



## Effect of ginger on zinc, lipid profile and antioxidants levels in blood and liver of streptozotocin induced diabetic rats fed on zinc deficiency diet

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Diabetes mellitus is a non-communicable disease affecting 463 million people across the world. Ginger has enormous health promoting potential effects in number of ailments including diabetes. So, the purpose of this study was to evaluate the beneficial effect of ginger (*Zingiber officinale* Roscoe) supplementation on carbohydrate metabolism, antioxidant status and tissue zinc in diabetic rats fed zinc deficient diet. Rats were divided into four groups. The first group was non-diabetic rats fed adequate zinc diet. The second was diabetic group fed also adequate zinc diet. While, the third and the fourth groups were diabetic fed zinc deficient diet, one non-treated and the other treated with ginger 2% diet. The findings showed an increase of blood glucose, transaminases, lipids profile and malondialdehyde levels, whereas insulin, liver zinc, alkaline phosphatase, lactate dehydrogenase, proteins, reduced glutathione and antioxidant enzymes were reduced in zinc deficient rats. However, treatment with ginger restored the previous parameters. The obtained results indicated that ginger has a powerful effect, which led to a reduction of diabetes development in zinc deficiency due to its antioxidant potential.

**Keywords:** Diabetes, Oxidative stress, Transaminases, Zinc deficiency, *Zingiber officinale*

Diabetes mellitus is a group of metabolic diseases, which is characterized by high blood glucose levels over a prolonged period. It results from defects in insulin secretion and/or insulin action. Globally, 463 million people are reported to be affected by diabetes and is expected to increase to 700 million by 2045. In South-East Asia, the current population of 88 million diabetes patients is estimated to increase by 74% i.e., to 153 million by 2045<sup>1</sup>. People with diabetes have been reported to suffer from elevated rates of depressive symptoms and there is correlation between depressive symptoms and diabetes complications<sup>2</sup>. The persistent hyperglycemia is often accompanied with excess generation of free radicals, which produce intensive oxidative stress and contribute in the development of this pathology<sup>3</sup>. In the context of severity of the disease, many researchers took up the challenge to understand the mechanism better and also look into potential natural sources for possible biomolecules<sup>4-7</sup>.

Zinc is an important metal required for different biological systems. It plays a crucial role in pancreatic  $\beta$ -cells as an essential element for insulin production<sup>8</sup> and acts as a co-factor for important antioxidant enzymes

involved in the antioxidant defense systems such as superoxide dismutase (SOD) activation, glutathione peroxidase (GSH-Px) and the expression of metallothionein<sup>9</sup>. As zinc important for insulin synthesis and antioxidant enzymes activities, its deficiency may disrupt their synthesis and promoting more oxidative stress<sup>10</sup>. However, there is clear evidence that any imbalances in the state of this metal are involved in the aggravation of diabetes and some of its complications<sup>11</sup>.

Ginger (*Zingiber officinale* Roscoe) has been widely used as dietary spice and in many traditional and alternative medicines worldwide<sup>12,13</sup>. It has been reported that ginger contains potent compounds including (6)-gingerol, shogaols, phenolic compounds, essential oils and oleoresin resins<sup>14</sup>, through them ginger exhibits its antioxidant properties<sup>15,16</sup>. In this study, we examined the modulator effect of ginger supplementation in preventing the development of diabetic pathology observed in zinc deficiency by evaluating body weight gain, zinc status, carbohydrate metabolism and antioxidant system in streptozotocin induced diabetic rats.

### Materials and Methods

#### Animals

Female Wistar albino rats weighing 190-230 g of 10 weeks of age were obtained from Pasteur Institute

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(Algiers-Algeria). The Animals were housed in individual plastic cages with bedding. Standard rat food and deionized water were made available *ad libitum*. Rats were acclimated for a period of two weeks to their surroundings. The temperature was maintained at  $22\pm 2^{\circ}\text{C}$  with a photoperiod of 12 h light/dark cycle. All animals were maintained and used in accordance with the Animal Welfare Act and the Guide form, the Care and Use of Laboratory Animals prepared by Annaba University, Algeria.

#### Induction of diabetes

Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of freshly streptozotocin (STZ) solution at dose of 55 mg STZ/kg body weight<sup>17</sup>, dissolved in cold citrate buffer (0.1M, PH 4.5). After 5 days of STZ injection, blood glucose level was measured on samples taken from the tail vein using a glucose-meter (ACCU-CHEK Active, Roche Diagnostics Mannheim, Germany). Only rats with glycaemia higher than 14 mmol/L were used as diabetic rats.

#### Plant preparation

Fresh ginger (*Zingiber officinale* Roscoe) rhizomes were purchased from local market (Ginger was imported from China), and were authenticated by Dr. Moncef Zaafour, Department of Biology, Faculty of Sciences, Badji Mokhtar University, Annaba, Algeria. The rhizomes washed several times, peeled, cut into small pieces, then shade dried and pulverized with a blender until a fine powder was obtained. The obtained powder was stored at  $22\pm 2^{\circ}\text{C}$  in airtight containers protected from light until further use.

#### Feed

Basal diet of animals was prepared as described by Southon *et al.*<sup>18</sup> and it consisted in g/kg diet as follows: Cornstarch 326 (32.6%), Sucrose 326 (32.6%), Protein (egg white solids) 168 (16.8%), Lipids (corn oil) 80 (0.8%), Fiber (cellulose) 40 (0.4%), Vitamin mix 20 (0.2%), and Mineral mix 40 (0.4%). The mineral mix was formulated to contain either adequate (54 mg/kg)<sup>16</sup> or in adequate (1.2 mg/kg) quantities of zinc as determined by atomic absorption spectroscopy. The mineral mix was supplied in (g/kg diet) by calcium hydrogen orthophosphate, 13; disodium hydrogen orthophosphate, 7.4; calcium carbonate, 8.2; potassium chloride, 7.03; magnesium sulfate, 4; ferrous sulfate, 0.144; copper sulfate, 0.023; potassium iodide, 0.001; manganese sulfate, 0.180; and zinc sulfate, 0.1. The low zinc diet contained no additional zinc sulfate.

