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Original Article

Changes in Oxidative Stress and Antioxidant Status in Streptozotocin-Induced Diabetic Wistar Albino Rats: Effects of Oral Zinc Supplementation

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Abstract

A major factor in the development and course of diabetes mellitus is oxidative stress. Overproduction of reactive oxygen species in the presence of concurrent deficient antioxidant mechanisms leads to enhanced oxidative stress. The objective of the current study was to assess the effect of zinc supplementation on oxidative stress and total antioxidant capacity in streptozotocin induced diabetic rats.

Thirty-two male Wistar albino rats were randomly divided into four equal groups: Group 1- the untreated control; group 2- the zinc-supplemented group; Group 3- the diabetic-STZ-induced group; and Group 4- the diabetic-STZ-induced group supplemented with zinc. A single intraperitoneal injection of streptozotocin (55 mg/kg body weight) was used to induce diabetes. All procedures and animal handling procedures were performed according to the Institutional Animal Care and Use Committee. Zinc sulfate was administered via gavage at a dose of 100 mg/kg body weight for 60 days. On the last day of the experiment, the rats were sacrificed under anaesthesia. Protein carbonyl (PC), total antioxidant capacity (TAC), thiobarbituric acid reactive substances (TBARS), and enzyme activities such as those of catalase (CAT) and superoxide dismutase (SOD) were measured in plasma. Comparisons between groups were performed using analysis of variance (ANOVA) for normally distributed variables or Bartlett's test for inequality of population variances. Differences were considered significant at $P<0.05$. Zinc supplementation leads to an increase in the total serum proteins of the antioxidant defense system, and the activities of CAT, SOD and TAC increase. On the other hand, the content of TBARS decreased and the concentration of PC decreased. Zinc supplementation has been shown to have positive effects on antioxidant capacity and glycemic control in diabetic rats. In conclusion, the results of this study demonstrate the beneficial effect of zinc supplementation on oxidative stress in diabetic rats.

Keywords: Antioxidant enzymes, Diabetes mellitus, Oxidative stress, Zinc supplementation.

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1. Introduction

In addition to environmental factors, diabetes mellitus (DM) is a multifactorial disease. Pathophysiological alterations in diabetes are typified by malfunctioning β-cells [1] and inflammation-dependent ROS produced locally

by tissue, and their downstream signalling pathways, which lead to an insufficient response to insulin levels [2, 3].

The disorder known as oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and the capacity of the antioxidant system to neutralize these substances. Damage to tissues results from increased oxidative stress due to increased production of free radicals and/or a reduced capacity to scavenge ROS. The pathophysiology of several diseases, such as vascular and diabetic disorders, is significantly impacted by oxidative stress [4].

Increased production of ROS and other free radicals is a result of hyperglycemia, and numerous studies indicate that complications from diabetes may arise from the increased production of ROS and free radicals. Increased oxidative stress results from the hyperglycemia that is associated with diabetes, which also lowers cellular antioxidant defense capacity and increases ROS production [5, 6].

Reactive oxygen species cause membrane lipid peroxidation, protein aggregation, and apoptosis in epithelial cells. Antioxidants such as glutathione, vitamin E, and ascorbic acid, as well as the enzymes catalase, superoxide dismutase, and glutathione peroxidase, counteract the harmful effects of ROS [7, 8]. Antioxidants also increase the function of the antioxidant defense enzyme system and prevent the production of ROS. Therefore, antioxidants may reduce inflammation, halt the progression of diabetes, and lessen the biological harm caused by oxidative stress [9].

Several animal studies have demonstrated that oxidative stress is caused by sustained high intracellular and extracellular glucose concentrations. In a reaction that is dependent on transition metals, glucose oxidizes to an enediol radical anion. It then undergoes further conversion into reactive ketoaldehydes and superoxide anions, which dismutate into hydrogen peroxide and produce extremely reactive hydroxyl radicals when transition metals are present. Cell redux and zinc levels have an impact on cellular signal transduction pathways. Increased oxidative stress and disturbances in Zn homeostasis have been linked to chronic diseases, such as diabetes mellitus resulting from pancreas ß-cell apoptosis, insulin resistance in type 2 diabetes, and diabetic cardiomyopathy [10, 11].

