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Effect of Amonium Vanadate Nano-Particles on Experimental Diabetes and Biochemical Factors in Male Spargue-Dawly Rats

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Abstract

***Background*:** Vanadium compounds have antidiabetic, but there is no study about the antidiabetic effects of ammonium monovanadate nono-particles. In this study, antidiabetic effect of ammonium monovanadate was compared with its nonoparticles.

***Materials and Methods*:** In this experimental and animal modeling study, 42 male Sprague Dawley rats were divided in 6 groups (each group 7 rats). Diabetes was induced by IP injection of 60 mg/kg streptozicin to five groups. One normal group and one diabetic group were kept without treatment as control groups. Other diabetic rats were treated with nonovanadate 25 mg/kg (NV25), ordinary vanadate 25 mg/kg (V25), nonovanadate 15 mg/kg (NV15) and water ordinary vanadate 25 mg/kg (WV25) groups for 10 days by gavage. Heparinized blood was collected at days 11 and 21 and centrifuged to separate plasma. Glycosylated hemoglobin, insulin, triglyceride (TG), total cholesterol (TC), HDL-c and LDL-c were measured at both time and compared between groups.

***Results*:** All treatment except in V25 decreased glycemia, but insulin increased only in NV25 group ($p<0.05$). All treatment decreased TG, TC and LDL-c significantly ($p<0.001$).

***Conclusion*:** Ammonium mono vanadate nonoparticle has better effects than ordinary ammonium mono vanadate on some biochemical factors in experimental diabetes.

Introduction

Vanadium is an essential microelement for the growth of mammalian cells in the culture medium [1]. Vanadium has a performance like insulin in diabetes [2, 3]. It reduces hyperglycemia and improves the functions of insulin secretion and storage in the beta cells [4, 5]. Vanadate ions imitates the insulin effects through post-receptory mechanism [6, 7]. One of the Vanadium mechanisms is the inhibition of protein tyrosine phosphatase enzyme which is important for the termination of insulin receptor signaling pathway [8]. Vanadium (including vanadyl and vanadate) increases glucose uptake, stimulation of glycogen synthesis, facilitation of the transport and oxidation of glucose, increase in the insulin receptor tyrosine kinase activity, inhibition of gluconeogenesis and glycogenolysis and activation of lypogenes in the rats [9].

It has been reported that the vanadium compounds provide some changes in the metabolism of proteins, lipids, enzymes and other metabolic activities [10]. Therefore it was suggested that vanadyl sulfate is considered as a pharmaceutical agent for the treatment of type 1 diabetes, because it may have an important role in the insulin activitiy [11]. However, the large increase of intracellular vanadium may cause metabolic disorders. For example, high concentrations of sodium vanadyl cause structural changes in the kidney, spleen and respiratory tissue. Therefore, due to the toxicity of Vanadium, its usage as a clinical treatment for diabetes has been delayed [10, 11]. Nanotechnology has been significantly developed in the field of pharmaceutical industry and nano-drugs are superior in different directions including medication side effects, lower doses of the drug, higher purity, higher selectivity in target tissue and easier to get, compared to the conventional therapy. Given that the effects of ammonium nono-vanadate have not been studied so far in diabetic animals, and its lower doses may be effective when it is in the form of Nano-particles and their toxicity may be reduced, therefore the aim of this study is to examine the effects of ammonium nano-vanadate in diabetic animals and to compare the hypoglycemic effects of some blood biochemical factors with the usual form of ammonium vanadate.

Materials and Methods

In an experimental study (animal model), 42 Male rats, from the Sprague-Dawley race, with a weight range of 20±180 g were purchased from the animal propagation and breeding laboratory of Jundishapur Medical Sciences University. During the study, animals were kept under 12 hours light, 12 hours dark at 23±2ºC. The animals were

kept for a week in this condition in order to adapt to environmental conditions and they had free access to food and water (compressed plate produces by Pars Tehran animal food production company). The rats were kept and killed based on animal rights laws. In order to create diabetes mellitus, 60 mg/kg of streptozotocin (made in Alexis Construction Company, USA) dissolved in citrate buffer was intraperitoneally injected to the rats and after 5 days, their diabetes (glucose greater than 300 mg/dl) were studied through measuring the blood glucose by glucometer (Bionium, Netherlands). The blood glucose of healthy rats was normal.