#### Experimental design

Rats were randomly divided into four groups (five each) as follow: the first group was no-diabetic rats (ND) fed adequate zinc diet. The second group: diabetic rats fed also adequate zinc diet (DAZ). The third group was diabetic rats fed zinc deficient diet (DZD) and the fourth group: diabetic rats fed also zinc deficient diet and treated with ginger 2% diet (DZD+Gg). The treatment period was continued for 27 days.

#### Blood collection and tissue samples preparation

At the end of the experiment, overnight fasted rats were decapitated by cervical cut and blood samples were drawn and used to determine serum insulin, total proteins, albumin cholesterol and triglycerides concentrations, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities. A fragment of liver and the right femur which removed from its connective tissue were excised, washed with isotonic saline, and blotted to dry. After that, they were weighed and dried at  $80^{\circ}\text{C}$  for 16 h, and zinc concentration in ash tissues was determined. The second fragment of liver was excised and washed with isotonic saline, and immediately conserved at  $-20^{\circ}\text{C}$  for assaying malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST) superoxide dismutase (SOD) and catalase (CAT).

#### Measurement of biochemical parameters

The biochemical parameters were measured using commercial kits from Spinreact Girona, Spain. Refs: total proteins-1001291, albumin-1001020, GOT-1001161, GPT-1001171, ALP-1001131, LDH-1001260, cholesterol-1001091 and triglycerides-1001311. Whereas, the concentration of insulin in serum was estimated using a SIEMENS Immulite 1000 systems INS (SIEMENS Healthcare Diagnostics), which is a solid-phase, two-site chemiluminescent immunometric assay.

#### Zinc tissue analysis

Dried livers and femurs were ashed into silica crucibles at  $480^{\circ}\text{C}$  for 48 h using a muffle furnace. After cooling, the ashed samples was dissolved with concentrated nitric acid (M 15.77), diluted with distilled water and filtered and then zinc concentration was determined using AA-7000 SHIMADUZ France atomic absorption spectrophotometer<sup>18</sup>. Standard reference materials: bovine liver and wheat flour were

used to check the accuracy of zinc recovery, which exceeded 96% in the reference materials. Zinc standards were prepared from 1.0 mg/mL zinc nitrate standard solution using 5% glycerol to approximate the viscosity characteristics. All tubes were soaked in HCL (10% v/v) for 16 h and rinsed with doubly distilled water to avoid zinc contamination from exogenous sources.

#### Measurement of antioxidant parameters

##### *Tissue preparation*

About 1.0 g of liver was homogenized in 2 mL of 0.1 M TBS buffer (TrisNaCl, pH 7.4). The homogenate was centrifuged at 10000 ×g for 15 min at 4°C and the resultant supernatant was used for determination of MDA, GSH, GSH-Px, GST, SOD and CAT<sup>19</sup>.

##### *Estimation of lipid peroxidation (MDA)*

The lipid peroxidation level in liver homogenate was measured as malondialdehyde (MDA), which is the end product of lipid peroxidation, and reacts with thiobarbituric acid (TBA) as a TBA reactive substance (TBARS) to produce a red coloured complex with a peak absorbance at 532 nm<sup>20</sup>.

##### *Determination of reduced glutathione (GSH)*

Liver glutathione was determined according to the method of Ellman<sup>21</sup> modified by Jollow *et al.*<sup>22</sup>. The method based on the development of a yellow colour when DTNB is added to compounds containing sulfhydryl groups. The GSH concentration (nmol GSH/mg protein) was obtained from the absorbance at 412 nm.

##### *Measurement of glutathione peroxidase (GSH-Px) activity*

The activity was determined by the procedure of Flohe & Gunzler<sup>23</sup>. The method based on the reaction between glutathione remaining after the action of GSH-Px and 5,5-Dithio-bis (2-nitrobenzoic acid) to form a complex that absorbs maximally at 412 nm.

##### *Measurement of glutathione-S-transferase (GST) activity*

The activity was measured by the method of Habig<sup>24</sup> using CDNB as electrophilic substrate that binds to GSH with the participation of the enzyme and forms a coloured GSH-substrate complex, detected at 340 nm.

##### *Measurement of superoxide dismutase (SOD) activity*

The activity superoxide dismutase (SOD) was determined according to the method described by Misra & Fridonich<sup>25</sup>. Ten micro liters of tissue homogenate were added to 970 µL of EDTA-sodium carbonate buffer (0.05M) at pH 10.2. The reaction

was started by adding 20 µL of epinephrine (30 mM) and the activity was measured at 480 nm for 4 min.

##### *Estimation of catalase (CAT) activity*

Liver CAT was determined according to the method of Aebi<sup>26</sup>. The activity was measured at 240 nm every 15 s for one minute.

#### Estimation of liver proteins:

Liver proteins concentration was determined using Bradford assay. Serum bovine albumin was used as a standard<sup>27</sup>.

#### Histopathological study

Fresh pancreas tissue was excised and fixed in 10% formalin. Then, tissues were washed and dehydrated by passing in ascending grade of ethanol series, leaned in xylene and embedded in paraffin. The paraffin sections were cut into 3.5 µm and stained with hematoxylin with eosin and examined microscopically<sup>28</sup>.

