The effects of dioxidovanadium (V) complex on cardiovascular metabolism in STZ-induced diabetic male Sprague Dawley rats

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Abstract
Aims/hypothesis
Insulin is an effective hyperglycemia agent, however hyperinsulinemia, as result of prolonged administration, has been shown to lead to cardiovascular disease (CVD) in DM. As a result, research into alternative therapies for the management of diabetes is needed. In our laboratory, a novel vanadium complex has been synthesized and has been shown to improve glycaemic control and liver function. The effects of this complex on cardiovascular metabolism, however, have not yet been established. Therefore, this study sought to investigate the effects of a dioxidovanadium (V) complex on cardiac muscle metabolism in STZ-induced diabetic rats. Vanadium complex was administered twice daily, and blood glucose concentration was monitored for 5 weeks. The animals were sacrificed, blood and hearts were collected for biochemical analysis (western blot (GLUT 1 and 4), pyruvate kinase (PK), acetyl-coA synthetase and ATP synthase) and microscopical analysis (TEM). After 5 weeks, untreated diabetic rats presented with hyperglycaemia compared to non-diabetic rats which was attenuated by vanadium complex administration. The administration of the complex showed an increase in the metabolic activity of enzymes, GLUT 1 and 4 expression. This was further supported by an increased number of mitochondria and their improved structure as shown by transmission electron microscopy. The administration of the dioxidovanadium (V) complex improved cardiovascular metabolism could be a vital hypoglycaemic agent in the management CVD and DM.

Keywords: Hyperglycaemia; Vanadium complex; Cardiovascular Metabolism; Diabetes; Hyperlipidaemia.

Introduction
Diabetes mellitus has been shown to increase the risk of hyperglycaemia-induced cardiovascular associated morbidity and mortality, with diabetic cardiomyopathy (DCM) being listed amongst the leading cardiovascular complications implicated in the deaths of approximately 16.9% of all DM patients [1, 2]. Cardiomyocyte death as a result of metabolic dysfunction and hyperglycaemia-induced myocardial ischaemia plays a key role in the development and progression of DCM and ultimately heart failure [2, 3]. Under normal physiological conditions the cardiac muscle is highly adapted to utilize all classes of metabolic substrates switching preference between free fatty acids and glucose in response to environmental changes [1, 4]. Cardiac cells have been shown to utilize free fatty acids at rest and glucose during stressful conditions such as pathological hypertrophy and hyperglycaemia induced-myocardial ischemia often seen in diabetes [1, 2]. Cardiomyocyte glucose entry is facilitated by two glucose transporters namely GLUT1 and GLUT4, however GLUT4 is predominant in the healthy adult heart [1, 4]. Hyperglycaemia causes a disruption in the physiological metabolic function of the heart as the resultant increased lipid formation, from excessive glucose, is overly infused in cardiomyocytes causing the repression and downregulation of GLUT4 gene expression [1, 5]. This will lead to a decrease in GLUT 4 transporter formation resulting in the reduced uptake of glucose by the cardiomyocytes [4, 6]. The repression of GLUT 4 will cause the compensatory upregulation of the limited GLUT 1 transporters in efforts to restore the metabolic flexibility of the cardiac muscle [5, 6]. Furthermore, the continuous cardiac inflexibility results in the significant destruction of mitochondrial structure and decline in mitochondrial volume and number [5]. Unfortunately, the lack of GLUT 1 and 4 as well as the impaired mitochondria lead to, respectively, a significant glucose decline depicted by a decrease in pyruvate kinase and acetyl-coA synthetase activity and a substantial reduction ATP production marked by a decrease ATP synthase activity [5]. The decreased ATP concentration can lead to apoptosis of cardiomyocytes which contributes to the development of DCM, cardiovascular dysfunction and heart failure [5-7]. The bolus intravenous administration of insulin in type 1 diabetes mellitus has been associated with hyperinsulinemia [8, 9]. Hyperinsulinemias also

