Antioxidant Status in STZ-induced diabetic rats treated with Vanadium pentoxide nanoparticles

K. Vijay*, R. Suresh, K. Loganathasamy, V. Narayanan, K. Pratheepa, K. Venkataraman and V. Pandiyan

Department of Veterinary Biochemistry, Madras Veterinary College, Chennai-600 007, Tamil Nadu, India.

Received: 08-08-2018 Accepted: 27-10-2018 DOI: 10.18805/ijar.B-3709

ABSTRACT

Antioxidant status determines the susceptibility of tissues to the oxidative stress associated with diabetes and its complications; hence in the present study antioxidant status was explored in streptozotocin-induced diabetic rats treated with vanadium in the form of vanadium pentoxide nanoparticles. Vanadium pentoxide and vanadium pentoxide nanoparticles at the dose rate of 5mg/kg were administered orally in STZ (50mg/Kg) induced diabetic rats for 30 days and glimepiride (reference drug) was administered orally at the dose rate of 800 μg/kg body weight. Vanadium pentoxide nanoparticles significantly reduced the blood glucose levels than the diabetic control and other treatment group of rats. On exploration of antioxidant status in liver, kidney and pancreas tissues, vanadium in the form of vanadium pentoxide nanoparticles outperformed the vanadium pentoxide by increasing the activities of the enzymes catalase, superoxide dismutase and glutathione peroxidase, and the concentration of reduced glutathione and by decreasing the lipid peroxide levels. The present study also showed that the restoration of antioxidant status by vanadium pentoxide nanoparticles is comparable with that of the reference drug. It can be concluded that vanadium pentoxide nanoparticles, due to its superior control over hyperglycemia and antioxidant properties, outperformed the vanadium pentoxide treatment in enhancing the antioxidant status in diabetic rats.

Key words: Antioxidants, Diabetes, Nanoparticle, Streptozotocin, Vanadium.

INTRODUCTION

Diabetes mellitus, a heterogeneous metabolic disorder, is characterized by hyperglycemia with disturbances in carbohydrate, lipid and protein metabolism (Patel et al., 2012). In diabetes mellitus, persistent hyperglycemia is associated with chronic oxidative stress due to the generation of reactive oxygen species (ROS) by the auto-oxidation of glucose and unsaturated lipids in plasma and membrane proteins (Rajarajeswari and Pari, 2011). Increased ROS level in diabetes could be due to increased production or diminished removal by antioxidants or both. Hence the level of non-enzymatic antioxidants like reduced glutathione (GSH) and the activities of enzymatic-antioxidant like catalase and SOD play a vital role in susceptibility of various tissues to oxidative stress and subsequent complication in diabetes (Lipinski, 2001).

Various types of insulin preparations and oral hypoglycemic drugs are in clinical use for the treatment of diabetes with limitations like physical pain, anxiety and cost effectiveness in case of insulin and oral hypoglycemic drugs like glimepiride imposing problems like gastrointestinal disorders and hypersensitive reactions. Hence there is a need for an alternative and effective drug in management of diabetes (Patel et al., 2012).

Vanadium, one of the thoroughly studied and effective trace mineral in reducing blood glucose, is a topic of debate as a hopeful anti-diabetic agent. Several sub-acute and handful of chronic studies on vanadium in the form complex concludes that vanadium exerts anti-diabetic effect by normalizing carbohydrate, protein and lipid metabolism with antioxidant properties (Kannan et al., 2016). Its biological potential as antidiabetic agent is hindered by its toxic effects like diarrhoea, dehydration and reduced feed and water intake (Domingo et al., 1995).

Altogether, very few studies have been done in experimental diabetic animals with metals like vanadium, chromium, and zinc in the form of nanoparticles. Though these metals have beneficial effect in the form nanoparticles as an anti-diabetic agent in rats, their toxicity profile and antioxidant properties need to be examined (Alkaladi et al., 2014). Vanadium in the form of nanoparticles didn’t exhibit any adverse effects in addition to better control over hyperglycemia and serological parameters (Keyshams et al., 2013 and Vijay et al., 2018).