#### Statistical analysis

Data were reported as mean ± SEM. Results comparison were carried out using one-way analysis of variance followed by student's *t*-test to compare means between the different groups. Differences were considered statically significant at  $P < 0.05$ . Statistical analysis was performed using Graph Pad Prism 7.

## Results

#### Body weight gain

This study showed that diabetes resulted in a significant ( $P < 0.001$ ) reduction in body weight of diabetic rats consuming sufficient zinc diet. Meanwhile, zinc deficiency affected more the bodyweight of the diabetic animals ( $P < 0.01$ ) as compared to those fed adequate zinc diet. However, ginger supplementation alleviated these changes (Table 1).

#### Tissues zinc concentration

Liver and femur zinc concentrations are also presented in Table 1. Induction of diabetes led to a decrease ( $P < 0.05$ ) in femur zinc contents. Moreover, diabetic rats fed low zinc diet have shown a significant reduction ( $P < 0.05$ ) in liver zinc concentration. Whereas, ginger supplementation restored zinc concentrations in the previous studied organs.

#### Biochemical parameters

As illustrated in Tables 1 and 2, diabetes affected most biochemical markers. After 27 days of treatment, blood glucose was increased significantly ( $P < 0.05$ ) in serum of diabetic rats fed an adequate

Table 1 — Initial body weight, final body weight, tissues zinc concentrations, blood glucose and insulin levels of non-diabetic rats (ND), diabetic adequate zinc rats (DAZ), diabetic deficient zinc rats (DZD), and diabetic deficient zinc rats given dietary ginger (DZD+Gg)

Parameters	Experimental groups			
	ND	DAZ	DZD	DZD+Gg
Initial body Weight (g)	199.44±3.57	204.22±4.61	195.66±2.98	196.44±3.07
Final body Weight (g)	215.75±3.88	173±4.31 <sup>a2</sup>	154.56±4.43 <sup>b</sup>	177.78±2.87 <sup>c1</sup>
Liver zinc(µg/g dry weight)	75.42±5.09	64.74±6.55	48.26±1.81 <sup>b</sup>	62.20±4.31 <sup>c</sup>
Femur zinc (µg/g dry weight)	85.73±4.11	74.58±1.67 <sup>a</sup>	71.95±2.56	89.37±1.62 <sup>c1</sup>
Blood glucose(mg/dL)	91.6±2.01	384±69.17 <sup>a1</sup>	389.6±42.2	126.16±11.16 <sup>c1</sup>
Insulin (µU/mL)	2.88±0.25	1.618±0.34 <sup>a</sup>	1.424±0.20	3.656±0.9 <sup>c</sup>

[Means ± SEM, number of animals = 5; <sup>a</sup>*P* ≤ 0.01, <sup>a1</sup>*P* ≤ 0.01, <sup>a2</sup>*P* ≤ 0.001: DAZ vs. ND; <sup>b</sup>*P* ≤ 0.05: DZD vs. DAZ; <sup>c</sup>*P* ≤ 0.05, <sup>c1</sup>*P* ≤ 0.01: DZD+Gg vs. DZD]

Table 2 — Serum total proteins, albumin, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), cholesterol and triglycerides of non-diabetic rats (ND), diabetic adequate zinc rats (DAZ), diabetic deficient zinc rats (DZD), and diabetic deficient zinc rats given dietary ginger (DZD+ Gg)

Parameters	Experimental groups			
	ND	DAZ	DZD	DZD+Gg
Total proteins (g/L)	67.4±1.03	57.4±2.68 <sup>a1</sup>	47.6±2.04 <sup>b</sup>	60.4±3.69 <sup>c</sup>
Albumin (g/L)	30±0.632	24.2±1.02 <sup>a1</sup>	19.6±0.69 <sup>b1</sup>	22.4±0.51 <sup>c</sup>
GOT (IU/L)	191.2±9.84	224±12.31	227.4±15.64	184.2±1.91 <sup>c</sup>
GPT (IU/L)	88±4.25	100±2.58 <sup>a</sup>	97.20±4.63	80±2.12 <sup>c1</sup>
ALP (IU/L)	366.8±40.1	338.75±4.84	258.25±21.11 <sup>b1</sup>	354.2±41.67
LDH (IU/L)	3159.4±123.8	2768.4±385.7	1786.6±178.1 <sup>b</sup>	2568.4±227.1 <sup>c</sup>
Cholesterol (g/L)	0.60±0.11	1.09±0.36 <sup>a</sup>	1.02±0.12	0.86±0.17 <sup>c</sup>
Triglycerides (g/L)	0.76±0.08	1.33±0.19 <sup>a2</sup>	2.07±0.46 <sup>b</sup>	0.71±0.19 <sup>c2</sup>

[Means ± SEM, number of animals = 5. <sup>a</sup>*P* ≤ 0.05, <sup>a1</sup>*P* ≤ 0.01, <sup>a2</sup>*P* ≤ 0.001: DAZ vs. ND; <sup>b</sup>*P* ≤ 0.05, <sup>b1</sup>*P* ≤ 0.01: DZD vs. DAZ; <sup>c</sup>*P* ≤ 0.05, <sup>c1</sup>*P* ≤ 0.01, <sup>c2</sup>*P* ≤ 0.001: DZD+Gg vs. DZD]

zinc diet and showed a decrease in insulin level. Meanwhile, ginger supplementation resulted in an increase of insulin (*P* < 0.05) and a decrease of blood glucose levels (*P* < 0.01). On the other hand, the rest of the biochemical parameters were generally altered under the diabetic state and showed more disturbances as a result of zinc deficiency. Nevertheless, most of these parameters variations were significantly ameliorated after ginger treatment.