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been shown to impair lipid and glucose metabolism as well as induce hypertrophy of the cardiomyocytes through enhancing the actions of various growth factors [TGF-B and IGF-1] which increase myocyte cellular growth resulting in cardiomyocyte hypertrophy and heart failure [8-10]. These complications warrant the continuous search for novel compounds which may provide glycaemic control and alleviate hyperglycaemia-induced cardiovascular complications [11-13]. Previous studies have shown that vanadium compounds act as anti-hyperglycaemic agents that exert numerous cardioprotective effects in myocardial ischemia, hypertension and myocardial hypertrophy; however, their use has been associated with kidney, brain and liver toxicity [11, 14, 15]. In our laboratory we have synthesized a novel vanadium complex namely dioxidovanadium(V)complex, cis-[VO2(obz)py] [Hobz=2-hydroxyphenyl-1H-benzimidazole and py =pyridine]] which is conjoined with organic heterocyclic ligands that provide thermodynamic stability and efficient vanadium transport to target tissues, rendering the complex safer, more potent and stable for use [16]. The effects of this complex have been tested on glucose homeostasis and has proved most effective in lowering blood glucose and improving glycaemic control [17]. However, the effects of dioxidovanadium(V) complex on the metabolic profile of cardiomyocytes has not yet been established hence the aim of my study is to investigate the effects of dioxidovanadium(V) complex on the metabolic profile of cardiomyocytes in STZ-induced diabetic rats.

Materials and methods

Drugs and chemicals

All chemicals and reagents used were purchased from standard pharmaceutical suppliers and were of analytical grade.

Vanadium Complex Synthesis

Vanadium complexes were synthesized in the Department of Chemistry at the University of KwaZulu-Natal, Pietermaritzburg, South Africa. The novel dioxidovanadium(V) complex, cis-[VO2(obz)py][Hobz=2-hydroxyphenyl-1H-benzimidazole and py =pyridine]] was successfully synthesized and verified using the UV-Vis, Emission, EPR, IR, V- and H NMR spectroscopy and crystal X-ray diffraction. [16]

Animals and Housing

Male Sprague-Dawley rats weighing 250-300g bred in the Biomedical Research Unit at the University of KwaZulu Natal were housed individually in Makrolon polycarbonate metabolic cages (Techniplast, Labotec, South Africa). The animals were acclimatized [duration: 5 days] before the commencement of the study. The animals experienced a 12hr day: 12 hr night cycle and were maintained in a standard laboratory environment (constant temperature and humidity). Animals had free access to water and rat chow daily. (Meadow Feeds, Pietermaritzburg, South Africa).

Use of experimental animals

The protocol for laboratory experimentation had been reviewed and approved by the Animal research ethics committee of UKZN (AREC/054/017D). Animals were constantly monitored for pain and discomfort according to the criteria of the Animal Research Ethics Committee’s humane end point document. The authors complied with ARRIVE guidelines when working with the mentioned animals.

Induction of diabetes

Type 1 diabetes was induced using a well-established protocol [18]. Briefly, animals were given a single intra-peritoneal injection of streptozotocin (60mg/kg) freshly prepared in 0.1 M citrate buffer (pH 4.5). The non-diabetic control group received the vehicle, citrate buffer through the same route. Animals showing glucosuria after 24 hours following a urine strip test (Rapidmed Diagnostics, Sandton, South Africa) were considered diabetic. After 7 days, blood glucose concentrations greater than 20 mmol/L in streptozotocin (STZ) - induced rats were considered to show stable diabetes.

Experimental design

The effects of dioxidovanadium(V) complex, cis-[VO2(obz)py] [Hobz=2-hydroxyphenyl-1H-benzimidazole and py =pyridine]] on cardiac muscle metabolism were investigated acutely and short-term in STZ induced male Sprague-Dawley rats.