Hence, the present study was undertaken to evaluate and compare the effects of vanadium in the form of vanadium pentoxide and vanadium pentoxide nanoparticles with the reference drug glimepiride on lipid peroxidation, enzymatic
and non-enzymatic antioxidant levels in streptozotocin-induced diabetic rats.

**MATERIALS AND METHODS**

**Chemicals:** Streptozotocin (STZ) and chemicals for antioxidant assay were purchased from M/s Merck Chemicals, Mumbai, India. Vanadium in the form of vanadium pentoxide (V$_2$O$_5$) and vanadium pentoxide nanoparticles (V$_2$O$_5$ NP) was synthesized, characterized and received as gift from the department of Inorganic Chemistry, University of Madras, Guindy campus (Suresh et al., 2014).

**Experimental animals:** This experiment was carried out after prior approval of Institutional Animal Ethical Committee (IAEC), MVC, Chennai – 7 (Approval No: 2172/ DFBS/B/2013). Forty 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. Forty animals were grouped randomly into five groups with mean body weight variation not exceeding 20%. Identification of rats was done by marking head / body / tail with picric acid / eosin / methylene blue. Rats were acclimatized for 3 weeks prior to the start of the experiment and they were maintained on standard rat feed supplied by Provimi Animal Nutrition India Private Limited, Bangalore, India. Rats were housed in cages with 12h light/dark cycle and fed ad libitum feed and water throughout the experimental period.

**Induction of diabetes in rats:** Rats showing blood glucose level < 110 mg/dL were administered intra-peritonially with a single injection of freshly prepared STZ (50 mg/kg body weight, dissolved in 0.1 M cold citrate buffer, pH 4.5) to induce diabetes. Control rats received citrate buffer instead of STZ. Up to 24h after injection the animals were allowed to develop hypoglycemia due to massive pancreatic insulin release caused by STZ (Kannan et al., 2016). After 72h of injection, blood glucose level was assessed by using MYLIFE PURA glucometer strips. The rats of treatment groups that showed blood glucose level more than 250 mg/dL were considered diabetic and the control were rats ruled out for spontaneous diabetes (blood glucose level < 110 mg/dL were included in the study). The day was considered as zero day of the experimental study.

**Experimental protocol:** Group I served as untreated normal control. Group II served as streptozotocin induced diabetic control. Groups III and IV were diabetic rats treated at the dose rate of 5 mg/kg body weight with V$_2$O$_5$ and V$_2$O$_5$ NP respectively. Group V was diabetic rats treated with standard hypoglycemic drug glimepiride at the dose rate of 800 µg/kg body weight for comparison (Kannan et al., 2016).

**Blood glucose:** Levels of blood glucose of control and treatment group of rats were assessed by using MYLIFE PURA glucometer strips on 0, 14th and 28th day of the experimental study.

**Antioxidant assay:** At the end of the experiment, animals were anaeasthetized and euthanized by CO$_2$ asphyxiation with steady state increase in CO$_2$ in the chamber. Liver, kidney and pancreas were isolated from all the experimental group of animals and were rinsed in normal saline, blotted to dryness, weighed and then homogenized in ice cold PBS, pH 7.4 using Teflon homogenizer. The homogenates were centrifuged in cooling centrifuge at 2500 rpm for 15 min to remove tissue debris and the aliquots of supernatants were used for the antioxidant assays as described below.

**Tissue Protein concentration:** Total protein concentrations of the tissues were estimated by the method as described by Lowry et al. (1951).

**I. Enzymatic antioxidants**

1. **Catalase (E.C.1.11.1.6):** Catalase activity was determined by monitoring the decrease in absorbance at 240 nm due to the decomposition of H$_2$O$_2$ (Caliborne, 1985).

2. **Superoxide Dismutase (E.C.1.15.1.1):** Superoxide dismutase (SOD) activity was assayed according to the method of Marklund and Marklund, (1974).

