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The effect dioxidovanadium complex (v) on hepatic function in streptozotocin-induced diabetic rats

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ABSTRACT

Diabetics are susceptible hepatic dysfunction risks due to hyperglycaemia and insulin therapy. Diabetes conventional treatments improve glycaemic control, however hepatic hazards associated with these agents remains a challenge. Accordingly, this study sought to investigate the effect of a dioxidovanadium complex (V) on the hepatic function in STZ-induced diabetic rats. Sprague-Dawley rats (240-250g) were divided into 4 groups (n=6) namely, non-diabetic control, diabetic control, insulin treated and vanadium complex group. The dioxidovanadium (10, 20, 40 mg/kg) was administered twice every 2\textsuperscript{nd} day for 5 weeks where blood glucose concentration was monitored weekly. At the end of the experimental period, all the experimental groups were sacrificed after which the lipid profile, liver superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA), plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and C reactive protein concentration were measured. The administration of dioxidovanadium significantly alleviated hyperglycaemia with concomitant attenuation in oxidative stress as evidenced by reduced MDA concentrations. Furthermore, vanadium complex abolished diabetes-induced dyslipidaemia. Lastly, vanadium complex administration attenuated the increase in AST, ALT and plasma CRP. These findings suggest that this metallo-compound (dioxidovanadium) may ameliorate liver dysfunction often observed in diabetes.

Keywords: hyperglycaemia, oxidative stress, liver dysfunction, C-reactive protein, liver function marker enzymes


**Introduction**

Liver dysfunction has been reported in diabetes mellitus and associated with oxidative stress and low grade inflammation (Amos et al. 1997). Hepatic oxidative stress is often marked with increases in lipid peroxidation and cellular damage (Lewis et al. 2002). The cell membrane disruption due to accumulation of oxidative stress products results in the release of hepatic function marker enzymes such as aspartate transaminase (AST) and alanine transaminase (ALT) (Hamed 2014). In diabetic patients the activity of serum ALT and AST is increased and strongly associated with signs of liver diseases (Harris 2005).

Some hypoglycaemic agents lower blood glucose in part via the liver. However, there is an ongoing controversy regarding the long-term effect of these therapeutic modalities on the hepatic function and injury. Troglitazone, an insulin sensitizer has been withdrawn mainly because of its hepatic hazardous effect which outweighed the benefits of the drug (Bilal et al. 2016). The risks and benefits of metformin use in patients with cirrhosis with diabetes are debated. The administration of subcutaneous insulin is the primary intervention used in type 1 diabetes (Boden and Shulman 2002). Despite the tight glycaemic control rendered by insulin, numerous and life threatening challenges exist (Saligram et al. 2012). Insulin is administered in high doses which precipitate increase in hepatic lipogenesis, fat accumulation and glycogen deposition in the liver resulting in hepatomegaly and risk of developing non-alcoholic fatty liver disease (NADFLD) (Anderwald et al. 2002). NADFLD is becoming common in type 1 diabetic and is often associated with elevated liver function enzymes such as AST and ALT. Additionally, this condition is also associated with hepatic inflammation. Other types of diabetes treatments administered to patients have been shown to possess undesirable hepatic effect, which result in tissue scarring and inflammation (Ishii and Takamura 2016). These challenges justify the continuous search for novel compounds which may provide a therapeutic effect with minimal hepatic hazards in diabetes. Additionally, it is imperative that any potential anti-diabetic agent is screened and evaluated for potential hepatic toxicological effect.