Short-term Effects

Post diabetes induction, the short-term effects of the novel vanadium complex (40 mg kg−1.p.o) on cardiac metabolism were investigated in STZ-induced diabetic rats. The experimental animals were divided into the following groups; non-diabetic (ND), diabetic control (DC), novel vanadium complex (40 mg kg−1.p.o) (VAN) and insulin (0,175 mg/kg-1, s.c) (INS) treated animals that served as positive control. The animals were housed in Makrolon polycarbonate metabolic cages (Techniplast, Labotec, South Africa). The vanadium complex was administered twice every third day at 09h00 AM and 15h00 PM by means of an 18-gauge gavage needle (Kyon Laboratories (Pty) LTD, Benrose, South Africa). The diabetic group which received H2O (3mL kg-1, p.o.) and insulin group (0,175 mg kg-1.s.c) acted as a negative control and positive control, respectively. Over the period of 5 weeks the blood glucose concentrations were monitored weekly, OneTouch select glucometer (Lifescan, Mosta, Malta, United Kingdom). This experimental protocol has been published in our laboratory as per [17].

Terminal studies

Blood and tissue collection

Blood was collected by cardiac puncture and then injected into individual pre-cooled heparinized containers while the rats were unconscious. EDTA tubes were used to collect plasma for (pyruvate kinase, ATP synthase) immunoassays and an acetyl-CoA synthetase ELISA. The blood was centrifuged for 15 minutes at 1000 × g (2-8°C). Cardiac muscle tissue was collected and 50% was stored in saline and the rest was stored in 2% glyceraldehyde.
for biochemical analysis for biochemical analysis and for
transmission electron microscopy.

**Biochemical analysis**

**Pyruvate kinase and ATP synthase**

Pyruvate Kinase and ATP synthase was measured using assays
following the manufacturer’s instructions (Elabscience and
Biotechnology, WuHan).

**Acetyl-coA synthetase**

Acetyl-coA synthetase was measured using an ELISA kit following
the manufacturer’s instructions (Elabscience and Biotechnology,
Wuhan).

**Western blot**

Western blot performed using a well-established protocol [31].
Briefly cardiac muscle tissues were harvested from untreated and
treated STZ-induced diabetic rats at the end of 5 week analysed
for, GLUT 1 and 4 using Western blotting. The tissues (0.1 g)
were homogenized on ice in isolation buffer (0.5 mM Na2EDTA,
0.1 M KH2PO4, 0.1 mM M dithiothreitol, 0.25 M sucrose) and then
centrifuged at 400 x g for 10 min (4°C). The protein content
was quantified using Bradford reagent. All the samples
were standardized to one concentration (1 mg/mL). The proteins
were then denatured by boiling in laemmli sample buffer (0.5 M Tris-HCl,
glycerol, 10% sodium dodecyl sulphate (SDS), 2-mercaptoethanol,
1% bromophenol blue) for 5 min. The denatured proteins were
loaded (25 µL) on prepared resolving (10%) and stacking (4%)
polyacrylamide gels along with molecular weight marker (5
µL). The gel was electrophoresed for 1hr at 200 V 400 mA in
electrode (running) buffer (Trisbase, glycine, SDS), pH 8.3).

Following electrophoresis, the resolved proteins was electro-
transferred to an equilibrated polyvinylidene difluoride (PVDF)
membrane for 1hr in transfer buffer (192 mM glycine, 25 mMTris,
10% methanol). After transfer, the membrane was blocked with
blocking buffer. The membranes were then immuno-probed with
antibodies GLUT 1 and 4 using Western blotting. The tissues (0.1 g)
treated STZ-induced diabetic rats at the end of 5 week analysed
for biochemical analysis for biochemical analysis and for
transmission electron microscopy.

