RETRACTED: Efficacy of Vanadyl Sulfate and Selenium Tetrachloride as Anti-Diabetic Agents against Hyperglycemia and Oxidative Stress Induced by Diabetes Mellitus in Male Rats

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Abstract: The use of metals in medicine has grown in popularity in clinical and commercial settings. In this study, the immune-protecting effects and the hypoglycemic and antioxidant activity of vanadyl sulfate (VOSO₄) and/or selenium tetrachloride (Se) on oxidative injury, DNA damage, insulin resistance, and hyperglycemia were assessed. Fifty male albino rats were divided into five groups, and all treatments were administered at 9:00 a.m. daily for 60 successive days: control, STZ (Streptozotocin; 50 mg/kg of STZ was given to 6 h fasted animals in a single dose, followed by confirmation of diabetic state occurrence after 72 h by blood glucose estimation at >280 mg/dl), STZ (Diabetic) plus administration of VOSO₄ (15 mg/kg) for 60 days, STZ (Diabetic) plus administration of selenium tetrachloride (0.87 mg/Kg), and STZ plus VOSO₄ and, after 1/2 h, administration of selenium tetrachloride at the above doses. The test subjects’ blood glucose, insulin hormone, HbA1C, C-peptide, antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, myeloperoxidase, and xanthine oxidase), markers of lipid peroxidation (MDA), and histological sections of pancreatic tissues were evaluated, and a comet assay was performed. Histological sections in pancreas tissues were treated as indicators of both VOSO₄ and selenium tetrachloride efficacy, either alone or combined, for the alleviation of STZ toxicity. The genotoxicity of diabetes mellitus was assessed, and the possible therapeutic roles of VOSO₄ or selenium tetrachloride, or both, on antioxidant enzymes were studied. The findings show that the administration of VOSO₄ with selenium tetrachloride reduced oxidative stress to normal levels, lowered blood glucose levels, and elevated insulin hormone. Additionally, VOSO₄ with selenium tetrachloride had a synergistic effect and significantly decreased pancreatic genotoxicity. The data clearly show that both VOSO₄ and selenium tetrachloride inhibit pancreatic and DNA injury and improve the oxidative state in male rats, suggesting that the use of VOSO₄ with selenium tetrachloride is a promising synergistic potential ameliorative agent in the diabetic animal model.

Keywords: diabetes mellitus; vanadyl sulphate; selenium tetrachloride; oxidative stress

1. Introduction

Diabetes is a metabolic disease characterized by insulin resistance and β-cell dysfunction [1]. Diabetes mellitus is caused by an insulin secretion dysfunction and the activation of oxidative injury [2]. A major marker of type II diabetes mellitus is dysfunction of the β-cells. Type II diabetes mellitus is a systemic, complex, chronic disease. Its prevalence has been growing over the last decades, due to increased obesity. Type II diabetes mellitus is accompanied by an elevated risk for several cardiovascular diseases, with heart failure as a more common initial presentation rather than myocardial infarction [3]. Better therapies are needed to improve insulin sensitivities and promote the survival and regeneration of pancreatic β-cells to improve the health of diabetic patients without inducing side effects such as hepatotoxicity, cardiotoxicity, and pancreatic tissue inflammation.
Type II diabetes mellitus patients are at high risk of cardiac stroke compared to the general population. A large meta-analysis showed kidney disease and cardiovascular diseases, as demonstrated by hazards for cardiovascular mortality, are higher among Type II diabetic patients throughout the whole spectrum of diseases. Such a marked cardiovascular risk significantly modifies the outcome of diabetic patients who often do not survive long enough to reach the natural fate of end-stage kidney disease. The importance of a multifactorial approach in type II diabetes mellitus has been emphasized by the analysis of Swedish National Diabetes and gender matched controls. In particular, type II diabetes mellitus patients with five risk-factors within target range showed either a small or any excess risk of death as compared with controls [4].

Antioxidants have been used excessively in diabetic experimental models to enhance the effects of free radical accumulation. Diabetes mellitus is characterized by vascular pathology that leads to vascular complications. Diabetes mellitus is also characterized by a lack of insulin [5].

Vanadium compounds act as insulin mimetics and have, since the 1970s, been known to be biologically important. Their physiological effects have been studied for their hypoglycemic action, and vanadium may act as a therapeutic agent against diabetes mellitus [6]. Vanadium has been extensively studied for its diabetes-fighting properties. Vanadium salts have a very low oral bioavailability, which lengthens their half-life due to bidentate coordination. This effect improves vanadium absorption and stability, resulting in a positive antidiabetic effect [6].