There is interest in the potential therapeutic health benefits of zinc supplementation because zinc deficiency is common worldwide and there is an obvious link between zinc and redux effects in various biochemical pathways. Zinc supplementation may have positive effects on the consequences of diabetes, such as cardiovascular disease. Zn supplementation may have antioxidant and cardioprotective benefits, and support a healthy lipid profile, according to several studies [12, 13].

Dyshomoeostasis of zinc results from uncompromised diabetes, and chronic zinc deficiency usually causes increased susceptibility to oxidative stress. In diabetic rats, zinc supplementation has been shown to control hyperglycemia and hypoinsulinemia by decreasing metabolic abnormalities, such as protein glycosylation, glucosuria, and proteinuria [14]. The enzymes known as NADPH oxidases, which are linked to the plasma membrane and catalyze the conversion

of oxygen into O2·, are inhibited by zinc. SOD a Cu and Zn-containing enzyme, catalyzes the dismutation of O2· to H2O2. The production of cysteine-rich metallothionein, an efficient ·OH scavenger, is known to be induced by zinc. Studies on zinc deprivation conducted to determine whether zinc plays a physiological role as an antioxidant, have generally shown that zinc deprivation increases an organism's susceptibility to damage caused by a range of oxidative stresses [15, 16].

Zinc is essential for maintaining the proper balance between pro- and antioxidants in cells because it is a component of the active center of the anti-oxidative enzyme superoxide dismutase (SOD) and a factor that lowers the activity of oxidases [17]. Furthermore, this component might increase the expression of genes related to antioxidants and the activation of antioxidant transcription factors [18].

Consequently, the search for a novel approach to both prevent and treat this crippling consequence is still ongoing. In this study, we assessed the possible preventive effects of zinc supplementation on Streptozotocin (STZ)-induced diabetes in rats. In the blood serum of these animals, we investing acted the impact of zinc supplementation on the production of oxidative parameters such as protein carbonyl (PC), thiobarbituric acid reactive substances (TBARS), to talantioxidant capacity (TAC), superoxide dismutase (SOD), and catalase (CAT) activity.

2. Materials and Methods

2.1. Experimental animals

The average weight of the rats was 140 to 200g and the rats were maintained in wire-bottomed cages. Rats had free access to food and water and were fed with a standard diet and kept at 25±2°C and 50–60% humidity, with a 12 hours' light and 12 h. dark cycle. The experimental protocol used was approved by the Institutional Animal Care and Use Committee (university Oran 1, Oran, Algeria; Number of Protocol: UO-CEEA-2016). The general councils concerning the animal experimentation: European Directive 2010/63/EU concerning the protection of animals used for scientific purposes and laws for the welfare of animals, and General guidelines for the care and use of laboratory animals recommended by the Council of the European Communities (1987). The council directive 86/609/EEC was followed strictly.

Thirty-two male Wistar albino rats (Pasteur Institute, Algiers, Algeria), aged 6 to 8 weeks, were randomly assigned to four groups (n=8 per group): (1) the untreated nondiabetic control group (C); (2) the treated nondiabetic group (C-Zn); (3) the untreated diabetic group (D); and (4) the treated diabetic group (D-Zn). Diabetes was induced by intraperitoneal injection of streptozotocin (55mg/kg body weight, dissolved in 0.1M citrate buffer solution pH 4.5). The glucose level was measured after 72 hours using a glucometer (ACCU-CHEK Active, Germany) by loading one drop of blood from a tail vein incision onto a glucometer strip. Rats with a glucose level of 250 mg/dl or higher were classified as diabetic based on the method outlined by Sadri [19].

The animals were administered Zn (zinc sulfate monohydrate ZnSO4.H2O, Sigma-Aldrich, Germany) in the drinking water. Zinc sulfate (1 ml/kg) was given to groups (2) and (4) via gastric gavage at a dose of 100 mg/kg body weight, every day, for 60 days. Zn

supplementation starts 3 days after STZ injection. At the end of the experimental period, the animals were anaesthetized with ether and sacrificed. Blood samples were collected through cardiac puncture. The serum was collected by centrifuging the blood for 10 min at 3000 rpm, and stored at -20°C until use.

