been used in humans as insulin-mimetic salt and thus controls both type 1 and type 2 DM. Vanadyl sulfate defends the beta cells of Islets of Langerhans thus endorsing insulin production and discharge, leading to improved blood sugar regulation (Ahmadi *et al*., 2010 and El-Shenawy *et al*., 2013). On the other hand, some studies found that vanadium is not effective against hyperglycemic states to the extent shown by some other researchers. Another recent study showed that vanadium is not effective against diabetes mellitus at all (Sanchez-Gonzalez *et al*., 2012).

There are also different opinions about the effect of vanadium on insulin. Some believe that vanadium increases insulin secretion, but others believe that this element increases the sensitivity of insulin, rather than increasing its secretion (Novelli *et al*., 2005 and Shah *et al*., 2016). PPAR-γ is mainly expressed in adipose tissues, which controls adipogenesis and lipid storage (Heidari *et al*., 2018). It seems that VS has important role in gene expression of adiponectin in rats' adipocytes by enhancing PPARγ receptors (Wu *et al*., 2013 and Zhao *et al*., 2013).

Due to the rising occurrence of diabetes especially type 2 diabetes, multiform study intended at aborting and remedy is one of the universal investigate priorities. The present study was designed because of conflicting results regarding the effect of vanadium on metabolic profiles. To the best of our facts, this is the first study to evaluate a new modified method for induction of type 2 diabetes in rats and so to estimate vanadyl sulfate effect on PPAR-γ and TNF-α gene expression and so related metabolic responses, simultaneously, *in vivo*.

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MATERIALS AND METHODS

All chemical materials used in this project were bought from Sigma-Aldrich Corporation.

Experimental Animals

Healthy male Wister rats, weighting about 180–210 g, were gotten from Experimental Animal Centre of Kashan University of Medical Sciences, kept at standard conditions 22-24C, 40-60% relative humidity and 12h light–dark cycle and fed with standard rat food and water *ad libitum*. The animals were cared in agreement with the ethics for Care and Use of Experimental Animals. The study protocol was permitted by the ethics committee of Kashan University of Medical Sciences (IR. KAUMS. REC.1395.29).

Induction of type 2 diabetes in rats

For the first time in literature, we used a typical diet of high fat (includes 400 grams of sheep fat, 200 grams of sucrose, 18 egg yolks and 5 egg whites and 400 grams of rat chow, well combined and homogenized) and high fructose (including 25% fructose in drinking water) (HFFD: high at and high fructose diet) for 12 weeks to generate type 2 diabetic model.

Studied groups design

40 rats were distributed between 4 groups equally. The first group was normal rats that received standard rat chow and water without any HFFD/vanadyl sulfate (VS), and considered as controls. The 2nd group received HFFD without any treatment (non-treated diabetic group), the 3rd group, received HFFD and so 25mg/kg vanadyl sulfate (diabetic treated with VS25) and finally, the 4th group received HFFD and 50mg/kg vanadyl sulfate (diabetic treated with VS50). Each group had 10 rats.

Sera and liver tissue preparation

After 12 weeks, at the end of their treatment periods, the animals were weighed and anesthetized using ether and killed by decapitation. For metabolic profiles assessment, sera were separated from blood cells by centrifugation (Hettich D-78532, Tuttlingen, Germany) at 3000 rpm for 15 minutes. Each rat's liver was instantly removed, washed by cold (+4ºC) saline solution and were dried up. All samples stored in -80ºC until analysis.

Metabolic profiles assay

Fasting blood glucose level and lipid profiles including triglycerides (TG), Total Cholesterol (TC), high density lipoproteins (HDL) and low density lipoproteins (LDL) were determined in serum using commercially available kits (Pars Azmun, Tehran, Iran) by BT-3000 auto analyzer. Insulin level was quantified by ELISA method using kit of DiaMetra Company (Spello, Perugia, Italy). To determine the HOMA-IR and the quantitative insulin sensitivity check index (QUICKI), the suggested formulas were used (Pisprasert *et al*., 2014).