Vanadium supplementation in the diet occurs mainly as V^{4+}. Vanadium ingested via the stomach also exists as V^{4+}, which affects the absorption of the ingested vanadium. The development of metal-based drugs with vanadium may be of interest for its pharmacological properties [7]. VOSO_{4} has an insulin-mimetic effect and may, like insulin, have sensitizing properties, as indicated in previous studies.

Diabetes mellitus was induced using STZ in experimental rats, as previously reported. As Vanadyl salts possess insulin-mimicking effects in the body, vanadium may be an alternative treatment for diabetic patients [8]. Vanadyl is able to induce hypoglycemia, as documented previously [9]. Studies of vanadyl salts primarily focused on improving insulin sensitivity and treatment for diabetes [10].

Micronutrients are dietary minerals required by the body in a very small quantity. They may interact with xenobiotics at several sites during absorption and excretion [11]. Selenium tetrachloride plays a vital role in human health and is a basic nutrient for humans; it is a vital microelement of antioxidant enzymes, including glutathione peroxidase (GPx). Selenium tetrachloride exhibits protective effects against symptoms and side effects, and studies have verified the essential role of selenium tetrachloride in the preservation of the metabolic and endocrine functions of the human body. It is vital for all cellular processes [14].

Selenium tetrachloride is an essential trace element for the maintenance of immune–endocrine function. However, its effect on glucose metabolism has not been well studied. Chronic hyperglycemia may produce reactive oxygen species (ROS) and induce oxidative stress, leading to diabetes-related pathological complications and a decline in Se levels. It has been suggested that patients with type II diabetes mellitus could benefit from supplementation with selenium tetrachloride, as it may affect glucose homeostasis and insulin sensitivity [15].
In the study presented herein, I sought to estimate the impact of vanadyl sulfate (VOSO₄) and/or selenium tetrachloride on alleviating oxidative stress and hyperglycemia induced by experimental diabetes mellitus.

2. Materials and Methods

2.1. Chemicals and Analyses

I purchased all chemicals, including VOSO₄, selenium tetrachloride, and STZ, from Sigma-Aldrich. All chemicals were used without purification.

2.2. Experimental Animals

Fifty mature male rats (two months old), weighing 170–180 g each, were kept in sensitized metal cages with free access to food and water in a room maintained at a temperature of 25 °C ± 2 °C with a 12 h light/dark cycle. The rats were obtained from the King Fahd Center for Medical Research (King Abdulaziz University, Jeddah, Saudi Arabia). The experimental protocol was approved by the Deanship of Scientific Research at Taif University Ethical Committee, encoded with approval number 40-31-0142, and in accordance with Animal Research: Reporting of In Vivo Experiments Guidelines (Kilkenny et al., 2010) [16]. Rats were sacrificed under ketamine/xylazine anesthesia, and efforts were made to reduce stress and pain.

2.3. Animals and Experimental Design

Fifty Wistar male Albino rats weighing 170–180 g each were divided into five groups for 60 days. Animals that were involved in the experiment and were expected to become diabetic rats were fed high-fat diets for about 3 weeks before the start of the experiment, based on a previous study [17]; then, experimental diabetes mellitus was induced by a single injection of STZ to mimic type 2 diabetes, which is affected greatly by high-fat diets and the mode of nutrition before the incidence of diabetes mellitus disease. Group I, the control group, was administered physiological saline by intraperitoneal (IP) injection. Group II, the STZ group, was given a single dose of STZ (50 mg/kg) by intraperitoneal (IP) injection [17]. Group III was a diabetic group given STZ and orally administrated 15 mg/kg of VOSO₄ [18]. Group IV was a diabetic group given an oral administration of 0.87 mg/kg of selenium tetrachloride, according to the method described by Johri et al. [19]. Group V was diabetic and was treated orally with both VOSO₄ and selenium tetrachloride successively, as shown in the experimental protocol (Figure 1).

2.4. Experimental Induction of Diabetes Mellitus

Freshly prepared STZ dissolved in PBS (Ph = 4.5) was used to induce diabetes. STZ at 50 mg/kg, freshly prepared in the early morning, was given by IP injection to animals that had fasted for 6 h [20], following the administration of high-fat feed (composed of 66.5% commercial feed, 13.5% artificial butter, and 20% sugar) for the 3 weeks prior to the experimental induction of diabetes mellitus by the single injection of STZ [21]. Seventy-two hours after STZ injection, blood glucose levels were measured to evaluate the diabetic status of the test animals. Subjects with blood glucose levels higher than 280 mg/dL were considered to be diabetic.