2.2 Measurements of lipid peroxidation

2.2.1 TBARS

Lipid peroxidation is based on the reaction of malondialdehyde (MDA) as an end product of the lipid peroxidation process. The levels of MDA, a marker of oxidative stress, were measured by thiobarbituric acid reactive substances (TBARS) according to the methods of Yagi [20]. For the assay, 15g of trichloroacetic acid, and 0.375g of thiobarbituric acid were mixed with 2 ml of HCl. Two millilitres of this mixture were added to 1 ml of serum, and heated at 95°C for 50 min. After incubation the tubes were cooled, and the mixture was centrifuged at 1000rpm for 10min. The absorbance of the coloured layer was measured at 530nm. The TBARS concentration was calculated using an extinction coefficient of 156 mM-1 cm-1 and expressed in nmoles/ mg from the following equation. $C(M)=A/1.56\times10^{5}$.

2.2.2 Carbonyl

Protein carbonyl (PC) levels were measured using dinitrophenylhydrazine (DNPH), a method described by Reznick and Packer [21]. The results are expressed in nmol of protein carbonyl per mg of protein, using a molar extinction coefficient (e-22 000 M/cm) for DNPH.

2.3 Measurements of antioxidant enzyme activities

2.3.1 SOD

SOD activity was determined based on the amount of enzyme required to inhibit photoreduction of nitroblue tetrazolium (NBT), as previously described in a colorimetric assay by the method of Kono [22]. One unit of SOD was defined as the amount of enzyme required to inhibit the photoreduction of NBT by 50%. The results are shown as units per milligram of protein, at 25°C.

2.3.2. CAT

CAT activity was assayed according to Aebi [23] in which CAT activity was measured following the rate of disappearance of peroxide. The reaction mixture (1 ml) included 20 µl of homogenate, 100mM H_2O_2, and 100mM phosphate buffer. The rate of disposal of H_2O_2 at 25°C was measured by the change in absorbance at 240 nm for 1 min. Enzyme activity was expressed as µmol H_2O_2 / mg protein.

2.3.3. Total Antioxidant Capacity

The Total Antioxidant Capacity Assay Kit allows the quantity of small molecule antioxidants to be measured alone or in combination with protein antioxidants. Cu2+ ions are converted to Cu+ by both small molecules and proteins. Nevertheless, the application of the protein mask inhibits the reduction of Cu2+ by proteins, permitting the examination of only small molecule antioxidants.

A broad absorbance peak at 570nm is produced when the reduced Cu+ ion chelates with a colorimetric probe; this peak is proportional to the total antioxidant capacity. Trolox

equivalents, which range from 4 to 20 nmol/well, are used to measure antioxidant capacity. An antioxidant standard was provided by Trolox, a water-soluble vitamin E analogue.

2.4. Statistical analysis

For each group, the data are expressed as the mean±standard error of the mean (SEM). Comparisons across groups were performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test [24] (version 4.1; statsoft, Tulska, Okla). Differences were considered statistically significant at P<0.05.

3. Results and Discussion

3.1. Growth parameters

The blood glucose and body weight data are presented in **Table 1**. Rats in the STZ-induced diabetes group had significantly greater glucose levels than did those in the control group (p<0.01). Glucose levels were 4.2- and 4.1-fold greater in the untreated diabetic group and treated diabetic group, respectively, than in the control group and untreated group. The glucose levels of diabetic rats supplemented with Zn were significantly lower than those of untreated diabetic rats (p<0.05). The rats in the nondiabetic group gained more weight than did those in the diabetic group. The body weights of the diabetic rats were significantly lower than those of the nondiabetic rats (p<0.01).

At the end of the experimental period, the STZ-treated control diabetic rats had significantly greater blood glucose levels (**Figure 1**).

Table 1. Effects of zinc on blood glucose and body weights values in STZ-induced diabetic rats**.**

Parameters	C	C-Zn	D	D-Zn
Blood glucose (mg/dl) baseline	111.8 ±4.26	113.6 ±3.78	111.3 ±3.97	109.2 ±4.31
Blood glucose (mg/dl) at the start of zinc administration	98.7 ±3.22	104.36±5.82	422.17±15.03	431.56±19.23
Blood glucose (mg/dl) at the end of the experiment	102.34±4.85	108.88±7.02	512.17±13.19*	436.28±17.16*,**
Body weight (g) baseline	165.22 ±8.9	176.42 ±4.68	171.27 ±5.35	168.29 ±4.11
Body weight (g) at the start of zinc administration	193.63 ±9.2	196.18 ±6.63	191.85 ±7.31	198.08 ±6.48
Body weight (g) at the end of the experiment	326.54±32.28	356.91±28.31	207.89±26.18*	229.22±17.15*

Data are presented as Mean ± SD; n08, p<0.01 *vs* C and C-Zn; ** p<0.05 *vs* D.

