

Anti-Diabetic Effects of Vanadium Pentoxide Nanoparticles in STZ-Induced Diabetic Rats

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ABSTRACT

The aim of the present study was to evaluate the anti-diabetic effects of vanadium pentoxide nanoparticles and to compare it with that of vanadium pentoxide on STZ-induced diabetic rats. Vanadium pentoxide and Nano-vanadium pentoxide each at the dose rate of 5 mg/kg body weight were administered orally in normal and experimentally induced diabetic rats for 30 days and glimepiride was used as a reference drug. Both the vanadium pentoxide and Nano-vanadium pentoxide significantly decreased the levels of serum glucose, triacylglycerol, total cholesterol, urea and creatinine and the activities of ALT and AST and significantly increased the serum protein and liver glycogen levels in diabetic rats. No adverse effect in gastrointestinal tract was observed. In the present study vanadium in the form of nanoparticle outperformed as an anti-diabetic agent than that of vanadium pentoxide in normalizing the biochemical parameters without any adverse effects.

Key words: Streptozotocin; Glucose; Vanadium; Nanoparticle; Hyperglycemia.

INTRODUCTION

Diabetes Mellitus is a chronic metabolic disorder characterized by hyperglycemia, due to insulin resistance or insulin insufficiency, and associated disturbance in carbohydrate, protein and lipid metabolism¹.

Vanadium, chromium, zinc, molybdenum and cobalt have all been studied and proposed as possible supplement in the treatment of diabetes mellitus. Of which vanadium is the thoroughly studied and the

most potent transition metal in diabetes mellitus treatment². However, vanadium complex that has specificity to the target site with improved pharmacokinetic properties is required to initiate it as a clinical drug.

Nanotechnology has been developing significantly in the field of pharmaceutical industry. Nano-drugs are superior to conventional therapy in terms of medication side effects, doses of the drug, purity, selectivity in target tissue and availability.

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Hence, nanoparticles are exploited in various disease conditions for the effective diagnosis or treatment⁴. However, a lifelong disease condition like diabetes has no complete treatment so far⁵.

Very few studies have been done in experimental animals with metals like vanadium, chromium and zinc in the form of nanoparticles. Though these metals have beneficial effect in the form nanoparticles in diabetic rats, their toxicity profile is yet to be examined³.

Hence, the present study was conducted to evaluate the antidiabetic effects of vanadium pentoxide nanoparticles (V_2O_5 NP) on the biochemical parameters that indicate the abnormality of carbohydrate, protein and lipid metabolism and associated damage in liver and kidney, in STZ-induced diabetic rats.

MATERIAL AND METHODS

Chemicals

All the chemicals used in this study were of analytical grade. Streptozotocin (STZ) was purchased from Sigma Chemicals Co., St. Louis, USA. Glimpiride was obtained *as gratis* from Orchid pharmaceuticals limited, Chennai. The serum diagnostic kits were purchased from M/s Agappe diagnostics, Ernakulum, Kerala, India.

Vanadium pentoxide nanoparticles

Vanadium in the forms of V_2O_5 and V_2O_5 NP used in the present study were standardized and obtained as gift from the department of Inorganic Chemistry, University of Madras, Guindy campus⁶.

Animals

Adult male Wistar rats, weighing about 150-200 g were obtained from Laboratory Animal Medicine Unit, Tamil Nadu Veterinary and Animal Sciences University, Chennai - 51, India. The animals were acclimatized for three weeks prior to the start of the experiment. They were maintained on standard rat feed supplied by Provimi Animal Nutrition India Private Limited, Bangalore, India. All animals were housed in cages with 12/12 hours light/dark cycle and were fed *ad libitum* feed and water throughout the experimental period.

The animal experiments were carried out after prior approval of Institutional Animal Ethical Committee (IAEC), Madras Veterinary College Chennai – 600007 (Ethical Committee No: 2172/DFBS/B/2013).

Induction of diabetes mellitus

The blood glucose level was assessed by MYLIFE PURA glucometer to rule out spontaneous diabetes in the rats. Those animals which showed normal blood glucose levels of 80-110 mg/dL, were selected for the study. Diabetes was induced in rats with STZ at the dose rate of 50 mg/Kg by the method as described by Ganda *et al*⁷ and Katsumata *et al*⁸.

Experimental protocol

Experiment was conducted for thirty days with forty male Wistar rats which were randomly divided into five groups, each consisting of eight animals. Group I served as untreated normal control. Group II served as STZ-induced diabetic control. Groups III and IV were diabetic rats treated at the dose rate of 5 mg/kg body weight with V_2O_5 and V_2O_5 NP respectively. Group V was diabetic rats treated with standard hypoglycaemic drug glimepiride at the dose rate of 800 μ g/kg body weight for comparison.