2.5. Blood Collection

Using capillary tubes, blood samples were taken from the eye plexus with light anesthesia for biochemical and physiological analyses. The rats were ethically decapitated. Pancreatic tissue samples were kept at −25 °C.
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2.6. Determination of the Blood Glucose Level

Blood glucose levels were evaluated using commercial kits (Bio-diagnostic co.).

2.7. Measurements of Serum Insulin, C-Peptide, and HbA1c

Serum insulin was evaluated using a rat ELISA kit (ALPCO Diagnostics). I used the C-peptide enzyme commercial immune assay (Sigma-Aldrich) and HbA1c kits according to the manufacturers’ protocols.

2.8. Preparation of Pancreatic Tissue Homogenates

A small pancreatic tissue sample was used to estimate the antioxidant biomarkers. Pancreatic tissue was homogenized in 5 mL of cold buffer per gram at 4 $^\circ C$ and centrifuged at 5000 rpm for 30 min; the resulting supernatant was kept at $-20 \, ^\circ C$.

2.9. Determination of Oxidative Stress Markers

Supernatant fluids centrifuged from the pancreatic tissue were used to evaluate myeloperoxidase (MPO) and xanthine oxidase (XO) [22]. Superoxide dismutase (SOD) was determined using the method by Litwack et al. [23]. Pancreas MDA levels were determined using the method by Ohkawa et al. [24]. Catalase (CAT) was determined according to the method by Beers and Sizer [25].

2.10. Single-Cell Gel Electrophoresis (SCGE) (Comet Assay)

Pancreas tissues were placed in a Petri dish with ice solution (Ca$^{2+}$, Mg$^{2+}$ free with EDTA), and the cell viability was determined.

2.11. Histological Analysis of the Pancreas Tissues

The pancreatic tissues were fixed in 10% neutral buffered formalin and were embedded in paraffin, then thin sections were cut and stained with hematoxylin and eosin (H&E) and examined using a light microscope.
2.12. Statistical Analysis

Data were analyzed as the mean ± SEM using the SPSS v.22 program (SPSS Inc., USA) via one-way ANOVA. The significance of mean differences was examined using the post hoc Duncan test [26].

3. Results

3.1. Blood Glucose Level, Insulin Hormone, and Fasting C-Peptide Serum

STZ induced a highly significant and marked increase in blood glucose levels accompanied by a significant reduction in insulin levels and serum fasting C-peptides in the diabetic untreated group as compared to the control group. As shown in Table 1, diabetic rats treated with VOSO₄ and/or selenium tetrachloride exhibited a non-significant increase in blood glucose levels as compared with the STZ group. They also demonstrated a significant decrease in blood glucose levels with elevated insulin hormones and serum fasting C-peptides as compared with the STZ group.

Table 1. Blood glucose level, insulin hormone, HbA1C, and fasting serum C-peptide of male rats treated with VOSO₄ and/or selenium tetrachloride or their combinations.

| Parameters        | Control group | STZ group | STZ plus VOSO₄ group | STZ plus selenium tetrachloride group | STZ plus VOSO₄ and selenium tetrachloride group |
|-------------------|---------------|-----------|----------------------|--------------------------------------|-----------------------------------------------|
| Blood Glucose (mg/dl) | 84.01 ± 3.25 c | 371.25 ± 4.02 a | 140.36 ± 4.03 b | 129.36 ± 2.75 c | 97.25 ± 4.26 a |
| Insulin Hormone (uIU/mL) | 25.36 ± 1.25 a,b | 4.40 ± 0.24 d | 19.36 ± 2.15 c | 20.03 ± 2.11 c | 23.65 ± 2.02 b |
| HbA1C (mmol/mol) | 3.02 ± 0.65 a | 9.51 ± 1.36 a,b | 5.02 ± 1.02 b | 4.01 ± 0.87 c,d | 3.54 ± 0.96 d |
| Fasting Serum C-Peptide (ng/mL) | 4.18 ± 0.69 a | 0.52 ± 0.05 d | 0.52 ± 0.05 d | 2.98 ± 0.87 c | 4.00 ± 0.96 a |

Means within the same column (mean ± SE) carrying different letters are significant at p ≤ 0.05 using Duncan’s multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

3.2. Oxidative Stress Enzyme Biomarkers

Tables 2 and 3 show that STZ induced a significant decrease in CAT, SOD, and glutathione peroxidase (GPx) enzymes while eliciting a highly significant increase in malondialdehyde (MDA), myeloperoxidase (MPO), and xanthine oxidase (XO) levels. Compared with the control group, diabetic rats treated with VOSO₄ and/or selenium tetrachloride, either alone or in combination, showed a marked increase in CAT, SOD, and GPx levels and a decrease in MPO, XO, and MDA levels. The group treated with VOSO₄ in conjunction with selenium tetrachloride presented the best results recorded.