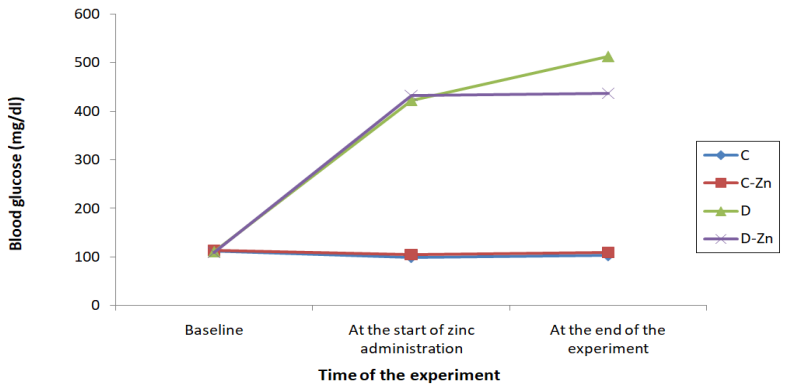

Figure 1. Blood glucose values of rats with Streptozotocin induced diabetes.

Glucose levels were 5- and 4-fold greater in the untreated diabetic group and treated diabetic group, respectively, than in the control group and untreated group. No differences in blood glucose levels were detected in diabetic rats treated with Zn at the end of the experiment (**Figure 1**). The body masses of diabetic rats were significantly lower than those of nondiabetic rats (**Figure 2**). Body masses were 1.57- and 1.55-fold greater in the control group and untreated group, respectively, than in the untreated diabetic group and treated diabetic group. The body weight of diabetic rats treated with Zn was lower than that of nondiabetic rats (**Figure 2**).

3.2. Oxidative stress markers

Our results showed that TBARS levels were 1.4-fold greater in untreated diabetic rats than in control rats (**Table 2**). Furthermore, the TBARS levels were 2.2- and 3.2-fold greater in the control group and untreated diabetic group, respectively, than in the treated diabetic group. However, they were 2.5-fold greater in the control group than in the treated nondiabetic group.

Table 2. TBARS and protein carbonyl content in plasma**.**

	TBARS (µmol/ml)	Carbonyl (nmol/mg)
C	0.391±0.025	3.73±0.5
C-Zn	0.152±0.007**	5.33±1.67
D	0.568±0.021****	16.84±6.44*,#,§
D-Zn	0.176±0.011*,***	12.59±5.64*,#

*p<0.01 *vs* C ; **p<0.01 *vs* C ; ***p<0.01 *vs* D ; ****p<0.05 *vs* C ; #p<0.01 *vs* C-Zn ; §p<0.05 *vs* D-Zn

STZ-induced diabetes provoked a significant increase (P<0.01) in protein carbonyl levels compared to those in the control group. Compared with untreated diabetic rats, diabetic rats treated with Zn exhibited a significant decrease (P<0.05) in protein carbonyl levels. Compared to those in the control group, protein carbonyl levels were 3.4- and 4.5-fold greater in treated diabetic and untreated diabetic rats, respectively. Similarly, compared to those in the treated nondiabetic group, carbonyl levels were 2.3- and 3.1-fold greater in the treated diabetic and untreated diabetic rats, respectively.