Blood samples were collected after 30 days of experiment and the serum were separated by centrifugation at 3000 rpm for 15 min and were used for the estimation of total protein, cholesterol, triacylglycerol, urea, creatinine, ALT and AST as per the protocol described in the standard kits. At the end of the experiment, animals were euthanized by carbondioxide asphyxiation with steady state of increasing the carbondioxide concentration in the chamber, liver samples were dissected out and pieces of liver were collected in sterile container for glycogen estimation in liver.

Statistical analysis

The results were expressed as mean \pm S.E. All the data were analyzed by SPSS package version 20, one way analysis of variance followed by Duncan's test multiple comparison test as described by Snedecor and Cochran⁹. A value of ($p < 0.05$) and ($p < 0.01$) were considered statistically significant.

RESULT

Anti-diabetic effects of V₂O₅ and V₂O₅ NP were studied in STZ-induced diabetic rats for 30 days and this effect was compared with that of standard drug glimepiride.

Body weight of control and experimental group of rats are shown in figure 1. Diabetic rats showed a significant decrease ($p < 0.01$) in the body weight when compared to normal rats. There was a significant ($p < 0.01$) improvement in body weight gain of diabetic rats after administration of V₂O₅ NP and V₂O₅ for 30 days, which is in accordance with glimepiride treated diabetic rats

Table 1 shows the levels of serum glucose, total protein, triacylglycerol and cholesterol of control and experimental group of rats. Diabetic rats showed a significant increase ($p < 0.01$) in serum glucose level. Administration of V₂O₅, V₂O₅ NP and glimepiride resulted in significant ($p < 0.01$) decrease in serum glucose level than untreated diabetic rats. Among the treatments V₂O₅ NP had a significant control over serum glucose level than V₂O₅ and glimepiride.

Significant increase ($p < 0.01$) in serum triacylglycerol and total cholesterol levels observed in diabetic rats were reduced by treatment with V₂O₅, V₂O₅ NP and glimepiride. Whereas, triacylglycerol level of V₂O₅ NP treated rats and cholesterol level of V₂O₅ NP and glimepiride treated rats were comparable to that of normal rats. Significant decrease in total protein concentration observed in diabetic rats was increased significantly in rats treated with V₂O₅ and glimepiride, while it was restored to near normal level in rats treated with V₂O₅ NP.

Table 2 shows the activities of AST and ALT and levels of serum creatinine and urea in control and experimental groups of rats. Diabetic rats showed a significant increase ($p < 0.01$) in activities of ALT and AST and in levels of creatinine and urea when compared to normal control rats. Treatment with V₂O₅, V₂O₅ NP and glimepiride resulted in significant ($p < 0.01$) decrease in activities of AST and ALT and levels of creatinine and urea. However, V₂O₅ NP and glimepiride treated rats showed significantly decreased

level of AST and ALT activities than that of V₂O₅ treated rats.

Liver glycogen level in control and experimental groups of rats are shown in Figure 2. The glycogen level significantly ($p < 0.01$) decreased in diabetic rats was not increased on treatment with V₂O₅. However, V₂O₅ NP and glimepiride treatments showed a significant ($p < 0.01$) increase in glycogen level when compared to diabetic rats.

DISCUSSION

The STZ-induced diabetic control rats showed a significant decrease in body weight. The decrease in the body weight may be due to increased catabolic reactions¹⁰, because of the inability to utilize carbohydrate as an energy source, leading to muscle waste¹¹. V₂O₅ and V₂O₅ NP treated diabetic rats showed an increase in body weight at the end of the experiment period, which could be attributed to its anti-diabetic effect followed by diminished protein and fat catabolism and increased oxidation of glucose as an energy source. These results were in accordance with previously reported studies by Kurt *et al*¹², Wei *et al*¹³ and Pillai *et al*¹⁴.

STZ, on administration to fasted rats, enters the β cells of pancreas *via* a glucose transporter (GLUT2) and causes alkylation of DNA and also DNA damage, which induces activation of poly ADP-ribosylation, which in turn leads to depletion of cellular NAD⁺ and ATP¹⁵. Depletion of the cellular energy results in β cell necrosis, insulin deficiency and hyperglycemia¹⁶. Treatment with V₂O₅ and V₂O₅ NP significantly reduced the blood glucose level when compared to diabetic control rats. Possible mechanisms are vanadium enhances glucose transport through an effect on GLUT4¹⁷. Vanadium, enhances the basal rates of glucose uptake and presumed metabolism of glucose by liver and muscle¹⁸. Activation of protein kinase B by vanadium results in inactivation of glycogen synthase kinase-3, leading to stimulation of the synthesis of glycogen from glucose¹⁹. Thus, vanadium also lowers the output of glucose from liver.