Rats were randomly divided into 6 groups and in all groups they developed diabetes except the control group that was healthy before the grouping. These four groups included healthy control, diabetic control, nano-vanadate 25 and nano-vanadate 15 (to assess the similar effects, lower doses of nanoparticles were selected). Vanadate 25 was gavaged in saline by 25 mg/kg ammonium nono-vanadate (prepared by grinding in industrial university of Isfahan, size of particles was determined as 40 nm by X-ray and electron microscopy), 15 mg/kg ammonium nono-vanadate and 25 mg/kg ammonium vanadate (Merck, Germany.) respectively. The group of vanadate in water was daily received 25 mg/kg ammonium vanadate in 0.4% sodium chloride solution. All studied groups were put under the above treatments during the first 10 days of the treatment period.

And on the 11th day, their blood samples were taken and all treatments were then stopped and again on 21st day, the animals were bloodletting. On the days 11 and 21 of this study and after 8 hours of fasting, rats were anesthetized by chloroform. On 11th day, the samples were taken from the ocular sinus using hairy tube and blood sampling from the heart was performed on 21st day. Heparinized blood was used to measure the glycosylated hemoglobin and serum plasma was used to measure insulin and lipid parameters. In order to confirm the development of diabetes, after induction of diabetes and after 8 hours of food deprivation on 11th and 12th days, the blood sugar levels were measured using glucomter on the blood taken from the tail of these rats. For the measurement of glycosylated hemoglobin, glycosylated hemoglobin kit (Biosystems, Spain) was used.

In this method, glycosylated hemoglobin was isolated using a cation exchange resin column and then its proportion to the total hemoglobin was determined (as percentage). Insulin was measured using rat insulin-specific kit (Mercodia, Sweden) and the levels of triglycerides, total cholesterol and HDL-c were enzymatically measured using commercial kits made by Pars Azmoon Company. Then the values of LDL-c were measured and the results were analyzed by SPSS-16 software.

For this reason, the results of the sampling in 11th and 21st days in each group were statistically analyzed using paired *t*-test and results of different groups were statistically analyzed using ANOVA test and they were compared by the Tukey test. Also for the comparison of the weight changes in each group, in successive weeks, repeated ANOVA test and Bonferoni test were used. In cases where $p<0.05$, differences were considered significant and the results were presented as mean±standard error.

Results

Development of diabetes increased the level of glucose in sampling on 11th day in the diabetic, vanadate and nano 15 control groups compared to the healthy control group 15. However, the level of glucose in nano 25 and vanadate in water groups showed no significant difference compared to the control group. While sampling on 21st day (10 days after treatment), glucose levels increased in all groups compared to the control group. Also, the level of glucose in the second stage of sampling increased in the groups of nano-vanadate 25, nano-vanadate 15 and vanadate in water but it was associated with a significant reduction in the healthy group (Fig. 1).

Development of diabetes causes glycosilated hemoglobin in the sampling of 11th day in all diabetic groups compared to the healthy control group. In the sampling of 21st day, the amount of glycosilated hemoglobin in the groups of nano 25, nano 15 and vanadate in water decreased compared to the previous sampling and reached to the level of healthy control group (Fig. 2).

In all diabetics groups except nano-vanadate 25, a significant reduction of insulin in the both stages of sampling was observed. Application of different treatments increased the amount of insulin, but insulin increase in the both stages was only considerable in the group of nano 25 compared to the diabetic control group (Fig. 3).

Induction of diabetes in the sampling of 11th day was not associated with the changes in triglyceride levels; however, the use of various forms of vanadate almost in a similar form greatly decreased the level of triglyceride compared to the normal and diabetic control groups. But in the second sampling, triglyceride levels in diabetic control group were higher compared to all other groups. Treatment cessation in the groups of vanadate 25 and vanadate in water increased the level of triglyceride; and in the group of nano vanadate 15, the increase in the level of triglycerides was relatively significant, but its increase in the diabetic control group was not significant.