Transition metals have been extensively investigated for their medicinal benefits and have displayed various effects including anti-oxidant, anti-inflammatory as well as anti-diabetic effects (Frezza et al. 2010 Fedorova et al. 2013). Vanadium is a transition metal that has also been reported to possess therapeutic effects including antioxidant and anti-diabetic effects (Fedorova et al. 2013; Reul et al. 1999). The anti-diabetic effect of vanadium is achieved through increase in glucose disposal in insulin target tissues. However, vanadium has been associated with toxicity to organs such as the liver and kidneys if administered as an inorganic salt (Sakurai 2002). Hence, in an effort to mitigate the vanadium associated toxicity, researchers are focusing on developing and evaluating organic vanadium complexes. The use of heterocyclic ligands in the synthesis of the vanadium complexes have been reported to provide stability and promote bio-availability (Goc 2006). Additionally, organic metal complexes are reportedly less toxic, more stable and effective compared to the vanadium salts. Research has shown that the organic ligand provides thermodynamic stability and efficient transport of vanadium to target locations in the body making the vanadium complexes safer, more effective and more stable than in their inorganic salt form (Sakurai et al. 2002).
In this study, we are interested in effect of the novel organic vanadium complex, 2-phenolate-1H-benzimidazole-dioxidovanadium which has been shown to attenuate hyperglycaemia in diabetic animals. Although, such beneficial effect has been reported, for further development, it is essential that the potential hepatic toxicological effect of this compound is elucidated. Therefore, this study sought to investigate the effects of 2-phenolate-1H-benzimidazole-dioxidovanadium on liver function markers in STZ-induced diabetic rats. We envisaged that measuring parameters such as oxidative stress markers, lipid profile, liver enzymes and plasma C-reactive protein (CRP) may provide a holistic insight on the hepatic function and injury in streptozotocin-induced diabetic rats.

Methods and materials

Chemicals and drugs

The following organic-, inorganic precursors and bio-reagents: insulin (NovoRapid pen refill, Novordisk Pty Ltd, Sandton, South Africa); streptozotocin, thiobarbituric acid (TBA), ammonium metavanadate (NH\textsubscript{4}VO\textsubscript{3}), 2-hydroxyphenyl-1H-benzimidazole and butylate hydroxyl toluene (BHT) (Sigma Aldrich Chemical Company, Missouri, St Louis, USA); Hydrochloric acid (HCl) (Merck Chemicals (PTY) LTD, Johannesburg, South Africa); phosphoric acid (H\textsubscript{3}PO\textsubscript{4}) (NT Laboratory supplies (PTY) LTD, Johannesburg, South Africa); USA Isofor inhalation anaesthetic (Safeline Pharmaceuticals (PTY) LTD, Weltevreden Park, Roodeport, South Africa) were used. Reagent-, analytical-grade and deuterated NMR solvents were procured from Merck SA. The metal complex, dioxidovanadium (V) \textit{cis}-[VO\textsubscript{2}(obz)py] was synthesized according to an experimental procedure previously reported. Purity confirmation of the compound was performed by \textsuperscript{51}V and \textsuperscript{1}H NMR spectroscopy as well as TOF-mass spectrometry.

Animals

Male Sprague-Dawley rats weighing between 250-300g, bred in the Biomedical Research Unit of the University of KwaZulu-Natal were used in this study. The animals were kept and maintained under constant laboratory conditions in terms of temperature and humidity, in a 12 hours days and 12 hours night cycle. The animals had access to water \textit{ad libitum}, and were given standard rat chow daily. All the animal experimentation protocols followed the University of KwaZulu-Natal (South Africa) animal ethics guidelines as per approved ethical clearance application (AREC/054/017D). The study adhered to the guidelines of the \textit{Guide for the Care and Use of Laboratory Animals} (8th edition, National Academies Press USA).
Diabetes induction

Diabetes type 1 was induced by a single intravenous injection of freshly prepared STZ (60mg/kg) in 0.1M of citrate buffer (pH 4.5). The control rats were only given an equal volume of citrate buffer. Animals with fasting blood glucose concentrations higher than 20 mmol/L in STZ induced rats were considered as a stable diabetes.

Experimental protocol

STZ-induced experimental animals were divided into three groups of (n=6 per group). Group one were the diabetic rats which remained untreated and acted as a negative control. Group 2 were diabetic rats which received cis-[VO₂(obz)py] (10, 20, 40 mg/kg). Group 3, were diabetic rats treated insulin (175 μg/kg, S.C) treated animals serving as a positive control. Non-diabetic rats served as absolute control. All the animals were housed individually in Makrolon polycarbonate metabolic cages (Techniplast, Labotec, South Africa). The vanadium complex was administered twice every 3rd day at 09h00 and 15h00 by means of 18-gauge gavage needle (Kyron Laboratories (Pty) LTD, Benrose, South Africa). Untreated control groups received distilled water through the same route. Blood glucose concentration was monitored every 3rd day using the OneTouch select glucometer (Lifescan, Mosta, Malta, United Kingdom). Body weight, water and food intake was recorded every 3rd day.