The serum triacylglycerol and total cholesterol levels were found to be increased in diabetic rats. Abnormally high concentrations of plasma lipids in diabetes are mainly due to an increase in the mobilisation of free fatty acids from the peripheral depots in the absence or deficiency of insulin. Hence, during diabetes the hepatic lipogenesis is decreased and lipolysis is increased¹⁸. Diabetes is also known to be associated with an increase in the synthesis of cholesterol, which may be due to the increased activity of HMG CoA reductase²⁰.

In the present study, administration of V₂O₅ and V₂O₅ NP to diabetic rats resulted in significant reduction in triacylglycerol and total cholesterol level, this might be due to enhanced glucose and lipid metabolism and decreased cholesterol synthesis by decreasing the activity of HMG-CoA reductase²¹. The findings are similar to the observations of Majithiya *et al*²², Gao *et al*²³ and Tas *et al*²⁴.

Insulin deprivation in diabetes causes a profound increase in protein catabolism, resulting in decrease in total protein level¹⁴. Vanadium due to its insulin like effect increases the utilization of glucose and thereby inhibits proteolysis resulting in the increased level of plasma proteins than that of diabetic rats, the results of the present study concurred with Clark *et al*²⁵ and Pillai *et al*¹⁴.

The increase in the activities of ALT and AST indicates the hepatotoxic effect of STZ in diabetic rats¹¹. V₂O₅ and V₂O₅ NP decreased the activities of ALT and AST, which indicate the tissue protective and non-toxic nature of the vanadium on the liver tissue¹³.

The elevated urea and creatinine levels in serum in the present study may be due to increased protein catabolism and kidney damage due to hyperglycemia¹⁴. Restoration of glucose and protein metabolism by V₂O₅ and V₂O₅ NP reduced the levels of serum urea and creatinine, which also accounts for the non-toxic nature of vanadium on the kidney tissue as reported by Clark *et al*²⁵.

The reduced glycogen level in diabetes is due to the insulin deficiency or impaired responsiveness to insulin²⁶. Vanadium stimulates glucose transport and increases glycogen synthesis²⁷ and being a potent inhibitor of phosphotyrosine phosphatases inhibits glycogenolysis²⁸. In addition, it activates protein kinase B resulting in inactivation of glycogen synthase kinase-3, leading to stimulation of the synthesis of glycogen from glucose¹⁹. This is attributed to the increased glycogen level in V₂O₅ NP treated rats.

In the present study, administration of V₂O₅ NP to diabetic rats resulted in significant decrease in levels of serum glucose and cholesterol and liver glycogen and activities of serum ALT and AST and significant increase in serum protein than that of V₂O₅ treatment. This may be due to the physical and biochemical changes in the behaviour of the nanoparticles, which efficiently allow drug accumulation at the target site²⁹. Thus the unwanted side effects and the toxicity of the V₂O₅ NP are reduced and the therapeutic efficacy is enhanced compared to V₂O₅ treatment which is concurrent with the study of Keyshams *et al*⁵.

Table 1: The levels of serum glucose, total protein, triacylglycerol and cholesterol of control and experimental group of rats after 30 days of experimental period

Group	Glucose (mg/dL)	Total Protein (g/dL)	Triacylglycerol (mg/dL)	Cholesterol (mg/dL)
Normal control	84.50 ^c ± 3.26	7.09 ^a ± 0.20	94.81 ^c ± 4.93	94.01 ^c ± 1.82
Diabetic control	580.38 ^a ± 6.58	5.21 ^c ± 0.10	421.64 ^a ± 30.69	149.46 ^a ± 4.40
Diabetic + V ₂ O ₅	361.00 ^b ± 12.54	5.85 ^b ± 0.14	288.53 ^b ± 15.50	120.24 ^b ± 5.03
Diabetic + V ₂ O ₅ NP	218.62 ^d ± 10.43	6.62 ^a ± 0.15	197.09 ^{bc} ± 39.78	93.88 ^c ± 7.13
Diabetic + glimepiride	283.38 ^c ± 24.69	6.06 ^b ± 0.21	239.03 ^b ± 20.89	101.73 ^c ± 3.54