Also, the level of triglycerides in all treated groups with various forms of vanadium was less than diabetic control group. Sharp increase in the total cholesterol in both the first and second sampling stages in diabetic control group was observed compared to the healthy control group. But in both stages, the cholesterol levels of other groups was the same as the level of cholesterol in the control group and they showed no significant difference with each other. Also in the both groups of nano vanadate 25 and vanadate 25, cholesterol levels in the second stage of sampling were reduced compared to the first stage of sampling. No significant changes in HDL-c level in samples of 11th and 21st days in the control diabetic

group was observed compared to the healthy control group. In the first-stage sampling, HDL-c levels in nano-vanadate 25 was reduced compared to the groups of healthy control, nano-vanadate 15 and vanadate in water and its value in the group of vanadate 25 was less than the group of vanadate in water. In the second stage sampling, HDL-c levels in the group of vanadate 25 were also less than the groups of vanadate in water and nano vanadate 15. Also the value of this parameter in the groups of diabetic control and vanadate 25 was also less than the group of nano-vanadate15. The value of this parameter in any of the groups indicated no significant differences between two sampling stages. The level of LDL-c at 11th and 21st days of sampling in diabetic control group had a sharp increase compared to the healthy control group. But LDL-c levels in all diabetic groups treated with various forms of vanadium in both stages of sampling were severely reduced compared to the diabetic control group and reached to the normal level. LDL-c levels in the second stage of sampling from the groups of diabetic control, nano vanadate 25 and vanadate 25 decreased compared to the first stage of sampling (Table 1).

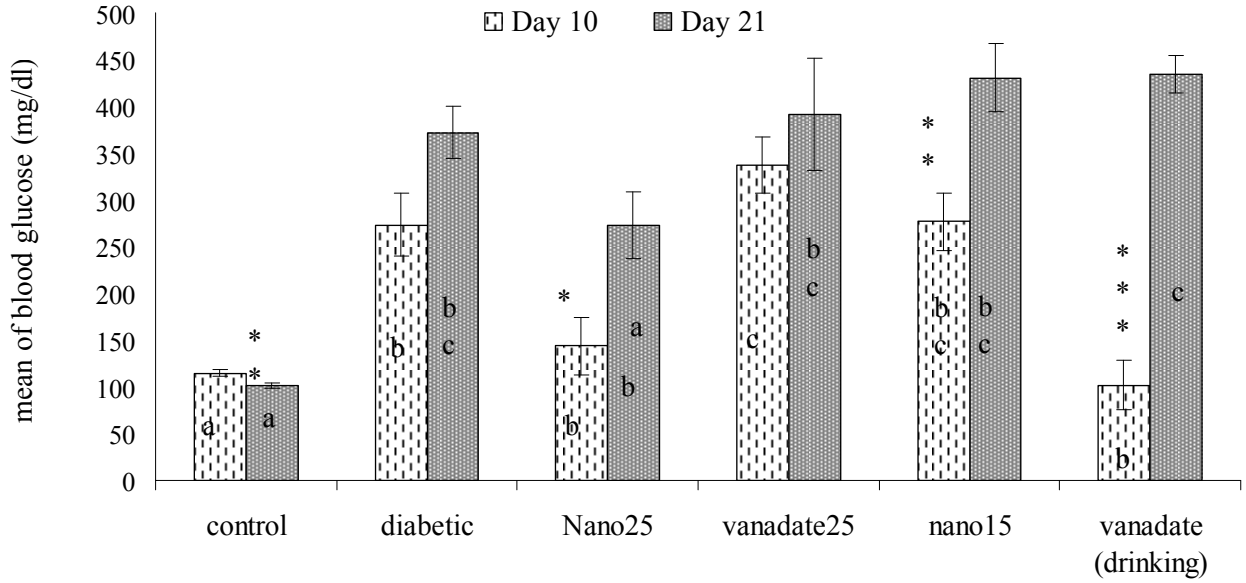

Figure 1. Mean±SEM of the blood sugar in different groups. Different Latin letters indicate significant difference with at least (p<0.05), between the different groups.* Indicates a significant difference between 11th and 21st days of sampling. (* p<0.05), (** p<0.01) and (*** p<0.001)