Tissue sampling harvesting

At the end of the experimental period, all the animals were anaesthetized by exposing them to Isofor for 3 min via a gas anesthetic chamber (100 mg kg⁻¹). The blood samples were collected by cardiac puncture into individual pre-cooled heparinized containers. The livers were removed, weighed, and snap frozen in liquid nitrogen for storage in Bio Ultra freezer (Snijers Scientific, Tilburg, Netherlands) at −80°C until use. For biochemical analysis, liver tissue homogenates were prepared in lysis buffer using an electric homogenizer.

Biochemical analysis

Plasma total cholesterol, triglycerides, LDL and HDL were measured by Global Clinical and Viral Laboratory in Amanzimtoti, South Africa. ALT and AST were measured using AST/ALT assay kit based on colorimetric method, following manufacturer’s instructions (ELabsciences). The liver SOD and GPx and plasma CRP were analysed using separate specific ELISA kits (Elabscience and Biotechnology, WuHan) according to manufacturer’s instructions.

MDA measurement

MDA concentration was measured using an established laboratory protocol [16]. Briefly, liver tissues (50 mg) were homogenized in phosphoric acid (500 μL, 0.2%) after which the homogenate was supplemented with phosphoric acid (400 μL, 2%). The homogenate was then separated into two glass tubes, each receiving equal volumes of the solution (200 μL). A further addition of phosphoric acid (200 μL, of 7%) into both glass tubes followed by the addition of butylated hydroxyl toulene (400 μL) into one glass tube (sample test) and
hydrochloric acid (HCl) (400 µl, 3 mM) into the second glass tube (blank). Both solutions were heated at 100°C for 15 minutes and then allowed to cool down to room temperature. Butanol (1.5 ml) was added to the cooled solutions. The sample solution was vortexed for 1 minute to ensure vigorous mixing and then allowed to settle until 2 liquid phases could be distinguished in the tube. The butanol phase (top layer) was transferred to Eppendorf tubes and centrifuged at 13200 xg in a Hermle Laborechnic GmBH centrifuge (Wehingen, Germany) for 15 minutes. The samples were aliquoted into wells of a 96-well microtiter plate in triplicate and the absorbance was read at 532 nm (reference λ 600nm) using a Nano spectrophotometer (BMG Labtech, Ortenburg, Baden-Wurttemberg, Germany). The absorbance was used to calculate the concentration of MDA using the Beer’s Law.

\[
\text{Concentration} = \frac{\text{Absorbance} \text{ final}}{\text{Absorption coefficient (156mmol}^{-1})}
\]

**Statistical analysis**

All data were expressed as mean ± SEM. The statistical analysis was performed using GraphPad prism Instant Software (version 5.00, Graphpad software, San Diego, California, USA). Two way analysis of variance followed by Bonferroni test was used to analyse the differences between the controls and experimental groups for blood glucose concentration. One way analysis of variance followed by Tukey-Kramer post-hoc test was used to analyse the discrepancies between the controls and experimental groups for following data set; liver and body weights, lipid profile, oxidative stress markers, liver function marks and plasma CRP Statistical significance was set at p<0.05 between the experimental groups. Two-way analysis was used for weekly blood glucose concentration

**Results**

**Blood glucose concentration**

Figure 1 presents the weekly blood glucose concentration in non-diabetic (ND), diabetic control (DC), diabetic rats treated with vanadium complex (VC) (10, 20 and 40 mg/kg) and subcutaneous insulin treated groups (SC) for 5 weeks. The untreated STZ-induced diabetic animals presented with hyperglyceamia over the period of 5 weeks by comparison with non-diabetic control (p<0.05, DC vs ND). The administration of vanadium complex attenuated the hyperglycaemia, showing a significant effect as from week 2 throughout the experimental period compared to the diabetic control (p<0.05, VC vs DC, Figure 1). Similarly, the administration of insulin attenuated hyperglycaemia in diabetic animals (p<0.05, SC vs DC).
**Liver and body weights**

Table 1 presents the liver tissue and body weights in non-diabetic (ND), diabetic control (DC), diabetic rats treated with vanadium complex (VC) (40 mg/kg) and subcutaneous insulin treated groups (SC) after the experimental period of 5 weeks. The induction of diabetes resulted in a significant decrease in the body weight in diabetic control compared to the non-diabetic control (p<0.05, DC vs ND), while no significant change was observed in the liver weights (Table 1). However, the administration of vanadium complex showed an increase in the body weights compared to the diabetic control (p<0.05, VC vs DC), yet no effect was observed in the liver weights. The administration of insulin showed significant increases in both the liver and body weights (p<0.05, SC vs DC).