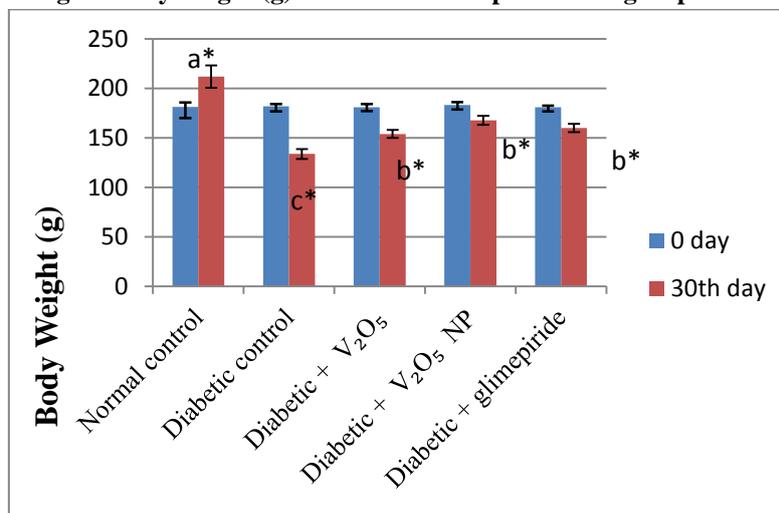
Values are statistically significant at $P < 0.001$

Table 2: Activities of AST and ALT and levels of serum creatinine and urea in control and experimental groups of rats after 30 days of experimental period

Group	AST (U/L)	ALT (U/L)	Creatinine (mg/dL)	Urea (mg/dL)
Normal control	61.18 ^d ± 1.93	27.02 ^d ± 0.86	0.53 ^b ± 0.04	43.42 ^c ± 2.09
Diabetic control	204.39 ^a ± 12.23	76.80 ^a ± 5.44	1.13 ^a ± 0.08	70.66 ^a ± 2.43
Diabetic + V ₂ O ₅	138.76 ^b ± 6.84	56.28 ^b ± 2.21	0.67 ^b ± 0.07	53.09 ^b ± 2.35
Diabetic + V ₂ O ₅ NP	107.15 ^c ± 4.24	41.06 ^c ± 2.02	0.63 ^b ± 0.08	51.52 ^b ± 3.04
Diabetic + glimepiride	120.10 ^c ± 6.42	44.84 ^c ± 1.50	0.68 ^b ± 0.17	55.52 ^b ± 2.82

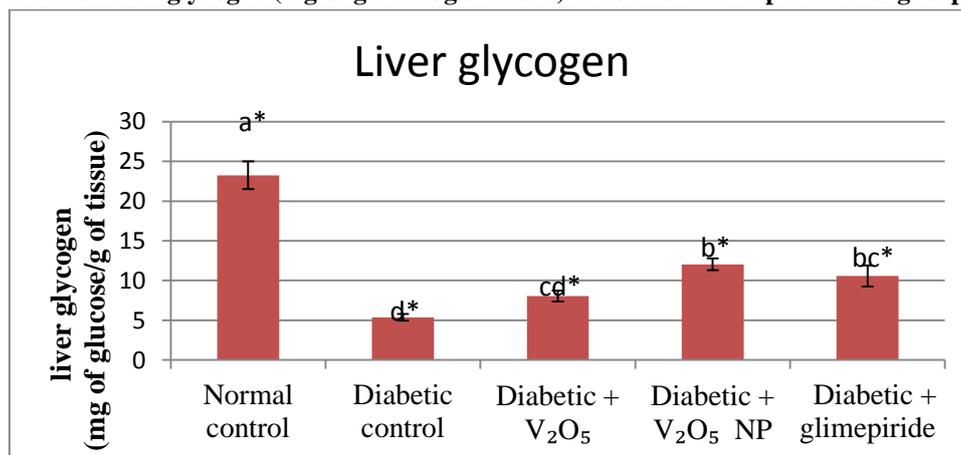
Values are statistically significant at $P < 0.001$

Fig. 1: Body weight (g) of control and experimental group of rats



Values are statistically significant at $*P < 0.01$; $#P < 0.05$.

Fig. 2: Level of liver glycogen (mg of glucose/g of tissue) in control and experimental groups of rats



Values are statistically significant at $*P < 0.01$; $#P < 0.05$.

CONCLUSION

In conclusion, the present study reveals that the oral administration of V₂O₅ NP and V₂O₅ evoked marked glucose lowering effects in diabetic rats and also restored the deranged carbohydrate, lipid and protein metabolism indicating the improved glucose homeostasis. Normalization of liver dysfunction, kidney dysfunction and loss of body weight in a holistic manner indicates the non-toxic nature of the particles. The observed anti-diabetic effect might be due to their insulin mimetic action. However, V₂O₅ NP out performed as an anti-diabetic agent compared to V₂O₅ due to the nature of particles with increased bioavailability and biological activity in the target organ. Further in-depth studies on mechanism and chronic toxicity studies would render an efficient and non-toxic hypoglycemic drug for diabetes treatment.

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