#### Oxidative stress parameters

The concentrations of hepatic MDA, GSH and enzymatic antioxidants parameters including GSH-Px, GST, SOD and CAT activities are illustrated in (Figs 1A & B; and Fig.1 C (a-c), respectively. Diabetic rats have a significant increase in MDA (*P* < 0.05) level, a decrease of GSH (*P* < 0.05) concentration and reduced activities (*P* < 0.05) of GSH-Px, GST, SOD and CAT as compared to non-diabetic rats. Interestingly, the GSH-Px and CAT activities were more affected in diabetic animals fed low dietary zinc. However, ginger supplementation resulted in improvements of the previous hepatic oxidative stress parameters.

#### Pancreatic histopathology results

The microscopic observation of pancreas sections revealed that non-diabetic rats exhibited normal

appearance of endocrine and exocrine structure (Fig. 2A). Diabetic rats fed adequate zinc diet showed a reduction in the size of islet cells (Fig. 2B). More abnormalities of the general architecture were observed in the diabetic group fed low zinc diet. β-cells were entirely lost. Acinar damage and thickening of the septa were also seen (Fig. 2C). However, diabetic rats received ginger treatment showed preserved structure of islet cells and attenuated the severity of atrophic change of acinar cells (Fig. 2D).

#### Discussion

Oxidative stress, which results from increased free radicals generation and/or decreased antioxidant capacity, plays an important role in the pathogenesis of diabetes mellitus<sup>29</sup>. However, a variety of plants and their active compounds have been shown a potent effectiveness for the treatment of this pathology. Several studies have demonstrated the beneficial effects of ginger on hyperglycemia by increasing insulin sensitivity, glucose uptake by tissues and reducing oxidative stress<sup>30</sup>. Hence, this study was designed to evaluate the ability of this spice to modulate effects of zinc deficiency in diabetes.

In the present study, the body weight was obviously reduced in diabetic rats in comparison with