3. **Glutathione peroxidase (E.C.1.11.1.9):** Glutathione peroxidase (GPx) was assayed as per the method of Rotruck et al. (1972).

**II. Nonenzymatic antioxidant**

1. **Reduced Glutathione (GSH):** Reaction of reduced glutathione with 5-5’ dithiobis-2-nitrobenzoic acid (DTNB) was estimated by the method of Moron et al. (1979).

**III. Lipid peroxidation:** Lipid peroxide levels in the homogenates were determined as thiobarbituric acid reactive substances (TBARS) by the method of Placer et al. (1966).

**Statistical analysis:** All the data were analyzed by SPSS package version 20, one way analysis of variance followed by Duncan’s test multiple comparison test (Snedecor and Cochran, 1994). A value of (p< 0.05) and (p< 0.01) were considered statistically significant.

**RESULTS AND DISCUSSION**

**Blood glucose level:** The levels of blood glucose of control and experimental group of rats are depicted in Table 1. Diabetic control and treatment group of rats showed significantly (p< 0.01) increased blood glucose level than the control rats on 0, 14th and 28th day. All the treatment group of rats significantly (p< 0.01) reduced the blood glucose level on 14th and 28th days of treatment than that of diabetic control rats. However, the magnitude of reduction in blood glucose levels are significantly (p< 0.01) different among the treatment and were in the order of V$_2$O$_5$ NP>Glimepiride>V$_2$O$_5$ This finding was concurrent with Keyshams et al., (2013) who had reported that Nanovanadium in addition to its hypoglycemic effect, it also
exhibits better control over glycaemic status than the regular kind vanadium in diabetic rats.

**Enzymatic antioxidant:** The levels of antioxidant enzymes critically influence the susceptibility of various tissues to oxidative stress and are associated with the development of complication in diabetes (Baynes 1991; Porte and Schwartz 1996). The activities of enzymes Catalase, SOD and GPx in liver, kidney and pancreas of control and experimental groups of rats are depicted in Tables 2 and 3.

**Catalase:** Catalase activity decreased significantly (p<0.05) in liver, kidney and pancreas of diabetic control rats. V$_5$O$_5$ NP and glimepiride treatments increased the catalase activities to near normal level in liver, kidney and pancreas. Treatment with V$_5$O$_5$ increased the catalase activity significantly (p<0.05) in pancreas, while no significant difference in liver and kidney was observed when compared with diabetic control rats.

The reduced activity of catalase in tissues of diabetic animals have been attributed to the increased production of superoxide radical that can themselves reduce the activity of these enzymes (Sekar et al., 1990) and also by direct glycation of this enzyme (Yan and Harding, 1997). Administration of vanadium normalizes the catalase activity by reducing the blood glucose level and thereby reducing the superoxide radical (Matsubara et al., 1995). Similarly the catalase activity was normalized by V$_5$O$_5$ NP, which might be due to its better control over hyperglycaemia and/or antioxidant activity.

**Superoxide dismutase:** In liver, kidney and pancreas of diabetic control rats, the activity of SOD decreased significantly (p<0.05) than that of control rats. The values were significantly (p<0.05) increased in liver and pancreas of all the treatment groups. However, the SOD activity was reversed only in V$_5$O$_5$ NP treatment.

The reduced activity of SOD in the present study could be due to its depletion or inhibition as a result of the increased production of free radicals (Yanardag et al., 2009) and also by direct glycation of this enzyme (Ramachandran et al., 2004). Vanadium, as a scavenger of oxy radicals and hydroxy radicals, attenuates superoxide radical-induced damage there by increasing the activity of superoxide dismutase (Matsubara et al., 1995). Similar result was observed in the present study which was concurrent with findings of Kannan et al., (2016).

**Glutathione peroxidase:** Decreased activity of GPx in liver, kidney and pancreas of diabetic rats was significantly (p<0.05) increased and reversed to near normal level in all the treatment groups except that the V$_5$O$_5$ treatment couldn’t reverse it in kidney of diabetic rats.