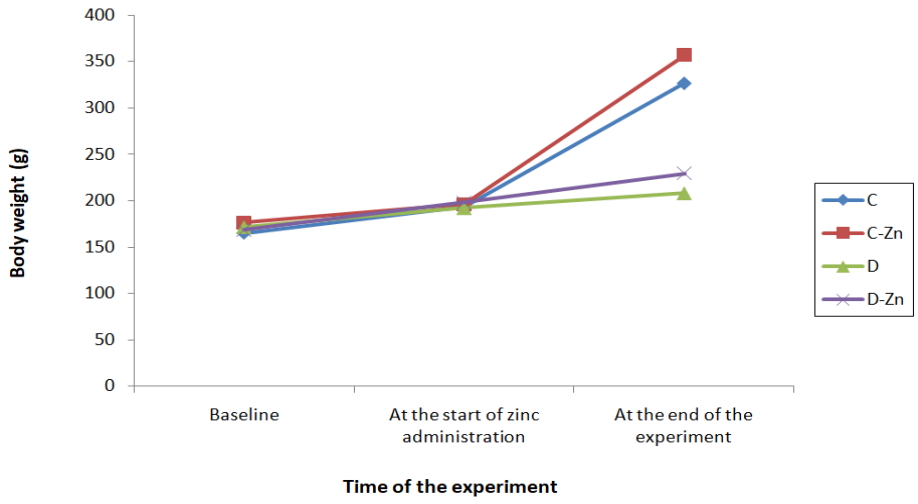

Figure 2. Body weight values of rats with Streptozotocin induced diabetes.

3.3. Antioxidant enzyme activities and total antioxidant capacity (TAC)

A significant decrease in the enzymatic activity of CAT was detected in diabetic animals compared with control animals (**Table 3**). CAT activity was reduced by 66% and 51% in treated diabetic and untreated diabetic rats, respectively, compared with that in controls. Zn supplementation increased enzyme activity by 46% in treated diabetic rats and 26% in treated nondiabetic rats.

Among the antioxidant enzymes, the activity of SOD decreased by 48% as a result of diabetes, in untreated diabetic rats compared with that in control untreated rats (Table 3). Furthermore, SOD activity was 2-fold greater in treated diabetic rats than in untreated diabetic rats. The administration of Zn improved SOD activity, by 77% in treated nondiabetic rats and 98% in treated diabetic rats.

Table 3. The activity of CAT and SOD**.**

	CAT µmol H2O2 /min/mg protein	**SOD units/mg protein**
C	569.77±21.76	147.95±42.92
C-Zn	721.71±39.72	263.07±82.26
D	188.14±34.05*	71.11±11.89***
D-Zn	276.55±35.13**	141.76±52.37****

*P< 0.01 versus C; **p<0.05 versus D; ***P< 0.01 versus C; ****P< 0.01 versus D

The TAC levels in the serum of the rats, supplemented with zinc were 1.9- and 1.8-fold greater in the treated nondiabetic rats and treated diabetic rats, respectively, than in the untreated control rats and untreated diabetic rats. Compared with those in the control group, the TAC levels in the untreated diabetic group were markedly decreased (-50%) (**Table 4**).

Table 4. The impact of zinc on TAC levels**.**

	TAC nmole/UL
C	3.966 ± 0.468
C-Zn	7,465±1,73*
D	1,994±0.34*
D-Zn	3.672 ± 0.43**,***

*P< 0.01 *vs* C; **P<0.05 *vs* D ; ***P< 0.01 *vs* C-Zn

Increased oxidative stress, free radical generation, and accelerated nonenzymatic glycation are all correlated with diabetes mellitus (DM). Reduced antioxidant defense is the outcome of local metabolic alterations caused by hyperglycemia [25]. Since zinc has been shown to regulate oxidative stress, it is possible that zinc is a physiological component of the antioxidant defense system. Zinc deficiency can cause oxidative stress and oxidant-mediated damage to several cell components [26].

Elevated levels of lipid peroxides (LPO) indicate increased lipid peroxidation [27]. On the other hand, an increased concentration of PC indicates oxidative modifications of proteins [28]. Zinc supplementation reduced the elevated levels of LPO and PC, which may indicate that zinc possesses antioxidant and free radical-scavenging properties. The capacity of zinc to compete with the transition metals Fe and Cu for binding sites on the cell membrane is another mechanism that can potentially support the antioxidant function of zinc. Lipid peroxides are produced by the catalysis of Fe and Cu ions, and substituting Zn for these metals in the plasma membrane may prevent LPO in diabetes [14].

PC levels are frequently employed as a biomarker for protein damage induced by oxidative amino acid residues related to stress

[29]. In our study PC levels were significantly greater in diabetic rats. Many studies have shown higher concentrations of PC in diabetic rats [30]. Our results validate the presence of elevated oxidative damage in DM and are consistent with earlier studies. In this work, the administration of zinc to diabetic rats resulted in the regression of oxidative stress, which in turn caused an increase in PC levels. Zinc has both hypoglycemic and antioxidant properties.