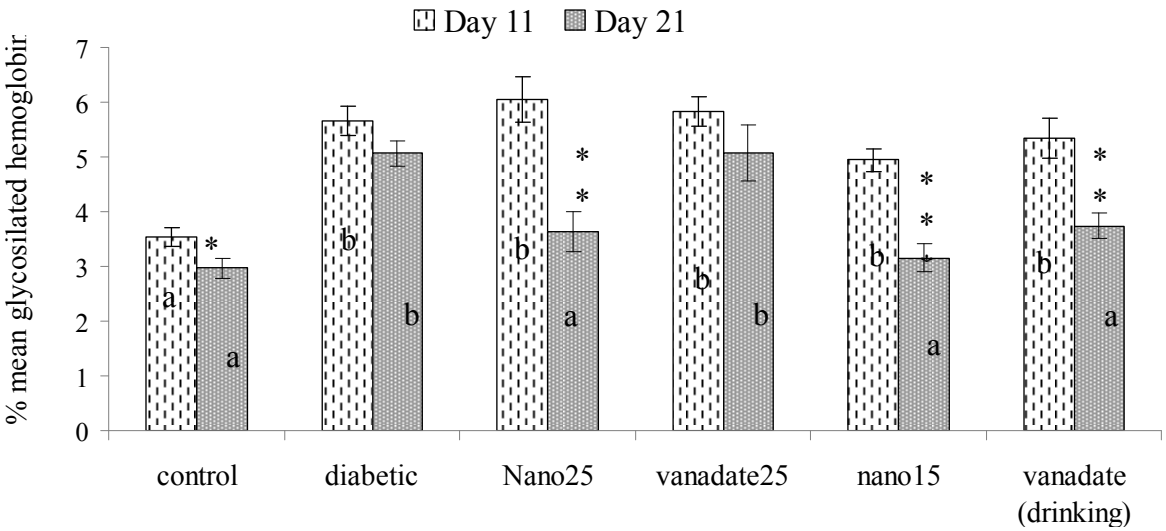

Figure 2. Mean±SEM of glycosilated hemoglobin in different groups. Different Latin letters indicate significant difference with at least (p<0.05), between the different groups (** p<0.01)