**Lipid profile**

Table 2 compares the week 5 plasma total cholesterol (TC), triglycerides (TG), HDL and LDL concentrations in non-diabetic control (ND), diabetic control (DC), diabetic animals treated with vanadium (10, 20, 40 mg) and diabetic animals subcutaneously administered with insulin (S.C). Diabetes induction resulted in increased TG and TC concentrations in comparison to non-diabetic control (p<0.05, DC vs ND). Diabetic control animals showed increased TC and TG, respectively when compared to ND animals ★ (DC vs ND, p<0.05). Diabetic rats treated with vanadium complex (10, 20, 40 mg/kg) decreased TC and TG concentrations in comparison to STZ-induced diabetic rats. However, an increase in TGs concentration was observed in insulin treated animals (p<0.05, SC vs DC). The diabetic rats presented with an increase in HDL concentration (p<0.05, DC vs ND) and no effect was observed during VC administration (Table 2).

**Oxidative stress markers**

Table 3 shows superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA) concentration measured in the liver tissues of non-diabetic (ND), diabetic control (DC), vanadium complex (40 mg/kg) (VC) and diabetic animals subcutaneously administered with insulin (S.C) groups after 5 weeks. SOD, GPx and MDA concentration in STZ-induced diabetic rats were significantly increased when compared to non-diabetic control. The induction of diabetes resulted in an increment of SOD, GPx and MDA concentration (DC vs ND, p<0.05, Table 3). Treatment with vanadium complex (40 mg/kg) resulted in a significant decrease in MDA concentrations (p<0.05, VC vs DC). Similarly, insulin administration showed a decrease in MDA concentrations (p<0.05, SC vs DC)).

**Liver function enzymes**

Figure 2 and 3 shows plasma alanine transaminase (ALT) and aspartate transaminase (AST) activities respectively in non-diabetic (ND), diabetic control (DC), vanadium complex (VC) (40 mg/kg) and subcutaneous insulin (SC) groups at the end of 5 weeks. The diabetic rats (DC) showed an increase in plasma
ALT and AST activities when compared to non-diabetic rats (p<0.05, DC vs ND, Figure 2 and 3). However, the administration of vanadium complex decreased plasma ALT activity (p<0.05, VC vs DC, Figure 2).

**Plasma CRP**

Figure 4 presents the plasma C-reactive protein (CRP) concentration in non-diabetic (ND), diabetic control (DC), diabetic rats treated with vanadium complex (VC) (40 mg/kg) and subcutaneous insulin treated groups (SC) at the end of 5 weeks. The untreated STZ-induced diabetic animals showed an increase in plasma CRP concentrations by comparison with non-diabetic control (p<0.05, DC vs ND). The administration of vanadium complex decreased the plasma CRP at the end of the experimental period (p<0.05, VC vs DC, Figure 4).

**Discussion**

Previous studies have reported the development of metal-based drugs with favourable pharmacological application and may offer unique therapeutic opportunities. Indeed, glycaemic control has been achieved through the administration of vanadium and zinc in the form of inorganic salts (Fedorova et al. 2013). Depending on the nature of ligand, vanadium complexes can be divided into inorganic and organic complexes (Sakurai et al. 2002). Vanadium complexes with organic ligands have proved to be less toxic, with improved solubility and lipophilicity (Goc 2006). The organic-ligand interactions are ubiquitous and play important roles in almost every biological process (Sakurai et al. 2002). Research has shown that the organic ligands provide thermodynamic stability and efficient transport of vanadium to target locations in the body, making the vanadium safer, more effective and stable than in the inorganic salt form (Sakurai et al. 2002). In this study, we have investigated the effects of dioxidovanadium (V) complex with more focus on the liver function in diabetes. This complex is fused with the organic ligands (phenol and benzimidazole) used in its synthesis. The benzimidazole and phenolate derivatives have been shown to possess anti-diabetic and anti-oxidant properties. Therefore, we envisaged that 2-phenolate-1H-benzimidazole-dioxidovanadium may have less potential hepatic hazards, which may be beneficial in this context as most hypoglycaemic agents prove to detrimental.