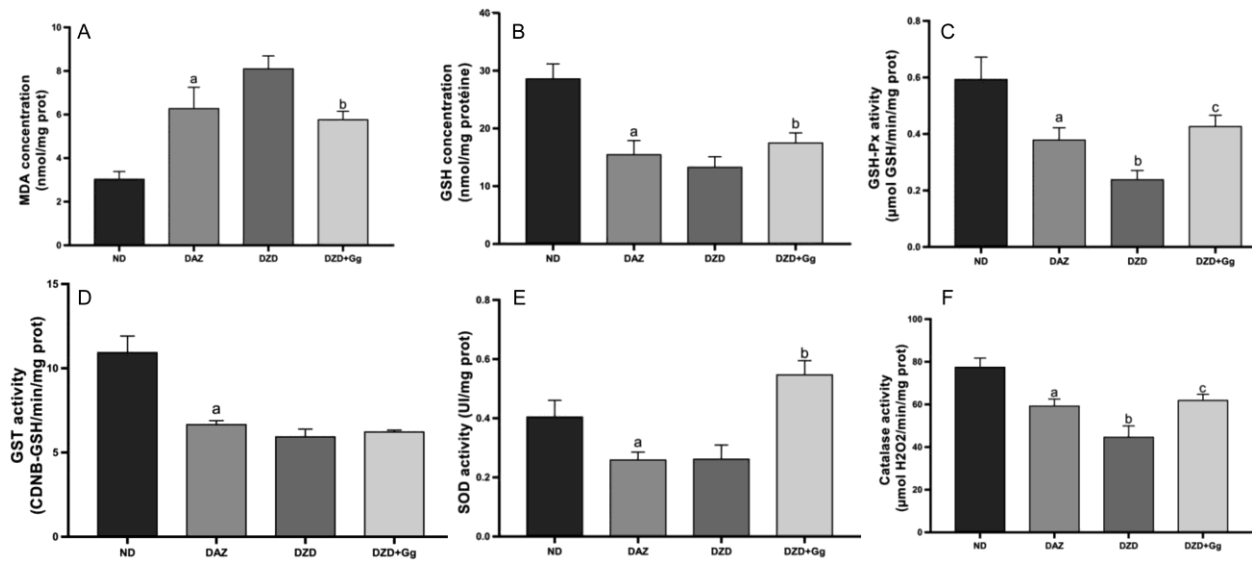


Fig. 1 (A) — Liver MDA level of non-diabetic rats (ND), diabetic adequate zinc rats (DAZ), diabetic deficient zinc rats (DZD), and diabetic deficient zinc rats given dietary ginger (DZD+ Gg). [Means  $\pm$  SEM, number of animals = 5. <sup>a</sup> $P \leq 0.05$ : DAZ vs. ND; <sup>b</sup> $P \leq 0.05$ : DZD+Gg vs. DZD], (B) — Liver reduced glutathione concentration of non-diabetic rats (ND), diabetic adequate zinc rats (DAZ), diabetic deficient zinc rats (DZD), and diabetic deficient zinc rats given dietary ginger (DZD+Gg)., (C) — (A) Glutathione peroxidase activity; (B) Glutathione transferase activity; (C) Superoxide dismutase activity; and (D) Catalase activity of non-diabetic rats (ND), diabetic adequate zinc rats (DAZ), diabetic deficient zinc rats (DZD), and diabetic deficient zinc rats given dietary ginger (DZD+Gg). [Means  $\pm$  SEM, number of animals = 5. <sup>a</sup> $P \leq 0.05$ : DAZ vs. ND; <sup>b</sup> $P \leq 0.05$ : DZD vs. DAZ; and <sup>c</sup> $P \leq 0.05$ : DZD+Gg vs. DZD], (D) — Glutathione transferase activity of non-diabetic rats (ND), diabetic adequate zinc rats (DAZ), diabetic deficient zinc rats (DZD), and diabetic deficient zinc rats given dietary ginger (DZD+Gg). [Means  $\pm$  SEM, number of animals = 5. <sup>a</sup> $P \leq 0.05$ : DAZ vs. ND], (E) — Superoxide dismutase activity of non-diabetic rats (ND), diabetic adequate zinc rats (DAZ), diabetic deficient zinc rats (DZD), and diabetic deficient zinc rats given dietary ginger (DZD+Gg). [Means  $\pm$  SEM, number of animals = 5. <sup>a</sup> $P \leq 0.05$ : DAZ vs. ND; and <sup>b</sup> $P \leq 0.05$ : DZD+Gg vs. DZD] (F) — Catalase activity of non-diabetic rats (ND), diabetic adequate zinc rats (DAZ), diabetic deficient zinc rats (DZD), and diabetic deficient zinc rats given dietary ginger (DZD+Gg). [Means  $\pm$  SEM, number of animals = 5. <sup>a</sup> $P \leq 0.05$ : DAZ vs. ND; <sup>b</sup> $P \leq 0.05$ : DZD vs. DAZ; and <sup>c</sup> $P \leq 0.05$ : DZD+Gg vs. DZD]

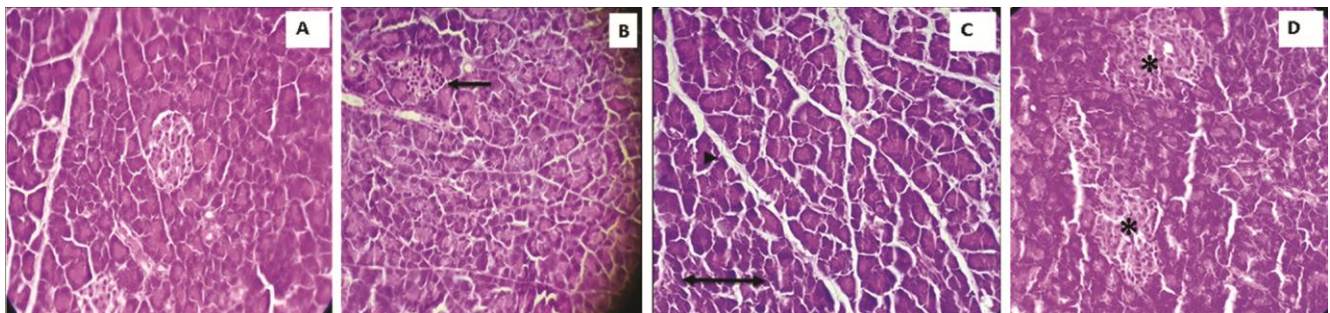


Fig. 2 — Effect of ginger on pancreas histopathological changes in studied groups. A: section of pancreas from non-diabetic group showing normal appearance of endocrine and exocrine structure. B: section of pancreas from diabetic adequate zinc rats showing reduced  $\beta$ -islets size ( $\rightarrow$ ). C: section of pancreas from diabetic deficient zinc rats showing entirely lost  $\beta$ -cells, acinar damage ( $\leftrightarrow$ ), and thickening of the septa ( $\blacktriangleright$ ). D: section of pancreas from diabetic deficient zinc rats treated with ginger (Gg) indicating restored  $\beta$  cell islets (\*) and reduced acinar cells damage (H & E, 400X).

non-diabetic rats, this finding is in agreement with the results of others studies<sup>15,17</sup>. It is undoubtedly high mobilization of protein and fat stores seem to be responsible for loss of body weight in diabetic rats<sup>31</sup>. Interestingly, it was also noticed a decrease of body weight of diabetic rats fed low zinc diet. This finding is supported by recent findings<sup>32</sup>. It is well accepted

that zinc deficiency brought taste disorders, frequently related to an impairment of gustine activity, a dependent zinc enzyme. Thus, these disorders result in reduced dietary intake and induce premature satiety with eating, which may further impair growth rate<sup>33</sup>.

Adversely, body weight was found higher in diabetic rats given low zinc diet and treated with

ginger than those non-treated diabetic rats. According to Madkor *et al.*<sup>15</sup>, increased body weight reflects the positive anabolic effect of this spice through improving glucose homeostasis, which reduces the degeneration of the adipocytes and muscle tissues. Zinc concentrations in liver and femur were significantly decreased in both diabetic rats fed either adequate zinc or deficient zinc diet. These findings are coinciding with previous reports<sup>32,34</sup>. The observed zinc depletion in those animals can be explained in part by insufficient intestinal absorption of this mineral and to excess urinary loss as a result of altered kidney function<sup>35</sup>. However, enhanced zinc concentrations in diabetic rats treated by additional ginger is possibly due to the hypoglycemic effect of ginger, which could be one of the factors contributing to the preservation of renal function against the deleterious effect of free radicals. Thus, it may result in the reduction of excessive urinary loss of zinc. As expected, diabetes induced a significant increase in blood glucose and decrease of insulin levels in diabetic rats fed sufficient zinc diet. These metabolic disorders might have resulted from the pancreatic  $\beta$ -cells depletion after STZ injection, which led to impaired insulin secretion<sup>36</sup>. On the other hand, the hyperglycemia is probably due to disturbance of the glucose utilization by tissues or to increased rate of endogenous glucose production<sup>37</sup>. Furthermore, the slight raise in blood glucose was observed in those animals given low zinc diet. This result is in agreement with other studies<sup>38,39</sup>, which reported that zinc deficiency may affect the ability of pancreas to produce and release insulin, which led to impair glucose homeostasis. Ginger supplementation significantly lowered blood glucose and enhanced insulin levels. This effect was proved by histopathological ameliorations in pancreatic cells, which showed restoration structural integrity of  $\beta$ -cells. The effect of ginger on glycemic control could be explained by the fact that gingerol, the most important bioactive compound of ginger, has a variety of mechanisms to up regulate blood glucose, it decreases hepatic glucose production and increases glucose transporter (GLUT<sub>4</sub>) translocation from intracellular compartments to the cells surface which result in increased glucose uptake into cells<sup>25</sup>. In addition, it is reported that ginger inhibits  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes in carbohydrate digestion<sup>40</sup>.