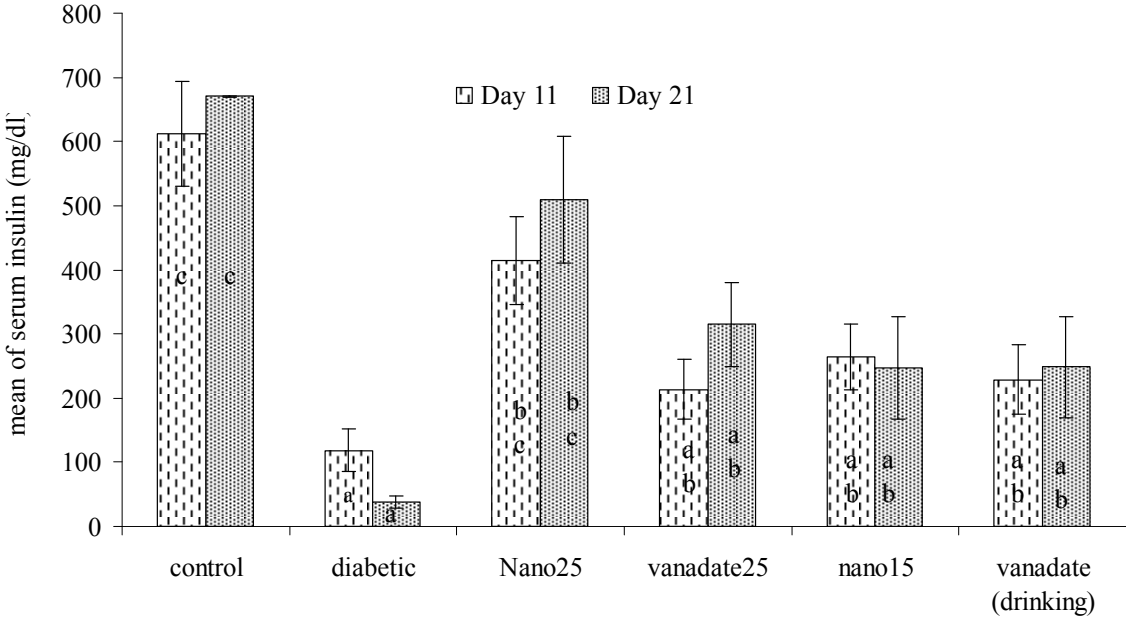

Figure 3. Mean ±SEM of serum insulin levels in different groups. Different Latin letters indicate significant difference with at least (p<0.05), between the different groups

Table 1. Mean ±SEM of the lipid parameters in different groups

parameter	day	control	Diabetic control	*p*-Value	Nano vanadate25	*p*-Value	Vanadate 25	*p*-Value	Nano vanadate15	*p*-Value	Vanadate in water	*p*-Value
TG	11	52±2	56±1	0.867	32±3	0.0001	37±3	0.0001	32±2	0.0001	28±2	0.0001
(mg/dl)	21	55±3	82±12	0.03	30±3	0.0001	47±5	0.007	36±2	0.0001	37±2	0.0001
Total cholesterol (mg/dl)	11	59±2	97±7	0.0001	59±2	0.0001	62±2	0.0001	60±5	0.0001	61±4	0.0001
	21	60±2	83±3	0.0001	51±3	0.0001	50±1	0.0001	61±4	0.0001	60±4	0.0001
HDL-c	11	17.5±1.2	16.4±1.2	0.971	12.5±0.8	0.142	14.1±0.3	0.685	18.1±1.1	0.872	18.1±1.3	0.858
(mg/dl)	21	16±1.6	14±1	0.974	14.7±1.1	0.997	12.6±1.1	0.970	20.3±1.6	0.007	18.1±0.8	0.152
LDL-c	11	30.5±2	69±6	0.0001	40±2	0.0001	41±3	0.001	35±4	0.0001	37±3	0.0001
(mg/dl)	21	34±2	52±3	0.0001	30±2	0.0001	28±2	0.0001	33±3	0.0001	35±3	0.0001

Discussion

The use of mono ammonium vanadate in drinking water and in the form of nano, with a dose of 25 mg/kg and with a lower dose in the group of nano vanadate 15 controlled the blood sugar. This blood sugar control was also associated with the improvements in the insulin levels, so we can conclude that the use of ammonium Vanadate and also ammonium nano vanavat in drinking water almost similarly led to the blood sugar control after 10 days of treatment and nano ammonium vanavat with a dose of 25 mg/kg has likely increased the Beta-cell secretory function. It should be noted that despite glycemic control (blood sugar control) in above groups, no recovery was observed in the status of glycosylated hemoglobin. In this study, vanadium could not have significant impact on glycosylated hemoglobin during treatment. This result differs from the results of some other studies [8], probably due to the differences in the prescribed manner or prescribed term of vanadium. It should be noted that despite the changes in blood glucose levels that are immediately affected by the amount of insulin or hypoglycaemic agents (drugs), glycosylated hemoglobin levels may change slowly over time, therefore, it can be argued that the improvement in blood glucose levels probably have occurred in the final days of treatment and consequently there was not an ample opportunity to return glycosylated hemoglobin levels. Following the cessation of treatment and in the second stage of sampling, as can be seen, blood glucose levels increased in all groups, blood glucose level of which was controlled in the previous sample. However, this increase was minimal in the group of nano 25 and maximal in the group of vanadate in water. But it still did not match the results of glycosylated hemoglobin, because the level of glycosylated hemoglobin in this stage of sampling in all treated groups was in the level of control group except the group of vanadate 25. This mismatch between the blood glucose levels and glycosylated hemoglobin can be attributed to the delayed response of glycosylated hemoglobin. Another noteworthy point is that after cessation of treatment, the amount of insulin did not decrease in any of the treated groups, while its amount decreased in the diabetic control group. Treatment of diabetic rats with vanadium salts, reduced hyperglycemia and in some cases has led to normoglycemia [12]. In most studies, vanadium salts were orally used in water and lowered the level of glucose, 2 to 4 days after treatment