The blood glucose concentrations were extremely high in the STZ-induced rats during the experimental period. Streptozotocin (STZ) administration selectively destroys the beta insulin-producing cells of the pancreas via DNA alkylation (Motala et al. 2008). However, in agreement with previous reports, the administration of vanadium complex in STZ-induced diabetic rats attenuated blood glucose concentration in
this study (Gurley et al. 2016). Vanadium administration has been shown to increase glucose transport and oxidation and insulin-receptor tyrosine-kinase activity (Goc, 2006). Furthermore, previous study has reported that vanadium inhibits microsomal glucose-6-phosphatase in STZ diabetic rats (Sakurai, 2002). The inhibition of glucose-6 phosphatase by vanadium is thought to increase hepatic glucose-6 phosphate which serves as substrate for glycogen synthesis (Goc 2006). The insulin-mimetic properties and anti-diabetic effects of vanadium compounds have been widely documented both in vivo and in vitro. Vanadium induces the recruitment of vesicles containing GLUT 4, by stimulating the tyrosine kinase activity of the β-subunit of the insulin receptor (Reul et al. 1999). Another mechanism of reduction of blood glucose concentration by vanadium compounds is the activation of PKB/Akt kinase leading to the increase of glucose uptake the cells via GLUT4 transporter (Sakurai 2002).

STZ-induced diabetes is associated with decreased body weight (Sibiya et al. 2017a). The body weight loss is attributable to the lack of insulin effect in diabetic animals (Musabayane et al. 2000). Insulin is a well-known anabolic hormone, promoting lipid storage and protein synthesis, hence it absence leads body weight loss (Musabayane et al. 2000). Indeed, the administration of insulin subcutaneously improved body weight. The ability of vanadium to protect against weight loss might be due to its glucose lowering effect and perhaps also other possible insulin mimetic effect (Juurinen et al. 2007). The liver has also been shown to be affected by hyperglycaemia toxicity (Harris 2005). In this study, the increase (hypertrophy) in the liver weights in diabetic control animals may be attributed to the increased influx of fatty acids into the liver and the low capacity of excretion of lipoproteins secretion from the liver resulting from deficiency of Apo lipoprotein B synthesis which is a protein involved in the metabolism of lipids (Khan and Newsome 2016).

Insulin is beneficial in restoring glycaemia and delaying the onset of diabetic complications, however, hepatic hazards have been reported (Ishii and Takamura 2016). Studies have documented that subcutaneous insulin is associated with hepatomegaly (Khan and Newsome 2016). Indeed, the animals on subcutaneous insulin presented with an increase in liver weights which may perhaps allude to increase in fat and glycogen deposition. Physiologically, when insulin is secreted by the beta-cells of the pancreas it is delivered to the liver for clearance via the portal vein, therefore, less insulin concentration reaches the circulation (Havel et al. 1970). However, in diabetic patients, subcutaneous insulin injections are introduced directly into the blood stream in high amounts, resulting in hyperinsulinaemia, since the first pass metabolism is by-passed (Havel et
al. 1970). Thus, hyperinsulinaemia may act as a major contributor to the progression of liver damage and dysfunction. The insulin-induced hepatomegaly is association with inflammation as a result of fatty deposition, which may progress to scarring of the liver tissue to cirrhosis (Brunzell et al. 1973). In this study, the administration of insulin subcutaneously, indeed resulted to hepatomegaly, further confirming the hepatic hazards associated with insulin injections. Treatments with vanadium complex in diabetic rats significantly restored liver and body weight to near normal (Sakurai 2002). Unlike insulin, vanadium has not been reported dysregulate hepatic lipogenesis. These observations are of therapeutic important considering insulin-induced hepatomegaly in diabetes.