Table 2. Oxidative/antioxidant parameters of antioxidant enzymes in pancreatic tissues of male rats treated with VOSO₄ and/or selenium tetrachloride or their combinations.

| Parameters        | Control group | STZ group | STZ plus VOSO₄ group | STZ plus selenium tetrachloride group | STZ plus VOSO₄ and selenium tetrachloride group |
|-------------------|---------------|-----------|----------------------|--------------------------------------|-----------------------------------------------|
| Pancreatic Catalase (U/g) | 1.88 ± 0.21 a | 0.26 ± 0.10 d | 1.42 ± 0.36 c | 1.63 ± 0.48 b | 1.74 ± 0.22 a |
| Pancreatic SOD (U/g) | 22.05 ± 1.15 a,b | 5.22 ± 1.35 d | 19.91 ± 1.58 c | 20.52 ± 2.16 b | 21.19 ± 2.25 b |
| Pancreatic MDA (U/g) | 3.05 ± 0.48 c | 81.15 ± 0.96 a | 20.42 ± 1.02 b | 12.26 ± 1.45 c | 8.78 ± 1.25 d |
| Pancreatic GPx (U/g) | 34.05 ± 1.85 a | 7.56 ± 1.18 e | 23.15 ± 1.15 d | 26.41 ± 1.28 c | 31.58 ± 1.58 b,c |

Means within the same column (mean ± SE) carrying different letters are significant at p ≤ 0.05 using Duncan’s multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.
Table 3. MPO and XO levels in the pancreatic tissues of male rats treated with VOSO₄ and/or selenium tetrachloride or their combinations.

| Groups                                      | Parameters | MPO (nmol/min/mL) | XO (U/g) |
|---------------------------------------------|------------|-------------------|----------|
| Control group                               |            | 15.16 ± 1.36      | 16.25 ± 1.25 |
| STZ group                                   |            | 25.16 ± 1.15      | 38.55 ± 1.16 |
| STZ plus VOSO₄ group                        |            | 18.24 ± 1.25      | 20.15 ± 1.25 |
| STZ plus selenium tetrachloride group       |            | 17.55 ± 1.19      | 19.65 ± 1.36 |
| STZ plus VOSO₄ and selenium tetrachloride group |     | 16.48 ± 2.16      | 17.16 ± 1.39 |

Means within the same column (mean ± SE) carrying different letters are significant at $p \leq 0.05$ using Duncan’s multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

3.3. Histological Examination

Figure 2: Photomicrographs of the pancreas showing (A) normal pancreatic parenchyma and normal appearance of islets of Langerhans; (B) the STZ-treated group, showing very reduced islets of Langerhans; (C) the STZ plus VOSO₄ group, showing high restoration of detached pancreatic parenchyma with mild-sized islets of Langerhans; (D) the STZ plus selenium tetrachloride group, showing normal pancreatic parenchyma with partially mild islets of Langerhans; and (E) the STZ plus VOSO₄ and selenium tetrachloride group, showing intact pancreatic parenchyma with enlarged islets of Langerhans.

Figure 2. Photomicrograph of pancreas showing (A) normal pancreatic parenchyma and normal appearance of Islets of Langerhans (black arrow) (H&E X400). (B) STZ treated group showing detached pancreatic parenchyma with reduction of islets of Langerhans (black arrow) (H&E X400). (C) STZ plus VOSO₄ group showing high restoration of the detached pancreatic parenchyma with mild sized islet of Langerhans (black star) (H&E X400). (D) STZ plus selenium tetrachloride group showing normal pancreatic parenchyma with appearance very little islet of Langerhans (black star) with smaller size than diabetic group treated with VOSO₄ (H&E X400). (E) STZ plus VOSO₄ and selenium tetrachloride group showing intact pancreatic parenchyma with enlarged islet of Langerhans (black star) which is the better effect (H&E X400).
3.4. Comet Assay

Figure 3. Comet images of cells derived from the pancreatic tissues. (A) Control group showed intact nuclei with normal round cell. (B) STZ group showed higher degree of damage with the presence of a lot of apoptotic cells. (C) STZ plus VOSO₄ group showing some of the intact nuclei with some apoptotic cells. (D) STZ plus selenium tetrachloride group showed less DNA damage, which is confirmed by a less damaged nuclei. (E) STZ plus VOSO₄ and selenium tetrachloride group showed less damaged DNA strands and less damaged nuclei.