Quantitative analysis of gene expression

The concentration of whole extracted RNA from hepatocytes, has been calculated based on OD 260/280 ratio measurements by Nano Drop UV-VIS-Spectrophotometer. To approve the reliability of the extracted RNA, it was electrophoresed. The cDNA was synthesized from the confirmed RNA with Moloney Murine Leukemia Virus enzyme (M-MLV Reverse Transcriptase, 200U/µl, Sigma-Aldrich Co., UK) conferring to the manufacture's protocol. In order to survey the mRNA expression quantities of the related genes, the achieved cDNA was applied as a template. To quantify the mRNA expression levels of these genes in the hepatocytes, real-time reverse transcriptase polymerase chain reaction (RT-PCR) has been carried out on a Bio-Rad iQ5 system (Bio-Rad Laboratories, Hercules, USA), using SYBR -Green quantitative PCR master mix kit (Sinaclon, Tehran-Iran). 18s rRNA gene was used as reference standard gene for all analyses to control the amount of the synthesized cDNA. PCR reactions were carried out in triplicate for each sample, and then the mean of the three readings was taken as fold-induction value. Fold change (X) values were calculated, using $X=2^{-\Delta\Delta Ct}$ equation, in which ΔCt represents the difference between the Ct values of the target genes and the Ct values of the reference standard genes, and ΔΔCt describes the difference between ΔCt value of each sample for each target gene and the average ΔCt of the reference standard gene. Primers were designed using Primer Express software (Applera Europe, Rotkreuz, Switzerland).

Specific primers used for real-time quantitative PCR

Genes	Primer
PPAR-gamma	F: GCACTGCCTATGAGCACTTC R: TCAGCTCTTGTGAACGGGAT
TNF-alpha	F: ACCAGGAGAAAGTCAGCCTC R: GCTGGGTAGAGAACGGATGA
18S rRNA	F: CGATGCGGCGGCGTTATTC R: TCTGTCAATCCTGTCCGTGTCC

STATISTICAL ANALYSIS

Data Analysis was accomplished using the Statistical Package for Social Science version 23 (SPSS Inc., Chicago, Illinois, USA). We conducted the Kolmogorov-Smirnov test to evaluate the normality of distribution of variables. Significance of differences was determined by ANOVA and posthoc Tukey's test. Results are demonstrated as the Mean Difference between groups and were measured statistically significant at $p \leq 0.05$.

Table 1: Effects of HFFD and different concentrations of vanadyl sulfate on body weight of studied groups.

(I) Group	(J) Group	Mean Difference (I-J)	*p-value*
Diabetics	Control	27.44	0.002*
VS25	Diabetics	-45.11	<0.001*
VS50	Diabetics	-49.22	<0.001*
VS50	VS25	-4.11	0.932

Table 2: Effects of HFFD and different concentrations of vanadyl sulfate on fasting blood glucose, Insulin, HOMA and QUICKI in experimental groups

Mean Difference (I-J) (p-value)					
(I) Group	(J) Group	Glucose (mg/dl)	Insulin(μIU/ml)	HOMA-IR	QUICKI
Diabetics	Control	122.67(P <0.001)*	0.93(P <0.001)*	0.91(P<0.001)*	-0.09(P<0.001)*
VS25	Diabetics	-62.11(P<0.001) *	-0.60(P: 0.012) *	-0.58(P<0.001) *	0.05(P: 0.003) *
VS50	Diabetics	-64.22(P<0.001) *	-0.92 (P<0.001) *	-0.73(P<0.001) *	0.06(P<0.001) *
VS50	VS25	-2.11(P: 0.999)	-0.31(P: 0.334)	-0.15(P: 0.671)	0.02(P: 0.547)

Table 3: Effects of HFFD and different concentrations of vanadyl sulfate on lipid profiles in experimental groups

Mean Difference (I-J) (p-value)					
(I) Group	(J) Group	TG	TC	LDL	HDL
Diabetics	Control	77.44(P <0.001)*	26.33(P <0.001)*	4.22(P <0.001)*	3.89(P: 0.321)
VS25	Diabetics	-54.33(P <0.001)*	-15.67(P:0.002)*	-3.56(P: 0.013)*	-1.22(P: 0.947)
VS50	Diabetics	-23.66(P <0.001)*	-17.78(P: 0.001)*	-2.00(P: 0.271)	-3.23(P: 0.484)
VS50	VS25	30.66(P <0.001)*	-2.11(P: 0.951)	-1.56(P: 0.487)	-2.00(P: 0.808)