Diabetes is generally accompanied with disturbance in protein profile. The lowered serum protein and

albumin levels in diabetic groups, as demonstrated in this study, are may be due to increase rate of proteins stores catabolism as a compensatory system to produce energy<sup>41</sup> or to micro proteinuria, which are important clinical markers of diabetes<sup>37</sup>. However, the normalized level of these parameters after ginger supplementation is mainly due to improved glycemic control. In this study a significant rise in serum GPT and slight increase of GOT activities in diabetic rats were also noticed, which could relate to excessive accumulation of amino acids (glutamic and alanine) in the blood of diabetic animals as a result of amino acid mobilization from protein stores and the conversion to keto acids assuring new source for energy under diabetes condition<sup>38</sup>. However, these elevations were prevented by ginger treatment.

Zinc is an important element for many metallo-enzymes including alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). In this study, PAL and LDH activities have been affected in serum of deficient zinc animals. Same results were found in earlier studies<sup>37</sup>. Simultaneously, treated diabetic rats with ginger have restored these enzymes activities. The mechanism by which ginger increased the activity of ALP and LDH still not clear, but it is probably due to improvement of zinc level. Moreover, the concentrations of cholesterol and triglycerides were found high in diabetic groups fed either on sufficient or deficient zinc feed. These findings might be as a result of lipids catabolism due to high demand of energy<sup>42</sup> and insulin deficiency affected the activity of lipoprotein lipase, an enzyme involved in the elimination of circulated triglycerides and resulting in hyper triglyceridemia<sup>43</sup>. On the other hand, these parameters were reduced in zinc diabetic rats given ginger. This might be due to the hypolipidemic activity of this spice to block the absorption of cholesterol in the gut and the increased faecal excretion<sup>44,45</sup>. Several studies have also suggested that ginger has an inhibition efficacy of cellular cholesterol synthesis, which results in augmenting the LDL receptor activity, leading to the elimination of LDL from plasma, thus modifying lipoprotein metabolism<sup>46</sup>.

Diabetes is characterized by high level of oxidative stress, which principally due to chronic hyperglycemia and impaired activity of antioxidant defense<sup>3</sup>. In the present study, the level of liver MDA was increased in diabetic rats. This is likely due to



high production of oxygen species (ROS) which are involved in the progression of diabetes. In other words, the effect of hyperglycemia, which increases polyunsaturated fatty acids attack, results in raised lipid peroxidation rate<sup>47,48</sup>. The reason of glutathione (GSH) depletion in diabetic rats could be explained by the increased rate of reduced glutathione oxidation to oxidized glutathione as a result of enhanced production of free radicals<sup>45</sup>. GSH-Px, GST, SOD and CAT activities were also significantly decreased in diabetic rats which indicated impairment in the antioxidant defenses system. This may be explained by the glycation of these proteins as a result of persistent hyperglycemia in diabetic state<sup>28</sup>. Furthermore, diabetic rats fed inadequate zinc feed have showed more significant decline in GSH-Px and CAT activities than adequate zinc diabetic group. Several reports have been confirmed the deleterious effect of zinc depletion on the function of these enzymes. Lima *et al.*<sup>9</sup> reported that zinc is a co-factor required for the action of various antioxidant enzymes and their regulation and its deficiency will affect the function of these enzymes. The treatment with ginger exhibited remarkably ameliorated activities of antioxidant enzymes, particularly GSH-Px, SOD and CAT. Diabetic rats treated with whole ginger or their bioactive components have been shown to improve the antioxidant defense system efficacy<sup>15</sup>.

The major component of ginger, 6 gingerol and 6 shogaol showed numerous pharmacological benefits, including improvement of glucose homeostasis and modulating insulin secretion by pancreatic  $\beta$ -cells through their protection against oxidative damages<sup>49</sup>. Also, the antioxidant compounds of ginger may potentially inhibit the production of ROS, decrease lipid peroxidation and improve GSH tissues in diabetic rats, which in turn ameliorate the GSH depending antioxidant enzymes<sup>50</sup>.

### Conclusion

From the findings obtained in this study, it is evident that ginger effectively reduced the complications caused by zinc deficiency in diabetes through attenuating hyperglycemia and maintaining insulin level, restored zinc concentrations and enhancing antioxidant defense systems. In this sense, the antidiabetic and antioxidant effects might be due to the bioactive compounds of this spice.

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### Conflict of Interest

Authors declare no conflict interests.