**Electron microscopy imaging**

**Cardiac muscle mitochondrial processing for transmission
electron microscopy imaging**

The hearts were removed quickly from the anaesthetized animals,
the atria and connective tissue was dissected and removed. For
ultrastructural examination, two hearts each from the diabetic
and the control group were fixed in 0.1 M phosphate buffer (pH
7.4) containing 2% glutaraldehyde at 4°C. Small tissue pieces
4-6 mm in size were taken from four different areas of mid-
myocardial layer of the free left ventricular wall between middle
of the chamber and the apex of the heart. These tissue samples
were immersed for 15 min in the aldehyde fixation solution, cut
into pieces of approximately 1mm3 and allowed to stand in the
solution for a total of 2 h for further fixation. In the experimental
as well as in the control group, heart muscle samples were also
fixed by the procedure of perfusion fixation described previously
(Singal et al., 1979). The tissue pieces were washed overnight in
cold phosphate buffer containing sucrose, post-fixed for 1hr with
1% osmium tetroxide, dehydrated in a graded alcohol series
and embedded in resin according to the method of Luft (1961).
Sections were cut with a diamond knife, stained with uranyl
cetate and lead citrate, and examined by a transmission electron
microscope.

**Myocardial mitochondria volume and number determination**

Mitochondria volume was measured using Imagej software
(version Java 8.00, Imagej software, Bethesda, Maryland, USA).

**Statistical Analysis.**

Data is expressed as means ± and standard error of means (SEM).
Statistical analysis was conducted using GraphPad Prism and
InStat software (version 5.00, GraphPad Software, San Diego,
California, USA). Terminal parameters were analysed using a
two-way ANOVA followed by the Bonferroni post hoc test, which
was used to analyse differences between the controls and the
experimental groups. Values of p< 0.05 indicated statistical
significance.

**Results**

**The effects of dioxidovanadium (V) complex on blood glucose**

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>ND</th>
<th>DC</th>
<th>INS</th>
<th>VAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.77±0.30</td>
<td>39.30±0.95#</td>
<td>27.55±1.51</td>
<td>29.11±0.95</td>
</tr>
<tr>
<td>1</td>
<td>5.05±0.27</td>
<td>35.30±0.87#</td>
<td>29.45±1.99</td>
<td>29.40±0.83</td>
</tr>
</tbody>
</table>

The effects of dioxidovanadium (V) complex on cardiovascular metabolism in STZ-induced diabetic male Sprague Dawley rats

Table

<table>
<thead>
<tr>
<th></th>
<th>Non-diabetic</th>
<th>Diabetic Control</th>
<th>Dioxidovanadium (40 mg/kg)</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5.05±0.36</td>
<td>34.30±1.27#</td>
<td>29.90±2.62★α</td>
<td>16.50±1.26★a</td>
</tr>
<tr>
<td>3</td>
<td>4.90±0.28</td>
<td>38.30±0.6#</td>
<td>30.98±1.15★α</td>
<td>16.00±0.70★α</td>
</tr>
<tr>
<td>4</td>
<td>5.75±0.46</td>
<td>39.30±0.68#</td>
<td>30.89±0.39★α</td>
<td>11.37±0.68α</td>
</tr>
<tr>
<td>5</td>
<td>5.33±0.05</td>
<td>35.08±0.93#</td>
<td>18.80±2.35★α</td>
<td>10.21±0.94★a</td>
</tr>
</tbody>
</table>

p<0.05 by comparison with normal control animals. ★ p<0.05 by comparison with diabetic control.
α p<0.05 by comparison with dioxidovanadium (V) complex treated animals.

The effects of dioxidovanadium (V) complex on triglyceride concentration

Figure 1 represents weekly triglyceride concentration in non-diabetic, diabetic control, as well as insulin and of vanadium (40mg/kg) treated diabetic rats (n=6) over a 5-week experimental period. The untreated STZ-induced diabetic animals showed significantly high triglyceride concentration over the period of 5 weeks in comparison with non-diabetic control (p=0.001). Treatment with vanadium complex and insulin showed a significant decrease in triglyceride levels by comparison with the diabetic control (p<0.05).