Figure 3. Comet images of cells derived from the pancreatic tissues. (A) Control group showed intact nuclei with normal round cell. (B) STZ group showed appearance of a lot of apoptotic cells (white head arrow) with large tail shadow in the form a comet-like shape. (C) STZ plus VOSO₄ group showing restoration of more intact nuclei (white star). (D) STZ plus selenium tetrachloride group clarified less damaged DNA strands (white arrow). (E) STZ plus VOSO₄ and selenium tetrachloride group which showed less damaged DNA strands and less damaged nuclei and more intact nuclei (white arrow).
4. Discussion

I sought to compare the benefits of VOSO$_4$ and selenium tetrachloride administration on STZ tissue injury by examining VOSO$_4$ and/or selenium tetrachloride treated diabetic groups. To my knowledge, research on the co-effect of VOSO$_4$ and selenium tetrachloride on STZ toxicity at the biochemical and histopathological levels in the pancreatic tissue of rats has not previously been conducted.

The ability of VOSO$_4$ in combination with selenium tetrachloride to counteract STZ-induced pancreatic toxicity in male rats was investigated in this study. Exposure to VOSO$_4$ and/or selenium tetrachloride in the STZ diabetic groups led to altered endocrine function and biochemical changes.

Concomitant administration of VOSO$_4$ and/or selenium tetrachloride with STZ significantly protected most of the biochemical variables altered by STZ, which suggests its efficacy as a protectant. The structure of the pancreatic tissues induced by STZ also improved due to these two antioxidant compounds and metals.

The untreated diabetic rats presented a marked increase in blood glucose levels. The results are in complete agreement with the previous literature [27] stating that STZ action is accompanied by severe hyperglycemia in β-cells. Hyperglycemia results in reduced insulin levels [28], and these changes include β-cell abnormalities.

Meanwhile, the diabetic groups treated with VOSO$_4$ exhibited hypoglycemia with insulin levels similar to those in previous studies [29]. This confirmed that the oral administration of vanadyl sulfate improved blood glucose levels and increased insulin sensitivity in type II diabetes, reducing (HbA1c) glycosylated hemoglobin while hepatic glucose was suppressed.

Vanadium compounds have been studied as potential therapeutic agents for the treatment of various major diseases. The translation of vanadium-related complexes and compounds into treatments for diseases, especially diabetes mellitus, remains unconfirmed due to the absence of a basic pharmacological understanding of these compounds [30].

VOSO$_4$ can be explained by examining vanadium’s effect on insulin in three critical areas [31]: (a) in the insulin-signaling network, where insulin receptors and insulin receptor substrates are affected by protein tyrosine phosphatases as +ve modulation and -ve regulators; (b) phosphoinositide protein kinases and mTOR as a +ve signal to protein kinases and as a -ve modulator; and (c) insulin receptors and mitogen-activated protein kinase with its own -ve modulations. Signaling loss implies a disturbance in the biological action of the insulin hormone. These findings were confirmed by previous studies on the administration of vanadium and other salts, which showed similar expression of insulin receptors when only using vanadium in low dosages.

Vanadium complexes can impact the healthy and the ill; therefore, vanadium salts may have multiple applications. The similarity between vanadate and phosphate allows for ease of phosphorylation in many signaling pathways. Additionally, vanadium salts are reported to mimic or enhance insulin [32]; these findings were confirmed in this study.

Oxidative stress is related to the decline in insulin secretion, and pancreatic tissues are more susceptible to oxidative stress because of their low antioxidant enzyme levels [33]. The treatment applied in our study elevated the antioxidant enzyme levels significantly when compared with those in STZ-treated groups, indicating that the current results support the previously obtained results. VOSO$_4$ is able to improve insulin resistance and restore high blood glucose levels to normal levels, as reported by Pepato et al. [32].

Oxidative stress and the accumulation of oxidative stress markers related to free radicals are the most deleterious effects of diabetes on cellular activities. The results showed that the combination of VOSO$_4$ and selenium tetrachloride successfully scavenged excess free radicals. As a result, lipid peroxidation marker (MDA) levels declined and the antioxidant enzyme capacities of CAT, SOD, and GPx increased, which improved hepatic function and improved blood glucose levels [30].

The current findings are in complete agreement with the findings of Treviño et al. [30], who confirmed that treatment of diabetic rats with vanadyl complexes in high doses elicited
a significant decline in MDA levels and resulted in significant improvement in antioxidant enzyme capacities in pancreatic tissue homogenates.