### References

- 1 International Diabetes Federation, IDF Diabetes Atlas, 9<sup>th</sup> edn., (Eds. Karuranga S, Malanda B, Saeedi P & Salpea P; International Diabetes Federation, Brussels, Belgium), 2019. <https://www.diabetesatlas.org>. Accessed on 23 February 2021.
- 2 Mary de Groot, 50 Years of Behavioral Science in Diabetes: A 2020 Vision of the Future. *Diabetes Care*, 44 (2021) 633. <https://doi.org/10.2337/dci20-0058>.
- 3 Lotfy M, Adeghate J, Kalasz H, Singh J & Adeghate E, Chronic complications of diabetes mellitus: a mini review. *Curr Diabetes Rev*, 13 (2017) 3.
- 4 Bansal K, Reddy KRC & Trigunayat A, Anti-hyperglycaemic study of Eladi Churna in streptozotocin (STZ) induced diabetic rats, *Indian J Tradit Know*, 18 (2019) 34.
- 5 Prabha LM & Subramanian S, Correlation between prolidase activity and spatial memory functions in streptozotocin-induced diabetic rats. *Indian J Exp Biol*, 59 (2021) 97.
- 6 Sharma S, Mishra V & Srivastava N, Protective effect of *Trigonella foenum-graecum* and *Cinnamomum zeylanicum* against diabetes induced oxidative DNA damage in rats. *Indian J Biochem Biophys*, 57 (2020) 15.
- 7 Yadav S, Hameeda IB & Jayadeep A, Neuroprotective and hepatoprotective effect of whole red rice forms against oxidative stress in streptozotocin induced diabetic rats. *Indian J Exp Biol*, 58 (2020) 151.
- 8 Capdor J, Foster M, Petocz P & Samman S, Zinc and glycemic control: a meta-analysis of randomized placebo controlled supplementation trials in humans. *J Trace Elem Med Biol*, 27 (2013) 137.
- 9 Lima VBS & Sampaio FA, Parameters of glycemic control and their relationship with zinc concentrations in blood and with superoxide dismutase enzyme activity in type 2 diabetes patients. *Arq Bras Endocrinol Metab*, 55 (2011) 701.
- 10 Alkaladi A, Abdelazim AM & Afifi M, Antidiabetic activity of zinc oxide and silver nanoparticles on streptozotocin-induced diabetic rats. *Int J Mol Sci*, 15 (2014) 2015.
- 11 Asadi S, Moradi MN, Khyripour N, Goodarzi TM & Marzieh M, Resveratrol attenuates copper and zinc homeostasis and ameliorates oxidative stress in type 2 diabetic rats. *Biol Trace Elem Res*, 177 (2016) 132.
- 12 Saravanan N, Patil MA, Kumar PU, Suryanarayana P & Reddy GB, Dietary ginger improves glucose dysregulation in a long-term high-fat high-fructose fed prediabetic rat model. *Indian J Exp Biol*, 55 (2017) 142.
- 13 Saain SN, Wahyudianingsih R, Afni M, Afifah E, Maesaroh M & Widowati W, Suppression of pro-inflammatory cytokines and mediators production by ginger (*Zingiber officinale* Roscoe) ethanolic extract and gingerol in lipopoly-saccharide-induced RAW 264.7 murine macrophage cells. *Indian J Nat Prod Res*, 11(2020) 260.