---

**Table 1:** Blood glucose levels of control and experimental group of rats on 0, 14th and 28th of experiments. (n=8, Mean ± SE). Means bearing different superscripts in a column differ significantly between groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>0 day</th>
<th>14th day</th>
<th>28th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>82.75 ± 3.58</td>
<td>80.00 ± 1.82</td>
<td>84.15 ± 3.08</td>
</tr>
<tr>
<td>II</td>
<td>524.13 ± 6.18</td>
<td>542.5 ± 8.59</td>
<td>570.4 ± 8.28</td>
</tr>
<tr>
<td>III</td>
<td>524.25 ± 15.3</td>
<td>458.63 ± 24.24</td>
<td>371.00 ± 10.2</td>
</tr>
<tr>
<td>IV</td>
<td>526.88 ± 12.53</td>
<td>284.75 ± 25.48</td>
<td>224.24 ± 10.18</td>
</tr>
<tr>
<td>V</td>
<td>521.88 ± 10.04</td>
<td>336.75 ± 22.26</td>
<td>284.26 ± 21.44</td>
</tr>
</tbody>
</table>

**Table 2:** Catalase and Superoxide dismutase activities in liver, kidney and pancreas of control and experimental groups of rats after 30 days of experiment (n=8, Mean ± SE). Means bearing different superscripts in a column differ significantly between groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>CATALASE (µmoles of H$_2$O$_2$ decomposed/min/mg of protein)</th>
<th>SOD (50% inhibition of pyrogallol autooxidation/min/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>I</td>
<td>34.22 ± 0.66</td>
<td>16.39 ± 1.20</td>
</tr>
<tr>
<td>II</td>
<td>22.16 ± 0.94</td>
<td>6.23 ± 0.96</td>
</tr>
<tr>
<td>III</td>
<td>22.71 ± 1.23</td>
<td>9.44 ± 1.34</td>
</tr>
<tr>
<td>IV</td>
<td>30.08 ± 1.16</td>
<td>13.23 ± 1.54</td>
</tr>
<tr>
<td>V</td>
<td>29.03 ± 5.88</td>
<td>15.27 ± 0.52</td>
</tr>
</tbody>
</table>

**Table 3:** Glutathione peroxidase activity and concentration of reduced glutathione in liver, kidney and pancreas of control and experimental groups of rats after 30 days of experiment (n=8, Mean ± SE). Means bearing different superscripts in a column differ significantly between groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>GPx (µmoles of glutathione (GSH) oxidized/min/mg of protein)</th>
<th>GSH (µmoles/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LIVER</td>
<td>KIDNEY</td>
</tr>
<tr>
<td>I</td>
<td>15.48 ± 3.31</td>
<td>158.60 ± 9.4</td>
</tr>
<tr>
<td>II</td>
<td>127.44 ± 5.05</td>
<td>121.77 ± 3.06</td>
</tr>
<tr>
<td>III</td>
<td>132.62 ± 2.67</td>
<td>134.79 ± 4.02</td>
</tr>
<tr>
<td>IV</td>
<td>150.22 ± 10.14</td>
<td>152.43 ± 6.95</td>
</tr>
<tr>
<td>V</td>
<td>149.78 ± 2.89</td>
<td>159.17 ± 5.04</td>
</tr>
</tbody>
</table>
Table 4: Lipid peroxide (LPO) levels in liver, kidney and pancreas of control and experimental groups of rats after 30 days of experiment (n=8. Mean ± SE). Means bearing different superscripts in a column differ significantly between groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>LIVER (nmole of MDA released / mg of protein)</th>
<th>KIDNEY (nmole of MDA released / mg of protein)</th>
<th>PANCREAS (nmole of MDA released / mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.77± 0.16</td>
<td>1.46± 0.11</td>
<td>0.74± 0.20</td>
</tr>
<tr>
<td>II</td>
<td>3.34± 0.29</td>
<td>4.93± 0.64</td>
<td>3.94± 0.33</td>
</tr>
<tr>
<td>III</td>
<td>2.21± 0.37</td>
<td>2.94± 0.23</td>
<td>1.88± 0.12</td>
</tr>
<tr>
<td>IV</td>
<td>1.94± 0.21</td>
<td>2.05± 0.31</td>
<td>1.67± 0.17</td>
</tr>
<tr>
<td>V</td>
<td>2.73± 0.33</td>
<td>2.37± 0.20</td>
<td>1.96± 0.12</td>
</tr>
</tbody>
</table>