I observed that increased lipid peroxidation in the STZ (untreated diabetic) group could include oxidative stress [33]. It was found that VOSO₄ combined with selenium tetrachloride protects pancreatic tissues from lipid peroxidation generation and from any changes in CAT, SOD, and MDA (a marker of peroxidation) levels.

STZ impacts the role of oxidative stress on pancreatic β-cells [34]. β-cells have a great affinity for free radicals, causing damage to β-cells and antioxidant enzymes. This effect was confirmed by the deterioration of the antioxidants in the pancreatic tissues [35]. The successively increasing insulin and selenium tetrachloride levels in the VOSO₄ treatment group and amelioration of pancreatic histological structures demonstrated that the β-cell structures under VOSO₄ enriched with selenium tetrachloride have antioxidant properties.

Combining selenium tetrachloride with VOSO₄ mitigated the harmful effects of STZ in all measured parameters. The observed levels were closely related to the normal control values in the nondiabetic group. These results were in accordance with those of Ithayarasi and Devi [36] and El-Demerdash [37], who found that selenium tetrachloride maintained antioxidant levels and antioxidant enzymes at near-normal levels, which enhanced their effects as antioxidants [38].

An important marker for the detection of DNA damage is the comet assay, which can detect lesions on DNA strands to show oxidative injury [39]. I used the comet assay to evaluate the incidence of DNA impairment [40,41]. The assay confirmed that STZ is highly genotoxic to pancreatic tissues. Greater amounts of DNA damage were found in the untreated STZ diabetic group, which may be due to the generation of reactive oxygen species that caused DNA strand delineation. The ROS declined in the diabetic group treated with VOSO₄ and selenium tetrachloride together. Thus, the comet assay confirmed high genotoxicity induced by STZ in the pancreatic tissue homogenates and the appearance of the tail comet of DNA in the STZ diabetic untreated group, whereas the group treated with STZ and VOSO₄ combined with selenium tetrachloride showed intact DNA strands with markedly alleviated DNA damage.

The current findings demonstrate that VOSO₄ and selenium tetrachloride increase insulin concentrations when β-cells have been damaged by STZ. This effect can be partially explained by the vital effect of selenium tetrachloride on insulin signaling. Selenium tetrachloride, as a major component of antioxidant enzymes, can prevent oxidative cellular damage by scavenging ROS. It may also cause a euglycemic balance in ROS production that maintains insulin sensitivity because ROS are also essential for insulin sensitization. There is growing evidence from previous studies on selenium tetrachloride’s potential benefits that should be balanced with its potential harmful effects [42].

5. Conclusions

In this study, experimental diabetes mellitus was induced in the islets of Langerhans; the results demonstrated that VOSO₄ and selenium tetrachloride may be helpful in the treatment of diabetes. The use of metals in enhancing insulin action and reducing cellular inflammation may have beneficial effects. The treatment of diabetes mellitus is challenged by the need to find safer compounds that have antidiabetic potency and can activate the β-cells. The results herein confirmed that the combination of VOSO₄ and selenium tetrachloride is more effective, that it is the best and safest treatment for hyperglycemia without genotoxicity, and that it may alleviate oxidative injury. With further study, this treatment may lead to a novel antidiabetic combination.