An increase in LPO may follow an increase in free radical concentrations. LPO is an indicator of lipid peroxidation. The plasma level of MDA was characterized as an index of lipid peroxidation [31]. Membrane fluidity, membrane-bound enzymes, and intracellular homeostasis can all be impacted by oxidative changes in lipids [32]. Zinc has been shown in numerous studies to have an antioxidant impact and to reduce diabetes by decreasing LPO, and protecting cells from oxidative damage [33]. The results of this investigation showed that the DM group had considerably greater LPO levels (represented as MDA) than did the control group. MDA generation was considerably reduced by Zn. The potential benefits of zinc supplementation in DM were demonstrated by a considerable decrease in plasma thiobarbituric acid reactive substances (TBARS).

The natural antioxidant enzymes SOD and CAT are responsible for eliminating harmful oxygen radicals from the body and are crucial for protecting cells from oxidative damage [34]. Zinc plays a crucial role in enhancing the oxidative state and lowering the rate of lipid peroxidation as a component ingredient of the antioxidant enzyme SOD [35]. Zn, together with copper, forms part of SOD and inhibits nicotinamide adenine dinucleotide phosphate oxidase. It also stimulates metallothionein, which lowers hydroxyl radicals, hence reducing the production of reactive oxygen species (ROS) [36, 37]. Hydrogen peroxide is a naturally occurring byproduct of cellular metabolism and a significant signal of oxidative cellular damage. This molecule is extremely reactive and harmful to the cell because of its strong oxidation characteristics. Under a typical physiological state, CAT deactivates H_2O_2. By preventing the overabundance of H_2O_2 in cells, CAT protects organisms from the damaging effects of H_2O_2 on lipids, proteins, and nucleic acids [38]. In the context of increased free radical production, any increase in SOD activity is advantageous. However, since SOD produces hydrogen peroxide as a metabolite that CAT must scavenge, an increase in SOD activity without a corresponding increase in CAT activity may be harmful [33].

The current investigation revealed a reduction in the activities of SOD and CAT in the serum of rats with diabetes. Reduced SOD and CAT activity in the serum of diabetic rats has been linked to elevated ROS generation in certain studies. An additional indicator of oxidative stress could be decreased SOD activity. Zinc may have the ability to scavenge free radicals. Similarly, this study revealed that increased Zn intake led to an increase in SOD activity. The elevated endogenous H_2O_2 may be the consequence of a high level of oxidative stress, as suggested by the decrease in CAT activity. In contrast, the activity of CAT was elevated in diabetic rats given Zn supplementation, which is consistent with the results of the current investigation.

The total antioxidant capacity (TAC) decreased and oxidative stress increased as a result of disruptions in the oxidative /antioxidative balance, which was reflected in increased ROS levels. The antioxidative state of the entire body is reflected in the serum TAC. The TAC of the cells increases when ROS generation is inhibited because more antioxidant enzymes remain intact in their reduced state [39].

Compared to those in the control group, the diabetic rats in this study had significantly lower serum TAC levels. Increased ROS production might be the result of increased ROS production from pathways such as the polyol pathway and glucose auto-oxidation. According to available data, oxidative stress is elevated in diabetes due to an excess of reactive oxygen species (ROS), and oxidative stress is indicated by decreased antioxidant activity efficiency in diabetic rats [40]. This study's findings are consistent. Consistent with this finding, rats treated with zinc exhibited an increase in antioxidant capacity, which was linked to the overexpression of antioxidant enzymes in response to oxidative stress caused by glucose.

4. Conclusion

In conclusion, this study investigated the physiopathology of DM from the standpoint of oxidative stress. The study also, showed that Zn protects against oxidative stress and its aftereffects, including oxidative damage to proteins and lipids in diabetic rats. The consumption of zinc does not increase the risk of an imbalance between oxidative and antioxidative processes. Given our research on how well Zn supplementation counteracts the detrimental effects of DM, it would seem reasonable to suggest that this important micronutrient may play a role in the potential management of diabetes.

Conflict of interest

The authors declare to have no conflict of interest.

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