- 14 Qian M, Xiao Y, Shi C, Ren G, Harold C, Trust B & Hua Li, Bioactive Compounds and Bioactivities of Ginger (*Zingiber officinale* Roscoe). *Foods*, 8(6) (2019)185.
- 15 Madkor HR, Mansour SW & Ramadan G, Modulatory effects of garlic, ginger, turmeric and their mixture on hyperglycemia, dyslipidemia and oxidative stress in streptozotocin-nicotinamide diabetic rats. *Br J Nutr*, 105 (2011) 1210.
- 16 Aborehab NM & Boshra SA, Hepatoprotective effect of ginger and grape seed, alone and in combination orally, in paracetamol induced acute liver toxicity in rats. *Indian J Exp Biol*, 57 (2019) 274.
- 17 Saleh D, BayoumiAR, El-Eraky WI & El-Khatib AS, Streptozotocin-induced vascular and biochemical changes in rats: Effects of rosiglitazone vs. Metformin. *Bull Fac Pharm Cairo Univ*, 51 (2013) 131.
- 18 Southon S, Kechrid Z & Wright AJA, Effect of reduced dietary zinc intake on carbohydrate and zinc metabolism in the genetically diabetic mouse (C57BL/ KsJdb+/db+). *Br J Nutr*, 60 (1988) 499.
- 19 Bouhalit S, Kechrid Z & EL-Feki, Effect of silymarin extracted from silybum marianum on nickel hematotoxicity in albino Wistar rats. *Int J Pharm Sci*, 9 (2017) 84.
- 20 Buege JA & Aust SD, Microsomal lipid peroxidation. *Methods Enzymol*, 105 (1984) 302.
- 21 Ellman GL, Tissue sulfhydryl groups. *Arch Biochem Biophys*, 82 (1959) 70.
- 22 Hollow DJ, Mitchell JR, Zampaglione Z & Gillette JR, Bromobenzene induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolites. *Pharmacology*, 11 (1974) 151.
- 23 Flohe L & Gunzler WA, Analysis of glutathione peroxidase. *Methods Enzymol*, 105 (1984) 114.
- 24 Habig WH, Pabst MJ & Jakoby WB, Glutathione S-transferase, the first enzymatic step in mercapturic acid formation. *J Biol Chem*, 249 (1974) 7130.
- 25 Misra HP & Fridovich I, Superoxide dismutase: Positive spectrophotometric assays. *Anal Biochem*, 79 (1977) 553.
- 26 Aebi H, Catalase *in vitro*. *Methods Enzymol*, 105 (1984) 121.
- 27 Bradford M, A rapid and sensitive method for the quantities of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*, 72 (1976) 248.
- 28 Houlod R, *Techniques d'histopathologie et de cytopathologie* (Editions Maloine), 1984, 225.
- 29 Said NS, Hadhoud KM, Nada WM & El Tarhouny SA, Superoxide dismutase, glutathione peroxidase and vitamin E in patients with diabetic retinopathy. *Life Sci J*, 10 (2013) 1851.
- 30 Akash MSH, Rehman K, Tariq M & Chen S, *Zingiber officinale* and type 2 diabetes mellitus: Evidence from experimental studies. *Crit Rev Eukaryot Gene Expr*, 25 (2015) 91.
- 31 Yang F, Li B, Dong X & Luo P, The beneficial effects of zinc on diabetes-induced kidney damage in murine rodent model of type1 diabetes mellitus. *J Trace Elem Med Biol*, 42 (2017) 1.
- 32 Derai EH & Kechrid Z, Combined effect of vitamins C and E on zinc status, carbohydrate metabolism and antioxidant values in diabetic rats fed zinc-deficient diet. *Med J Nutrition Metab*, 7 (2014) 55.
- 33 Yu Q, Sun X, Zhao J, Zhao L, Chen Y, Fan L, Li Z, Sun Y, Wang M & Wang F, The effects of zinc deficiency on homeostasis of twelve minerals and trace elements in the serum, feces, urine and liver of rats. *Nutr Metab (Lond)*, 167 (2019) 73.
- 34 Prasad AS, Effects of zinc deficiency on immune functions. *Trace Elem Exp Med*, 13 (2002) 1.
- 35 Kechrid Z, Hamdi M & Naziroğlu M, Vitamin D supplementation modulates blood and tissue zinc, liver glutathione and blood biochemical parameters in diabetic rats on a zinc deficient diet. *Biol Trace Elem Res*, 148 (2012) 371.
- 36 Galetti V, Zinc deficiency and stunting. In: *Handbook of famine, starvation and nutrient deprivation*. (Eds. Preedy V & Patel V, Springer, Cham), 2018, 1. [https://doi.org/10.1007/978-3-319-40007-5\\_93-1](https://doi.org/10.1007/978-3-319-40007-5_93-1)
- 37 Gupta S, Kataria M, Gupta PK, Murganandan S & Yashroy RC, Protective role of extracts of neem seeds in diabetes caused by streptozotocin in rats. *J Ethnopharmacol*, 90 (2004) 185.
- 38 Derouiche S & Kechrid Z, Zinc supplementation overcomes effects of copper on zinc status, carbohydrate metabolism and some enzyme activities in diabetic and non-diabetic rats. *Can J Diabetes*, 40 (2016) 342.
- 39 Hamdiken M, Bouhalit S & Kechrid Z, Effect of *Rutachalepensis* on Zinc, lipid profile and antioxidant levels in the blood and tissue of streptozotocin-induced diabetes in rats fed zinc-deficient diets. *Can J Diabetes*, 42 (2017) 356.
- 40 Fatmi W, Kechrid Z, Naziroğlu M & Flores-Arce M, Selenium supplementation modulates zinc levels and antioxidant values in blood and tissues of diabetic rats fed zinc- deficient diet. *Biol Trace Elem Res*, 152 (2013) 243.
- 41 Li Y, Tran VH, Duke CC & Roufogalis BD, Gingerols of *Zingiber officinale* enhance glucose uptake by increasing cell surface GLUT4 in cultured L6 myotubes. *Planta Med*, 78 (2012) 1549.
- 42 Kechrid Z, Amamra S, Dahdouh F & Bouzerna N, The Effect of CaNa2-EDTA on metabolism of zinc and carbohydrate as well as some biochemical factors in experimental diabetes. *Iran J Public Health*, 36 (2007) 15.
- 43 Girija K, Lakshman K, Udaya C, Sabhya SG & Divya T, Anti-diabetic and anti-cholesterolemic activity of methanol extracts of three species of *Amaranthus*. *Asian Pac J Trop Biomed*, 1 (2011) 133.
- 44 Han L, Gong X, Kawano S, Saito M, Kimura Y & Okuda H, Antiobesity actions of *Zingiber officinale* Roscoe. *Yakugaku Zasshi*, 125 (2005) 213.
- 45 Hassan M, Tariq NA, Khan S & Gul F, Antidiabetic and antihyperlipidemic effects of *Artemisia absinthium* L., *Citrullus colocynthis* (L.) Schrad. and *Gymnema sylvestris* (Retz.) R.Br. ex Sm. on type II diabetes hyperlipidemic patients. *Indian J Tradit Know*, 17 (2018) 233.
- 46 Ramada SK, Mllikarjuna K & Kesireddy SR, Efficacy of ethanolic extract of ginger on kidney lipid metabolic profiles in diabetic rats. *Int J Diabetes Dev Ctries*, 31 (2011) 97.



- 47 Mistry KN, Dabhi BK & Joshi BB, Evaluation of oxidative stress biomarkers and inflammation in pathogenesis of diabetes and diabetic nephropathy. *Indian J Biochem Biophys*, 57 (2020) 45.
- 48 Videla LA, Fernandez V, Ugarte G & Valenzuela A, Effect of acute ethanol intoxication on the content of reduced glutathione of the liver in relation to its lipoperoxidative capacity in the rat. *FEBS Lett*, 111 (1980) 6.
- 49 Mahluji S, Ostadrahimi AR, Mobasseri M, Ebrahimzade AV & Payahoo L, Anti-inflammatory effects of *Zingiber officinale* in type 2 diabetic patients. *Adv Pharm Bull*, 3 (2013) 273.
- 50 Shanmugam KR, Mallikarjuna K, Kesireddy N & Reddy KS, Neuroprotective effect of ginger on antioxidant enzymes in streptozotocin-induced diabetic rats. *Food Chem Toxicol*, 49 (2011) 893.