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References
1. Wang, M.; Song, L.; Strange, C.; Dong, X.; Wang, H. Therapeutic Effects of Adipose Stem Cells from Diabetic Mice for the Treatment of Type 2 Diabetes. Am. Soc. Gene Cell Ther. 2018, 26, 1921–1930. [CrossRef]
2. Fukunaka, A.; Fujitani, Y. Role of Zinc Homeostasis in the Pathogenesis of Diabetes and Obesity. Int. J. Mol. Sci. 2018, 19, 476. [CrossRef]
3. Palmiero, G.; Cesaro, A.; Vetrano, E.; Pafundi, P.; Galiero, R.; Caturano, A.; Moscarella, E.; Gragnano, F.; Salvatore, T.; Rinaldi, L.; et al. Impact of SGLT2 Inhibitors on Heart Failure: From Pathophysiology to Clinical Effects. Int. J. Mol. Sci. 2021, 22, 5863. [CrossRef]
4. Sasso, F.C.; Pafundi, P.C.; Simeon, V.; De Nicola, L.; Chiodini, P.; Galiero, R.; Caturano, A.; Moscarella, E.; Gragnano, F.; Salvatore, T.; Rinaldi, C.; et al. Efficacy and durability of multifactorial intervention on mortality and MACEs: A randomized clinical trial in type-2 diabetic kidney disease. Sasso et al. Cardiovasc. Diabetol. 2020, 20, 145. [CrossRef]
5. Hamza, R.Z.; El-Megharbel, S.M.; Altalbi, T.; Gobouri, A.A.; Alrogi, A.A. Hypolipidemic and hepatoprotective synergistic effects of selenium nanoparticles and vitamin E against acrylamide induced hepatic alterations in male albino mice. Appl. Organomet. Chem. 2020, 34, e5458. [CrossRef]
6. Pessoa, J.C. Thirty years through vanadium chemistry. J. Inorg. Biochem. 2015, 147, 4–24. [CrossRef] [PubMed]
7. Crans, D.C. Antidiabetic, Chemical, and Physical Properties of Organic Vanadates as Presumed Transition-State Inhibitors for Phosphatases. J. Org. Chem. 2015, 80, 11899–11915. [CrossRef]
8. Domingo, J.L.; Gómez, M. Vanadium compounds for the treatment of human diabetes mellitus: A scientific curiosity? A review of thirty years of research. Food Chem. Toxicol. 2016, 95, 137–141. [CrossRef] [PubMed]
9. Ghasemi, F.; Rezvani, A.R.; Ghasemi, K.; Graiff, C. Glycine and metformin as new counter ions for mono and dinuclear vanadium (IV) complexes. J. Inorg. Biochem. 2011, 105, 1345–1352. [CrossRef]
10. Carpéne, C.; Garcia-Vicente, S.; Serrano, M.; Marti, L.; Belles, C.; Royo, M.; Galitzky, J.; Zorzano, A.; Tastar, X. Insulin-mimetic compound hexaquis (benzylammonium) decavanadate is antilipolytic in human fat cells. J. Mol. Struct. 2018, 1154, 319. [CrossRef]
11. Al-Harbi, M.S.; Hamza, R.Z. Potential Ameliorative Effects of Selenium and Chromium Supplementation Against Toxicity and Oxidative Stress in Streptozotocin Diabetic Rats. Int. J. Pharmacol. 2016, 12, 483–495. [CrossRef]
12. Abuelzahab, H.; Hamza, R.; Montaser, M.; El-Mahdi, M.M.; Al-Harthi, W.A. Antioxidant, antiapoptotic, antigenotoxic, and hepatic ameliorative effects of L-carnitine and selenium on cadmium-induced hepatotoxicity and alterations in liver cell structure. Int. J. Mol. Sci. 2018, 19, 1154–1168. [CrossRef]
13. Wei, J.; Zeng, C.; Gong, Q.Y.; Yang, H.B.; Li, X.X.; Lei, G.H.; Yang, T.B. The association between dietary selenium intake and diabetes: A cross-sectional study among middle-aged and older adults. Nutr. J. 2015, 14, 18. [CrossRef]
14. Tsave, O.; Yavropoulou, M.; Kafantari, M.; Gabriel, C.; Yovos, J.; Salifoglou, A. Comparative assessment of metal-specific adipogenic activity in zinc and vanadium-citrates through associated gene expression. J. Inorg. Biochem. 2018, 186, 217–227. [CrossRef]
15. Hamza, R.Z.; Al-Motaan, S.E.; Malik, N. Protective and Antioxidant Role of Selenium Nanoparticles and Vitamin C Against Acrylamide Induced Hepatotoxicity in Male Mice. Int. J. Pharm. 2019, 15, 664–674. [CrossRef]
16. Kilkenny, C.; Browne, W.J.; Cuthill, I.C.; Emerson, M.; Altman, D.G. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. PLOS Biol. 2010, 8, e1000412. [CrossRef]
17. El-Meghrab, S.M.; Hamza, R.Z.; Gobouri, A.A.; Refat, M.S. Synthesis of new antidiabetic agent by complexation between vanadyl (II) sulfate and vitamin B1: Structural, characterization, anti-DNA damage, structural alterations and antioxidative damage studies. Appl. Organomet. Chem. 2019, 33, e4892. [CrossRef]
18. El-Meghrab, S.M.; Hamza, R.Z.; Refat, M.S. Synthesis, spectroscopic, structural and thermal characterizations of vanadyl (IV) adenine complex prospective as anti-diabetic drug agent Spectrochim. Acta Part A 2015, 135, 850. [CrossRef]
19. Johri, S.; Shukla, S.; Sharma, F. Role of chelating agents and antioxidants in beryllium induced toxicity. Indian J. Exp. Biol. 2002, 40, 575–582. [CrossRef]
20. Litwack, G.; Bothwell, J.W.; Williams, J.N.; Elvehjem, C.A. A Colorimetric Assay for Xanthine Oxidase in Rat Liver Homogenates. J. Biol. Chem. 1953, 200, 303. [CrossRef]
21. Furman, B.L. Streptozotocin-induced diabetic models in mice and rats. Curr. Protoc. Pharmacol. 2015, 70, 5–47. [CrossRef]
22. Suzuki, H.; Ota, S.; Sasagawa, T.; Sakatani, T.; Fujikura, Assay method for myeloperoxidase in human polymorphonuclear leukocytes. *Anal. Biochem.* 1983, 132, 345. [CrossRef]