![Figure 1](image1.png)

The effects of dioxidovanadium (V) complex on pyruvate kinase concentration

Figure 2 shows pyruvate kinase (PK) concentrations in non-diabetic, diabetic, dioxidovanadium and insulin treated groups at the end of the 5-week experimental period. STZ-induced diabetic animals' PK concentrations were significantly decreased in comparison with non-diabetic control group. Interestingly, administration of dioxidovanadium (40 mg/kg, p.o) attenuated the diabetic associated decrease in PK concentration and significantly (p<0.05) increased PK concentrations by comparison to STZ-induced diabetic control rats at the end of the 5-week experimental period. As expected, insulin also significantly (p<0.05) increased PK concentrations.

![Figure 2](image2.png)

The effects of dioxidovanadium (V) complex on acetyl-coA synthetase concentration

Figure 3 shows acetyl-coA synthetase concentrations in non-diabetic, diabetic, dioxidovanadium and insulin treated groups at the end of the 5-week experimental period. STZ-induced diabetic animals’ acetyl-coA synthetase concentrations were significantly decreased in comparison with non-diabetic control group. Interestingly, administration of dioxidovanadium (40 mg/kg, p.o) attenuated the diabetic-associated decrease in acetyl-coA synthetase concentration and significantly (p<0.05) increased acetyl-coA synthetase concentrations by comparison to STZ induced diabetic control rats at the end of the 5-week experimental period.

Figure 3: Shows effects of dioxidovanadium on acetyl-coA synthetase activity in STZ-induced diabetic rats at the end of the 5-week experimental period. Values are presented as means and vertical bars indicate SEM (n=6 in each group). # p<0.05 by comparison with normal control animals. ★ p<0.05 by comparison with diabetic control.

The effects of dioxidovanadium (V) complex on ATP synthase concentration

Figure 4 shows ATP synthase concentrations in non-diabetic, diabetic, dioxidovanadium and insulin treated groups at the end of the 5-week experimental period. STZ-induced diabetic animals’ ATP synthase concentrations were significantly decreased in comparison with non-diabetic control group. Interestingly, administration of dioxidovanadium (40 mg/kg, p.o) attenuated the diabetic-associated decrease in ATP synthase concentration and significantly (p<0.05) increased ATP synthase concentrations by comparison to STZ-induced diabetic control rats at the end of the 5-week experimental period. As expected, insulin also significantly (p<0.05) increased

Figure 4: Shows effects of dioxidovanadium on ATP synthetase activity in STZ-induced diabetic rats at the end of the 5-week experimental period. Values are presented as means and vertical bars indicate SEM (n=6 in each group). # p<0.05 by comparison with normal control animals. ★ p<0.05 by comparison with diabetic control.

Western blot

Figure 5 below illustrates the effects of dioxidovanadium (V) complex on GLUT4 and GLUT1 expression in the non-diabetic group and the STZ-induced diabetic groups of this study. A strong expression is indicated by a stronger band intensity. The untreated diabetic control had a significant decrease in GLUT 4 and GLUT 1 expression by comparison to the non-diabetic control. The group treated with the dioxidovanadium (V) complex cis-[V(O2)(dbz)py] showed a significant increase in GLUT4 expression compared to the untreated diabetic control group. As expected, the insulin treated group showed significant increase in GLUT4 expression by comparison to the diabetic control.
Figure 5: Shows effects of dioxidovanadium on GLUT 1 and 4 expression in STZ-induced diabetic rats at the end of the 5-week experimental period. Values are presented as means and vertical bars indicate SEM (n=6 in each group). # p<0.05 by comparison with normal control animals. ★p<0.05 by comparison with diabetic control.

The effects of dioxidovanadium (V) complex on GLUT 1 and 4

Figure 6: Shows the transmission electron microscopy (TEM) assessment of 5 µm heart sections.