[12]. Dose of vanadium salts were different in various studies and the 0.5 mg/ml concentration of this salt dissolved in water lowered the blood sugar. Of course, its effect on lowering blood sugar depends also on the duration of treatment. 2 to 4 days after the cessation of drug intake, the animals developed hyperglycemia [13], but in another study, normoglycemia was continued 13 weeks after treatment [14]. Ding et al. found that low-dose and high-dose of meta ammonium vanadate (10 and 100 g/mlμ) leads to the reduction in the level of glucose and glycosylated hemoglobin and improvement of diabetic conditions in diabetic rats [15]. Vanadate is a PTP inhibitor that can react with insulin signaling pathways in three ways: 1. Imitating the metabolic action of insulin 2. Increasing the insulin sensitivity 3. Prolonging the biological response to the insulin.

Vanadate and its compounds such as praxovanadium have clearly a function similar to the function of insulin [16]. Wang et al. found that long term treatment (3 weeks) with vanadium significantly preserved the insulin level in Zuker rats. They attributed this condition to the improving glucose homeostasis and consequently preventing from the destruction of beta cells due to the glucose toxicity [17]. Wang et al. supported this hypothesis that says Vanadium acts as a protective agent for the beta cells in type 2 diabetes [17]. In our study, a portion of improvement in the level of insulin can be attributed to the blood glucose control and thus the reduction in the level of glucose toxicity in pancreatic beta cells. Based on our study, nano ammonium vanadate not only has hypoglycaemic effects, but also its hypoglycaemic effects are much better than the effect of regular kind of ammonium vanadate. Diabetes increased the level of triglycerides (TG) (second stage of sampling), total cholesterol (TC) and LDL-C which is consistent with the results of many other studies [15]. The use of various forms of vanadium in the present study significantly decreased the amount of TG, so that the level of TG in treated groups was even lower than its level in healthy control groups. Also, all the treatments lowered the level of total cholesterol and LDL-C.

It should be noted that although the amount of LDL-C in diabetic groups was lowered following the use of various forms of vanadium, but given that the HDL-C levels decreased in the first two groups (which used higher doses of vanadate or nano vanadate as gavage) and because the cessation of treatment in these two groups was associated with the considerable reduction in the level of LDL-C, the high levels of ammonium vanadate appears to be causing lipid abnormalities, although nano-vanadate 15 and also ammonium vanadate in water were never been associated with such adverse effects. In a study conducted by Willsky et al. in addition to the blood glucose, diabetic hyperlipidemia was significantly decreased following the use of Vanadyl sulfate, while serum lipids in normal animals had no specific changes after application of vanadyl sulfate [8]. Ding et al. found that ammonium vanadate lowered the high levels of cholesterol, triglycerides and HDL-C in diabetic rats [15]. Kim et al. observed the hypoglycemic effect of vanadium on diabetic dogs with alloxan monohydrate and found that the TG and cholesterol levels in diabetic dogs were reduced compared to the healthy dogs [18].

Willsky et al. studied the reducing-effect of vanadyl sulfate on lipid parameters (free fatty acids, cholesterol, and triglycerides) in diabetic rats [8]. Although the results of the present study were promising in experimental model, but other administrated or prescribed methods, and different doses of vanadium nanoparticles and preparation of one of its forms that can be consumed in drinking water are among other aspect of this issue that must be addressed. Also it seems that at the long-term use, perhaps lower doses have fewer side effects that need more studies. Based on the conducted study, nano ammonium vanadate has hypoglycaemic effects comparable to the usual form of ammonium vanadate in drinking water and in addition, it could increase the level of insulin, while the use of ammonium vanadate gavage, almost had no proper effect. This may be due to the physical and biochemical changes in the behavior of particles which was likely affected the biological, insulin-like and insulinotherapic properties of ammonium vanadate.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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