All groups were fed with HFFD except controls, for 12 weeks.
Treated groups received different concentrations (25 and 50 μM) of vanadyl sulfate (VS25 & VS50) in addition to HFFD for the same time.
Data have been shown as Mean Difference, n=10.
The mean difference is significant at the 0.05 level.
*indicated statistically significant difference between groups.
P: *P-Value*

RESULTS

Effect of different concentrations of vanadyl sulfate on body weight of diabetic rats

Just as displayed in table 1, the average ultimate body weight of non-treated diabetic group (2nd group) was significantly increased by 8.9% compared to the normal control animals (*p-value:* 0.002). Different concentrations of vanadyl sulfate reduced the body weight significantly (*p-value:* 0.000) by 13.4% and 14.7% in 3rd and 4th groups respect to the 2nd group. But these reduction was not vanadyl sulfate dose-dependent.

Changes in fasting blood glucose, Insulin, HOMA-IR and QUICKI in experimental groups

There was statistically significant increase (*p-value:* 0.000) in glucose, insulin and HOMA-IR levels in 2nd group compared to controls (102.7%, 81.6% and 267.5% enhancements respectively). Treatment with the different doses of vanadyl sulfate decreased glucose, insulin and HOMA-IR and increased QUICKI significantly in 3rd and 4th groups in comparison to the 2nd (*p-value:* 0.000). These variations were vanadyl sulfate dose- independent, (table 2).

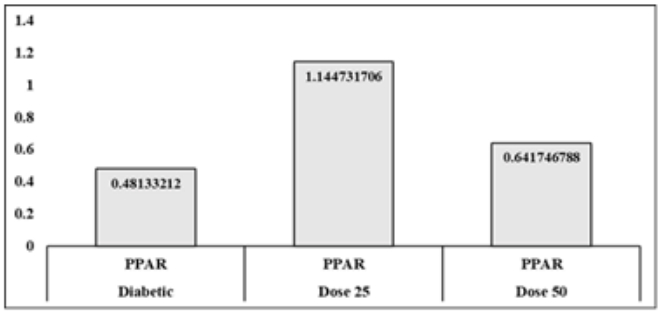
Effects of VS-treatment on lipid profiles in serum of rats

Just has been shown in table III, respect to control group, lipid profiles including TG, TC and LDL were increased statistically significant by 125.6%, 42.5% and 19.3% in 2nd group. Vanadyl Sulfate significantly (*p-value:* 0.000) reduced TG and TC in the 3rd (39.1% & 17.7%, respectively) and 4th (17% & 20.1%, respectively) groups compared to the 2nd. LDL concentration was significantly (*p-value:* 0.013) reduced by VS25, However, VS50 had no significant (*p-value:* 0.271) effect on LDL level. It was very interesting that the effect of VS25 on the reduction of triglyceride was significantly greater than that of VS50 (*p-value: 0.000*). There was no significant difference in HDL level between all four groups.

Effects of treatment with vanadyl sulfate on PPAR-γ and TNF-α gene expression

Up to our knowledge there were no documents regarding the effects of VS on PPAR-γ and TNF-α genes expression in liver of type 2 diabetic rats. We showed that VS could increase gene expression of PPAR-γ in liver of treated groups compared to diabetics. The increased ratio was higher in VS25 related to VS50 (fig. 1). The effect of VS on TNF-α expression was also evaluated. This effect was a diminutive expression for TNF-α in a dose dependent manner (fig. 2).