The effects of dioxidovanadium (V) complex on myocardial mitochondria structure (TEM)

Figure 6 Depicts mitochondria in Sprague Dawley rat cardiomyocytes. (A) Shows thin longitudinal sections of Sprague Dawley rat myocytes. Mitochondria are longitudinally arranged (green arrow) in the spaces between the myofibrils (blue arrows), often in single file, constituting ~40% of the cell volume. Most mitochondria are cylindrical in shape with a variable diameter and length. (B) Represents the STZ-induced diabetic group and the diagram exhibits larger rounder mitochondria with infrequently extending protrusions (red arrows). These represent fatty acid infusion present in DM. Insulin treatment represented by (C) displays some vascular restitution (green arrow) in comparison to (B) however not complete restitution as there are still remnants of fatty acid infusion present (red arrow). Oral treatment with the dioxidovanadium (V) complex (D) however shows significant vascular structural improvement with more cylindrically shaped mitochondria arranged longitudinally in between myofibrils, (D) also depicts a significant diminution of fatty acid infusion.
STZ-induced male Sprague Dawley rats (B) revealed striking mitochondrial abnormalities, with a 45% decrease of mitochondria volume and an apparent increase of vacuolated mitochondria compared with those of controls (A). Treatment with the dioxidovanadium (V) complex (D) significantly increased mitochondria volume and decreased vacuolation/lipid infusion as compared to the STZ-induced diabetic group (B).

The effects of dioxidovanadium (V) complex on mitochondria volume and number.

Figure 7 shows mitochondria volume percentages in non-diabetic, diabetic, dioxidovanadium (V) complex, and insulin treated groups at the end of the 5-week experimental period. STZ-induced diabetic animals’ mitochondria volume percentages were significantly decreased in comparison with non-diabetic control group. Interestingly, administration of dioxidovanadium (40 mg/kg, p.o) attenuated the diabetic-associated decrease in mitochondria volume percentages and significantly (p<0.05) increased the mitochondria volume percentages by comparison to STZ-induced diabetic control rats at the end of the 5-week experimental period.

Discussion

In our laboratory we have synthesized a novel vanadium complex namely dioxidovanadium(V) complex, cis-[VO2(obz)py](Hobz=2-hydroxyphenyl-1H-benzimidazole and py =pyridine) which includes organic heterocyclic ligands that provide thermodynamic stability and efficient vanadium transport to target tissues, rendering the complex safer, more potent and stable for use [16, 17]. The effects of this complex have been tested on glucose homeostasis and liver function and have proven most effective in lowering blood glucose and improving glycaemic control in STZ-diabetic rats [16, 17]. However, the effects of dioxidovanadium (V) complex on the metabolic profile of cardiomyocytes has not yet been established. The current study therefore investigates the effects of the novel dioxidovanadium (V) complex on the metabolic profile of cardiomyocytes in an STZ-induced rodent model. Mechanisms underlying the progression and the development of diabetic cardiomyopathy have been widely documented and involve various multifactorial influences [18]. Hou et al., (2017) states that in DM, hyperglycaemia, hyperinsulinemia, and hyperlipidaemia are central to cellular and molecular changes that induce cardiac metabolic inflexibility and cardiovascular dysfunction. Vanadium compounds have been shown to improve cardiovascular function by managing hyperglycaemia and its adverse effects on cardiovascular metabolism, however the mechanisms by which these changes come to pass have not yet been elucidated [19]. This study therefore explores the effects of the dioxidovanadium (V) complex on various metabolic structures and pathways in efforts to establish various mechanisms by which vanadium improves myocardial metabolism [19, 20]. Previous studies suggest the vanadium lowers hyperglycaemia via the recruitment of GLUT 4 vesicles to the plasma membrane facilitating myocardial cellular glucose entry [20]. Furthermore vanadium administration has been shown increase protein tyrosine phosphatase (PTPase) phosphorylation, glucose transport and oxidation by stimulating the insulin receptor henceforth tyrosine kinase activity leading to the downstream events that occur in the insulin signalling pathway [19, 20]. Cardiac muscle metabolism has been shown to be one of the key components responsible for regulating essential cardiac function [21]. To maintain appropriate metabolism the cardiac muscle uses both lipids and glucose for the efficient production of ATP[21]. Cardiac muscle glucose entry is facilitated by two glucose transporters namely GLUT 1 and GLUT 4, however GLUT 4 is predominant in the healthy adult heart [2, 24]. Chronic hyperglycaemia and hyperlipidaemia as a result of DM, have previously been documented to cause calamitous effects on cardiac muscle metabolism resulting in impaired cardiovascular function [22]. This occurs via disruptions in the physiological metabolic profile of the heart leading to an increase in lipid production as shown in (Fig 2) of this study [23]. The lipid over-infusion observed in cardiomyocytes causes the repression
and downregulation of GLUT 1 and 4 gene expression, affecting cardiac metabolism [21, 25]. Regulating hyperglycaemia and hyperlipidaemia is therefore imperative in ameliorating cardiovascular dysfunction in DM [24, 26].