Previous studies showed that vanadium treatment potentially ameliorate lipid metabolism derangement often seen in diabetes (MacArthur et al. 2007). Reports suggest that dyslipidaemia is a frequent complication in all types of diabetes which can range from hypercholesterolemia to hypolipoproteinaemia (Brunzell et al. 1973). Hyperlipidaemia could be a factor for fatty liver development and has also been observed in diabetic patients on insulin therapy (Lewis et al. 2002). In the present study, serum TG and TC concentrations were elevated in the diabetic group compared to those in the control group, which is consistent with other studies and further correlated with increases in liver weight. In the absence of insulin, lipolysis is increased thus leading to an aggravated circulating triglycerides and cholesterol in the blood (Kowluru et al. 1999). However, the elevated serum TG concentration in diabetic rats was significantly decreased after treatment with vanadium complex. This result also suggest that liberation of fats as energy source was reduced, since tissues particularly skeletal and adipose tissue could be able to utilize glucose as the energy source facilitated by dioxidovanadium administered. This is in agreement with the evidence that decreasing high concentrations of serum and hepatic TGs is often observed after treatment with vanadium compounds such as vanadium dipicolinate, vanadyl sulphate and vanadate containing compounds (Sakurai 2002). The increase in the plasma triglycerides seen in insulin injected animals may indicate increases in hepatic lipogenesis and may further explain the hepatomegaly observed. Subcutaneous insulin administration have been shown to increase the expression of sterol regulatory element-binding transcription factor 1 (SREBP1c). The SREBP1c is a major transcription factor which regulates de novo lipogenesis through direct activation from AKT) in insulin signalling pathway. The observations may further illuminate the potential cardiovascular risks associated insulin injection.
Oxidative stress is the main cause for the development and advancement of liver dysfunction in diabetes (Lewis et al. 2002). Hyperglycaemia evokes oxidative stress through activating of the following pathways; polyol, hexosamine and protein kinase C pathway. In this study, the ability of vanadium complex to attenuate hyperglycaemia might had a positive outcome in attenuating oxidative stress, as tight glycaemic control has been shown to inactivate the above mentioned pathways (Sibiya et al. 2017b). Studies have indicated an increase in liver antioxidant enzymes in response to elevated free radicals (Sakamaki et al. 1999). The results of our study agree with other studies showing that induction of diabetes increase GPx and SOD activities (Sakamaki et al. 1999). A decrease in SOD and GPx in the liver upon vanadium administration may be an indication of vanadium complex’s ability to counteract free radical generation. Free radicals results in lipid peroxidation and MDA has over the years been utilized as a prominent marker for lipid peroxidation. The reduction of MDA concentration may owe to the improvement in glycaemic control and or the direct antioxidant capacity of vanadium complex. The phenolate component in our vanadium complex is envisaged have counter-acted the free radicals. The antioxidant therapy is amongst the treatment strategies for prevention and slowing the progression of diabetic complications such including hepatic damage (Maritim et al. 2003). Therefore, the ability of dioxidovanadium to alleviate oxidative stress may be beneficial in diabetes.

In diabetes mellitus, hepatocytes damage is inevitable, resulting in an increase in the circulating liver function marker enzymes, such are aspartate transaminase (AST) and alanine transaminase (ALT) (Hamed 2014). The increase in serum ALT and AST in diabetic patients is commonly associated with signs of hepatic diseases such as NALFD (Frezza et al. 2010). The deleterious effect of hyperglycaemia in the liver of diabetic rats observed in the present study was evidenced by elevation of liver damage biomarkers. In this study, animals receiving insulin showed high levels of ALT and ALT thus indicating hepatic damage, these observations also correlated with increase in liver mass which may perhaps indicate liver dysfunction and injury. The hepatoprotective effects of vanadium is demonstrated by a significant reduction of serum ALT and AST. Additionally, C Reactive protein CRP has been shown to be released by the hepatocytes in response to inflammation or hepatic injury (Musabayane et al. 2000). CRP and diabetes associated inflammatory cytokines such as tumour necrosis factor (TNF α) contribute to liver dysfunction and are associated with low grade inflammation in diabetes. The elevation of liver enzymes is associated with higher CRP concentrations in diabetic control as evidenced in this study, however administration of vanadium complex decreased plasma
CRP at the end of 5 week experimental period. The attenuation of both liver enzymes and CRP may perhaps be the outcome of glycaemic and oxidative stress attenuation by the vanadium complex administered. Additionally, these observations may also suggest that our vanadium complex exert no or little toxicity to the hepatocytes, unlike other oral hypoglycaemic agents such as troglitazone. Addionally, such observations indicate that the incorporation of organic ligands mitigate the toxicity associated with inorganic vanadium compounds. Perhaps such observations allude to the effective, non-toxic vanadium complex doses and dosing schedule used in the study. Furthermore, the reduction in CRP concentration may also allude to the cardio protective effect provided by vanadium, since CRP is also a prominent marker for screening cardiac injury (Musabayane et al. 2000). Taken together, these results suggest that 2-phenolate-1H-benzimadizole-dioxidovanadium possess no hepatic hazards and improves hepatic function in streptozotocin-induced-diabetes. Moreover, the observations from this study further alludes and put more emphasis on the hepatic hazards associated with subcutaneous insulin as evidence by high increase in liver mass, triglyceride, ALT, AST and CRP.