The decreased GPx activity in diabetic rats may be due to increased oxidative stress in diabetes, which inhibits the activity of glutathione peroxidase and it may also be due to decrease in the concentration of GPx, the substrate for GPx (Yanardag et al., 2009). Administration of V2O5 NP increased the activity of GPx, which was similar to the findings of Ramachandran et al., (2004) and Yanardag et al., (2009), who had reported that the reduced GPx activity in the tissues of diabetic rats was increased after the administration of vanadium complex. The increased level of GSH in diabetic rats treated with vanadium may also in part cause increase in GPx activity.

Non-enzymatic antioxidant: The non-enzymatic antioxidant, reduced glutathione (GSH) levels in liver, kidney and pancreas are shown in Table-3. GSH concentration decreased significantly (p< 0.05) in liver, kidney and pancreas of diabetic control rats. V2O5 NP treatment increased the GSH levels significantly (p< 0.05) in liver, kidney and pancreas of rats. But GSH level in V2O5 NP treatment was increased only in pancreas and that of glimepiride was increased only in liver and pancreas when compared with diabetic control rats.

GSH is one of the important nonenzymatic antioxidant in the antioxidant defense system. Decreased level of GSH observed in the tissues of diabetic rats was normalized on treatment with nano vanadium pentoxide, which concurs with the results of Koyuturk et al., (2005), who had reported that the treatment with vanadium increases the activity of glucose-6-phosphate dehydrogenase, which in turn enhances NADPH levels and the activity of glutathione reductase (GR). Increased activity of GR due to enhanced NADPH level replenishes GSH level.

Lipid peroxidation: Lipid peroxide levels in liver, kidney and pancreas of control and experimental groups of rats are depicted in Table-4. Elevated lipid peroxide levels in liver, kidney and pancreas of diabetic rats were significantly (p< 0.05) reduced in that of V2O5, V2O5 NP and glimepiride treated rats. All the treatment groups differed significantly from diabetic control groups.

Lipids when react with free radicals undergo peroxidation to form lipid peroxides. Lipid peroxides are supposed to cause the destruction and damage of cell membranes leading to changes in membrane permeability and fluidity, enhancing the protein degradation rates (Klepac et al., 2005). Maintenance of persistent normoglycemia by the administration of vanadium complex may attenuate lipid peroxidation in tissues and thus prevents tissue damage (Das et al. 2000) and the vanadium itself acts as scavenger of oxyradicals and prevents liver dysfunction (Koyuturk et al., 2005). Decreased Lipid peroxide levels found in the present study shows the protective nature of vanadium in the form of nanoparticles, which concurs with the result of Kannan et al., (2016).

In conclusion, vanadium in the form of nanoparticles outperformed the vanadium and glimepiride treatments in restoring enzymatic and non-enzymatic antioxidants and in reducing the lipid peroxide levels. The current study concurred with the findings of previous studies done with vanadium complexes, which shows that the vanadium pentoxide nanoparticles have desirable antioxidant property which may be attributable to its better control over hyperglycemia and enhanced target action like vanadium complexes. Further chronic toxicity study is needed to find out its antioxidant activity in chronic diabetes.

ACKNOWLEDGEMENT

We thank Dr. Selvasubramanian (late), the member of the advisory committee of this study, for his support and guidance to carry out this study successfully. We also thank Tamilnadu Veterinary and Animal Sciences University for its financial assistance to carry out this study.

REFERENCES