In this study, DM was induced using STZ which selectively destroys the pancreatic beta cells resulting in hyperglycaemia and hyperlipidaemia as shown by significantly elevated glucose and lipid profile in the STZ-induced diabetic animals in comparison to the non-diabetic group (see Fig 1 and 2) [17, 24]. The elevated blood glucose concentration and alterations to the lipid profile of STZ-diabetic animals was associated with the significant reduction in the protein expression of GLUT 1 and GLUT 4 transporters in comparison to that of non-diabetic animals. This led to a decrease in GLUT 1 and 4 transporter formation and the significant reduction in cardiomyocyte glucose [24, 26]. This is due to the hyperglycaemia-induced metabolic inflexibility which causes the predominant hyperlipidaemia and alter the cardiac muscle metabolic substrate preference to lipids over glucose causing lipid deposition, cardiac metabolic instability, and a low ATP production quota [27]. Treatment with insulin and the dioxidovanadium (V) complex however ensued a significant decrease in lipid and blood glucose concentrations from weeks 3 to 5 of the study in comparison to the diabetic animals (see Table 1 and Fig 1). This was associated with an increase in the expression of GLUT 1 and GLUT 4, respectively (see Fig 5). The improved expression of GLUT 1 and GLUT 4 may be attributed to the dioxidovanadium (V) complex’s insulin mimetic action as it acts on the insulin receptor and various substrates such as p38K in the insulin signalling pathway [23, 24, 27]. The insulin mimetic action leads to a significant decrease in hyperglycaemia and hyperlipidaemia resulting in the significant increase of GLUT 1 and 4 gene expression and transporter proteins [23, 24, 27]. This causes an increase in cardiomyocyte glucose entry and the improvement in ATP production [27, 28]. Furthermore, the continuous cardiac metabolic inflexibility observed in the STZ-induced diabetic group resulted in the significant destruction of mitochondrial structure and the decline in mitochondrial volume and number [28]. This occurs via mitochondrial damage due to increased lipid in fusion and the excessive activation of the electron transport chain (ETC) [25, 28]. This causes injury to mitochondrial structures and a decline in mitochondrial volume and number [25, 26, 28]. In our study myocardial mitochondrial structure, volume and number were evaluated using TEM. As anticipated, there were significant alterations in the mitochondrial structure, with a significant decrease in mitochondrial volume and number in the STZ-induced diabetic group in comparison to the non-diabetic group (see Fig 6 and 7). This is due to vacuolation from the prevailing hyperlipidaemia caused by hyperglycaemia-induced metabolic inflexibility which results in impaired β-oxidation and the accumulation of triglycerides (see Fig 2) [27, 28]. However, upon treatment with the dioxidovanadium (V) complex, significant improvements in mitochondrial structure, volume and number were observed (see Fig 6 and 7). This can be attributed to the complex’s ability to manage hyperglycaemia in turn reducing the hyperglycaemic insult to the mitochondria by decreasing lipid infusion, vacuolation and unnecessary ETC stimulation [28]. This, therefore, reinstates the physiological function of the mitochondria and myocardial β-oxidation resulting in a significant decrease in triglycerides and a subsequent increase in ATP production [28, 29]. Unfortunately, the lack of GLUT 1 and 4 as well as the impaired mitochondria structure lead to diminished enzyme function in the glycolysis and citric acid cycle metabolic pathways [27-29]. This impairment is distinctly marked by the plunger intake of pyruvate kinase, acetyl-coA synthetase and ATP synthase rate limiting enzymes [29]. These enzymes play a significant role in fulfilling the ATP quota and ramifications to these pathways cause a significant decline in ATP production [29]. To confirm this observation pyruvate kinase, acetyl-coA synthetase, ATP synthase were measured using assays and an ELISA. Indeed, the activities of rate limiting enzymes PK, acetylcoA synthetase and ATP synthase were significantly lower in the STZ-induced diabetic group in comparison to those of the non-diabetic group (Fig 2, 3, 4). This is due to hyperglycaemia affecting cardiovascular glucose entry and lipid utilization which alters the glycolytic and the citric cycle enzyme activities as well as intermediate substrates production, leading to a decline in ATP and cardiomyocyte injury [29]. However, treatment with the dioxidovanadium (V) complex resulted in a significant increase in the concentrations of these enzymes in comparison to the STZ-diabetic control (see Fig 3, 4, 5). This suggests that the complex played a significant role in reducing the hyperglycaemic injury henceforth restoring enzyme function, improving cardiovascular metabolism and overall ATP production [30]. Furthermore, in this study, we have provided direct evidence that dioxidovanadium (V) complex administration can alleviate metabolic abnormalities associated with hyperglycaemia, significantly lowering hyperglycaemia, upregulating GLUT 1 and 4 gene expression and improving important enzyme function in metabolic pathways. These changes reinstated ATP production, and cardiomyocyte function and therefore lowered the developmental risk of DCM, CVD, and heart failure in STZ-induced diabetic dioxidovanadium (V) complex-treated rats.