**Conclusion**

The administration of dioxidovanadium complex attenuates hyerglycaemia, oxidative status, and lipid profile disturbances in streptozotocin-induced diabetic animals. Additionally, dioxidovanadium administration exert no apparent hepatic toxicological effect in diabetes induced animals. Taken together, the observations from this study encourages further research and developments, regarding the use of vanadium therapy as an alternative remedy for diabetes while highlighting the hepatic hazards associated insulin injections.

**Conflict of interest**

The authors declare that there is no conflict of interest.

**Acknowledgement**

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Table 1: Liver tissue and body weights at the end of 5 weeks in non-diabetic (ND), diabetic control (DC), diabetic rats treated with vanadium complex (VC) (10, 20 and 40mg/kg) and subcutaneous insulin treated groups (SC) for 5 weeks.

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<th>Experimental groups</th>
<th>Liver tissue weights (g)</th>
<th>Body weights (g)</th>
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<tr>
<td>ND</td>
<td>10.8 ± 0.32</td>
<td>345.2 ± 14.9</td>
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<tr>
<td>DC</td>
<td>11.5 ± 0.62</td>
<td>170.0 ± 7.40★</td>
</tr>
<tr>
<td>VC (10 mg/kg)</td>
<td>11.2 ± 0.38#</td>
<td>224.6 ± 10.0#</td>
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<tr>
<td>VC (20 mg/kg)</td>
<td>10.8 ± 0.50#</td>
<td>228.8 ± 9.60#</td>
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<tr>
<td>VC (40 mg/kg)</td>
<td>10.5 ± 0.39#</td>
<td>229.7 ± 5.90#</td>
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<tr>
<td>INS SC</td>
<td>11.3 ± 0.28</td>
<td>253.7 ± 6.50#</td>
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★ p < 0.05 when comparing DC and ND, # p<0.05 when comparing vanadium complexes with DC and INS SC vs DC.
Table 2: The lipid profile was assessed in non-diabetic control (ND), diabetic control (DC), diabetic animals treated with vanadium complex (VC) (40mg/kg) and diabetic animals subcutaneously administered with insulin (SC). Data is expressed as mean ± SEM (mean of samples per group, n=6).

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Triglycerides (mmol/L)</th>
<th>Cholesterol (mmol/L)</th>
<th>HDL (mmol/L)</th>
<th>LDL (mmol/L)</th>
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<td>ND</td>
<td>1.43 ± 0.07</td>
<td>4.08 ± 0.07</td>
<td>0.6 ± 0.2</td>
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<tr>
<td>DC</td>
<td>2.50 ± 0.2★</td>
<td>5.65 ± 0.20★</td>
<td>1.3 ± 0.1★</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>40 (mg/kg)</td>
<td>1.00 ± 0.14#</td>
<td>3.30 ± 0.01#</td>
<td>1.5 ± 0.1</td>
<td>2.0 ± 0.1#</td>
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<tr>
<td>SC INS</td>
<td>3.00 ± 0.26#</td>
<td>4.3 ± 0.8#</td>
<td>0.9 ± 0.1</td>
<td>2.1 ± 0.1#</td>
</tr>
</tbody>
</table>

★ p < 0.05 when comparing DC to ND, # p<0.05 when comparing vanadium complexes to DC and INS SC vs DC
Table 3: The oxidative stress markers in liver tissues of non-diabetic control, diabetic control (DC), diabetic rats treated with vanadium complex (VC) (40mg) and subcutaneous insulin treated groups (SC) at the end of 5 weeks experimental period. Values are expressed as mean ± SEM.