23. Litwack, G.; Bothwell, J.W.; Williams, J.N.; Elvehjem, C.A. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *J. Biol. Chem.* 1953, 200, 303. [CrossRef]

24. Ohkawa, H.; Ohishi, N.; Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 1979, 95, 351. [CrossRef]

25. Beers, J.R.; Sizer, I.W. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* 1952, 195, 133. [CrossRef]

26. Snedecor, G.W.; Cochran, W.G. *Statistical Methods*, 6th ed.; Oxford & IBH Co.: Bombay/New Delhi, India, 1967.

27. Hamza, R.Z.; Al-Baqami, N.M.; Khojah, E.; Mansour, A.M.A.; Al-Motaani, S.E.; Al-Salmi, F.A.; El-Megharbel, S.M. Possible Antioxidant and Antidiabetic Effects of Combretum Molle Extract in a Diabetes Mellitus Experimental Model in Male Rats. *Nat. Prod. Commun.* 2021, 16, 1–10.

28. West, E.; Simon, O.R.; Morrison, E.Y. Streptozotocin alters pancreatic beta-cell responsiveness to glucose within six hours of injection into rats. *West Indian Med. J.* 1996, 45, 60.

29. Treviso, S.; Díaz, A. Vanadium and insulin: Partners in metabolic regulation. *J. Inorg. Biochem.* 2020, 208, 111094. [CrossRef]

30. El-Megharbel, S.M.; Al-Salmi, F.A.; Al-Harthi, S.; Alsalami, K.; Hamza, R.Z. Chitosan/Selenium Nanoparticles Attenuate Dielofenac Sodium-Induced Testicular Toxicity in Male Rats. *Crystals* 2021, 11, 1477. [CrossRef]

31. El-Megharbel, S.M.; Al-Salmi, F.A.; Refat, M.S.; Hamza, R.Z. Selenium/Chitosan-Folic Acid Metal Complex Ameliorates Hepatic Damage and Oxidative Injury in Male Rats Exposed to Sodium Fluoride. *Crystals* 2021, 11, 1354. [CrossRef]

32. Steibrenner, H.; Sies, H. Protection against reactive oxygen species by selenoproteins. *Biochim. Biophys. Acta* 2009, 1790, 1478–1485. [CrossRef]

33. Refat, M.S.; Hamza, R.Z.; Adam, A.M.A.; Saad, H.A.; Gobouri, A.A.; Al-Harbi, F.S.; Al-Salmi, F.A.; Althalhi, T.; El-Megharbel, S.M. Quercetin/Zinc complex and stem cells: A new drug therapy to ameliorate glycometabolic control and pulmonary dysfunction in diabetes mellitus: Structural characterization and genetic studies. *PLoS ONE* 2021, 16, e0246265. [CrossRef] [PubMed]

34. Stróżyk, A.; Osica, Z.; Przybyłak, J.D.; Kołodziej, M.; Zalewski, B.M. Effectiveness and safety of selenium supplementation for type 2 diabetes mellitus in adults: A systematic review of randomised controlled trials. *J. Hum. Nutr. Diet.* 2019, 32, 635–645. [CrossRef] [PubMed]

35. Hamza, R.Z.; Diab, A.E.A.A. Testicular protective and antioxidant effects of selenium nanoparticles on Monosodium glutamate-induced testicular structure alterations in male mice. *Toxicol. Rep.* 2020, 7, 254–260. [CrossRef] [PubMed]

36. Kida, K.; Utsuyama, M.; Takiwaa, T.; Thurlbeck, W.M. Changes in lung morphologic features and elasticity caused by streptozotocin-induced diabetes mellitus in growing rats. *Am. Rev. Respir. Dis.* 1983, 128, 125–131. [CrossRef]

37. Parkman, J.K.; Sklioutovskaya-Lopez, K.; Menikdiwela, K.R.; Freeman, L.; Moustaid-Moussa, N.; Kim, J.H. Effects of high fat diets and supplemental tarr cherry and fish oil on obesity and type 2 diabetes in male and female C57BL/6j and TALLYHO/Jng mice. *J. Nutr. Biochem.* 2021, 94, 108644. [CrossRef] [PubMed]