**Conclusion**

In summary, we have found that the novel dioxidovanadium (V) complex exhibits cardioprotective effects in STZ-induced diabetic male Sprague Dawley rats by improving cardiomyocyte metabolism through the physiological enhancement of important metabolic enzyme activities, upregulating myocardial GLUT 1 and 4 expression as well as the improvement in mitochondrial structure, volume and number. Therefore, due to the observed results of the current study the dioxidovanadium (V) complex could be a vital hypoglycaemic agent in the management of CVD and DM.

**Ethics approval and consent to participate**

All animal experimentation was reviewed and approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal, South Africa.
The effects of dioxidovanadium (V) complex on cardiovascular metabolism in STZ-induced diabetic male Sprague Dawley rats

Natal (AREC/054/017D). The animals were monitored for pain, discomfort and distress using the criteria listed in the university’s Animal Research Ethics Committee’s humane endpoint document.

Author Disclosure Statement

Nombuso Xulu: I have nothing to disclose
Phikelelani Ngubane: I have nothing to disclose
Andile Khathi: I have nothing to disclose
Ntethelelo Sibiya: I have nothing to disclose
Patrick Mangundu: I have nothing to disclose

Data Availability

The datasets used and/or analysed during the current study are available from the correspond author on reasonable request.

Conflicts of Interest

The authors declare that they have no competing interests.

Funding Statement

NRF and CHS Funded- Funding was used to acquire animals (Sprague Dawley rats), equipment and machinery to perform analytical techniques (Blood analysis and ELISA’s).

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