<table>
<thead>
<tr>
<th>EXPERIMENTAL GROUPS</th>
<th>SOD (ng/ml)</th>
<th>GPx (ng/ml)</th>
<th>MDA (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
<td>7.45 ± 0.06</td>
<td>1.61 ± 0.02</td>
<td>0.99 ± 0.02</td>
</tr>
<tr>
<td>DC</td>
<td>8.51 ± 0.08★</td>
<td>1.94 ± 0.02★</td>
<td>2.10 ± 0.01★</td>
</tr>
<tr>
<td>VC (40 mg/kg)</td>
<td>8.76 ± 0.08</td>
<td>1.83 ± 0.02#</td>
<td>1.09 ± 0.01#</td>
</tr>
<tr>
<td>SC INS</td>
<td>8.61 ± 0.12</td>
<td>1.97 ± 0.03</td>
<td>1.11 ± 0.01#</td>
</tr>
</tbody>
</table>

★ p < 0.05 when comparing DC and ND, # p<0.05 when comparing vanadium complexes with DC and INS SC vs DC.
**Figure 1:** The weekly blood glucose concentration in non-diabetic (ND), diabetic control (DC), diabetic rats treated with vanadium complex (VC) (10, 20 and 40mg/kg) and subcutaneous insulin treated groups (SC) for 5 weeks. Values are expressed as mean ± SEM, n=6 in each group. ★ p < 0.05 by comparison with diabetic control.

**Figure 2:** Shows the liver function marker enzymes (ALT) activity in non-diabetic (ND), diabetic control (DC), diabetic rats treated with vanadium complex (VC) (40mg/kg) and subcutaneous insulin treated groups (SC) at the end of 5 weeks experimental period. Data is expressed as mean ± SEM (mean of samples per group, n=6) ★ p < 0.05 when comparing DC and ND, # p<0.05 when comparing VC and SC with DC.

**Figure 3:** Shows the liver function marker enzymes (AST) activity in non-diabetic (ND), diabetic control (DC), diabetic rats treated with vanadium complex (VC) (40mg/kg) and subcutaneous insulin treated groups (SC) at the end of 5 weeks experimental period. Data is expressed as mean ± SEM (mean of samples per group, n=6) ★ p < 0.05 when comparing DC and ND, # p<0.05 when comparing VC and SC with DC.

**Figure 4:** Shows the comparison of plasma CRP concentration in STZ-induced diabetic rats treated with vanadium complex (40mg/kg) with positive control (SC), non-diabetic (ND) and untreated diabetic animals (DC). Data is expressed as mean ± SEM (mean of samples per group, n=6) ★ p < 0.05 when comparing DC and ND, # p<0.05 when comparing VC and SC with DC.
Figure 1: The weekly blood glucose concentration in non-diabetic (ND), diabetic control (DC), diabetic rats treated with vanadium complex (VC) (10, 20 and 40mg/kg) and subcutaneous insulin treated groups (SC) for 5 weeks. Values are expressed as mean ± SEM, n=6 in each group. ★ p < 0.05 by comparison with diabetic control.

193x109mm (300 x 300 DPI)
Figure 2: Shows the liver function marker enzymes (ALT) activity in non-diabetic (ND), diabetic control (DC), diabetic rats treated with vanadium complex (VC) (40mg/kg) and subcutaneous insulin treated groups (SC) at the end of 5 weeks experimental period. Data is expressed as mean ± SEM (mean of samples per group, n=6) ★ p < 0.05 when comparing DC and ND, # p<0.05 when comparing VC and SC with DC.

125x79mm (300 x 300 DPI)
Figure 3: Shows the liver function marker enzymes (AST) activity in non-diabetic (ND), diabetic control (DC), diabetic rats treated with vanadium complex (VC) (40mg/kg) and subcutaneous insulin treated groups (SC) at the end of 5 weeks experimental period. Data is expressed as mean ± SEM (mean of samples per group, n=6) ★ p < 0.05 when comparing DC and ND, # p<0.05 when comparing VC and SC with DC.
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