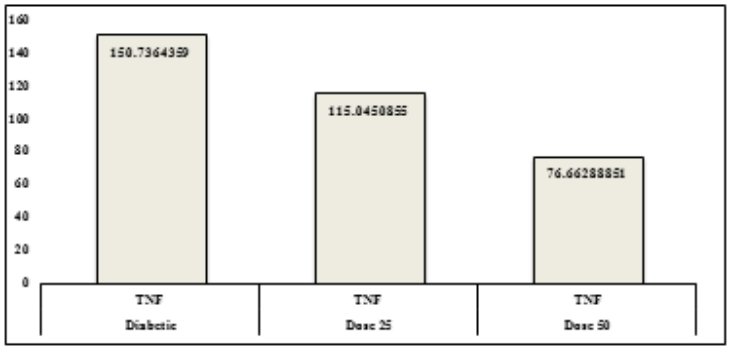
	Gene	Ratio=Fold change target/Fold change reference
Diabetic	PPAR-γ	0.48133212
VS25	PPAR-γ	1.144731706
VS50	PPAR-γ	0.641746788



VS could increase gene expression of PPAR-γ in liver of treated groups compared to diabetics. The increased ratio was higher in VS25 related to VS50.

Fig. 1: PPAR-γ gene expression in non-treated diabetic group and VS-treated diabetic groups

	Gene	Ratio=Fold change target/Fold change reference
Diabetic	TNF-α	150.7364359
Dose 25	TNF-α	115.0450855
Dose 50	TNF-α	76.66288851



VS had significant effect on TNF-α gene expression in liver of treated groups compared to diabetics. This effect was a diminutive expression for TNF-α in a dose dependent manner.

Fig. 2: TNF-α gene expression in non-treated diabetic group and VS-treated diabetic groups

DISCUSSION

In current research, for the first time in literature, we estimated the outcome of vanadyl sulfate on metabolic parameters and the genes expression in type 2 diabetic model, simultaneously. The first series of findings showed that compared to controls, in non-treated diabetic rats, weight, glucose, TG, TC, insulin and insulin resistance were increased significantly (*p-value <0.05*) that indicated induction of type 2 diabetes in rats. Further, the results of vanadyl effect on metabolic profiles and gene expression showed that vanadyl sulfate significantly reduced weight, insulin secretion, TNF-α genes expression, lipid profiles except HDL that we couldn't find any significant change and increased PPAR-γ gene expression in VS-treated diabetic rats compared to the non-treated diabetic group. Although the exact mechanism for reducing effects of VS on metabolic profiles stay ambiguous, but it may be proposed that the inhibitory power of this compound on metabolic responses may be owing to their useful effect on the gene expression. Contrary to our findings, some studies demonstrated that vanadyl sulfate did not modify insulin sensitivity (Jacques-Camarena *et al*., 2008) but increased triglyceride concentrations (Jacques-Camarena *et al*., 2008 and Imura *et al*., 2013) and other lipid parameters or failed to improve dyslipidemia in diabetic rats (Shahi *et al*., 2011). In consistent to our findings, there are a number of studies which supported benefit of vanadium supplementation for perfection of lipid complaints (Zhang *et al*., 2014 and Park *et al*., 2013). Soveid, *et al*. showed that oral vanadium administration to diabetics had lowering effects on lipid parameters, but unlike us, they showed that HDL-c levels have been increased with unaltered TG levels (Soveid 2013). Our results, demonstrated that vanadyl sulfate reduced fasting

glucose, insulin secretion and HOMA-IR, but increased insulin sensitivity significantly in VS- treated groups compared to non-treated diabetic rats. These outcomes were similar to Missaoui and *et al* that showed vanadyl sulfate normalized plasma glucose and ameliorated insulin performance in STZ-experimental diabetes and prompted beta cells reproduction and/or regeneracy (Missaoui *et al*., 2014). Further, the influence of vanadyl administration were evaluated on PPAR-γ and TNF-α genes expression in this study. The results were in line with other studies indicating the positive effect of this metal on the genes (Wu *et al*., 2013 and Zhao *et al*., 2013). But the difference between our work and others was that this was done in the liver tissue, for the first time. Our study had some limitations. The main restriction of our study was the absence of measurements of circulating levels of vanadyl sulfate in animals due to financial limits. Really, owing to limited financial resource for this projects, we did not assess some markers. Therefore, measurement of inflammatory cytokines and biomarkers of oxidative stress after intervention are reasonable in forthcoming studies.

CONCLUSION

It may be concluded that vanadyl sulfate should be imperative and beneficial molecule for the expansion of the therapeutic materials for improvement of metabolic profiles and inflammatory markers in diabetic patients. Undoubtedly, more studies are required to disclose the potential and/or likely toxicity of this compound in long-term use.

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