

Original Article

CHARD (*BETA VULGARIS VAR. CICLA*) EXTRACT MODULATES ZINC STATUS, GLUCOSE LEVEL AND ANTIOXYDANT VALUES IN DIABETIC RATS FED ZINC DEFICIENCY DIET

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ABSTRACT

Objective: Oxidative stress which comes from hyperglycemia, it accelerates the development of cellular and vascular damage complications in diabetes, but the antioxidants may play a beneficial role in its prevention. Several plants extracts have an antioxidant activity and the ability to reduce oxidative stress in diabetes. Thus this study was conducted to investigate the effect of *Beta vulgaris var cicla* extract on zinc status, glucose concentration and antioxidant parameters in streptozotocin-diabetic rats fed zinc deficiency diet.

Methods: Twenty-eight male albino (Wistar) rats were divided into four groups: two groups fed a zinc-sufficient diet one non-diabetic and the other diabetic, while the others two diabetic groups were fed a zinc-deficient diet, one non-treated group and the other treated with the extract of *Beta vulgaris var cicla*. After 21 d of dietary manipulation, fasting animals were scarified. Blood glucose, tissues zinc (femur, liver, kidney), malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GSH-Px) and glutathione-S-transferase (GST) were evaluated.

Results: Body weight gain of zinc-deficient diabetic animals was lower than that of zinc-adequate diabetic animals. It was noticed also that inadequate dietary zinc intake increased glucose and MDA levels. In addition, zinc deficiency diet led to a decrease in zinc tissues, GSH concentration both GST and GSH-Px activities. However, Oral administration of *Beta vulgaris* extract significantly decreased both serum glucose and MDA ($p < 0.001$) levels, with a significant increase in body weight gain ($p < 0.001$), GSH concentration ($p < 0.05$, $P < 0.001$), GST ($p < 0.05$, $p < 0.001$) and GSH-Px ($p < 0.001$) activities.

Conclusion: The present study showed that *Beta vulgaris var cicla* supplementation presumably acting as an antioxidant, and it can be a natural source for the reduction of diabetes development caused by zinc deficiency.

Keywords: Diabetes, Rat, Zinc deficiency, *Beta vulgaris var cicla*, Antioxidant

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INTRODUCTION

Diabetes mellitus is a hereditary, chronic disorder in the endocrine system that constitutes a major public health problem throughout the world [1]. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism [2, 3]. As a consequence of the metabolic derangements in diabetes, many complications develop including coronary artery disease, nephropathy, retinopathy, and neuropathy [4]. Oxidative stress is thought to play a major role in the development of most of these complications [5]. Oxidative stress may occur when antioxidant mechanisms are not working properly as in dietary deficiencies of vitamin E, vitamin C or the essential elements such as selenium, zinc, and manganese among others. Another important cause of oxidative stress is the excessive endogenous production of free radicals by diseases progression as in diabetes mellitus and cancer [6]. Elevated glucose levels were recognized as a pathogenic factor of chronic diabetic complications by generating reactive oxygen species (ROS) and attenuating the antioxidative machinery via glycation of the antioxidant enzymes. The major ROS sources in the diabetic complications were: autooxidation of glucose, the activation of polyol pathways, mitochondrial respiratory chain deficiencies, xanthine oxidase activity, NAD (P)H oxidase, advanced glycation end products (AGEs) and nitric oxide synthase (NOS) [7]. Zinc is an essential component of numerous proteins, which play crucial roles in growth and development. It showed potent antioxidant and anti-inflammatory properties and was involved in the defence against oxidative stress [8]. Diabetic patients often suffer from zinc deficiency at the late stage, particularly in the patients whose glucose was poorly controlled [9]. In addition, zinc has been reported to exert antidiabetic effects in various experimental

models [10]. Thus, it seems that zinc deficiency increases the risk for several chronic disease states such as diabetes [11], and this risk may be associated with an increased vulnerability to oxidative stress [12]. *Beta vulgaris var. cicla* (chard) is an herbaceous biennial leafy vegetable cultivated in many parts of the world. This plant belongs to *chenopodiaceae* family. It is a low cost plant that has widespread use in many traditional dishes. Modern pharmacologists addressed the importance of bioactive molecules from *Beta vulgaris var cicla* extracts and demonstrated their antidiabetic, anti-inflammatory, antioxidant and anticancer activities. Different types of oral hypoglycemic agents such as *biguanides* and *sulphonylurea* are available along with insulin for the treatment of diabetes mellitus, but have side effects associated with their uses [13]. Therefore, there is an urgent need to search for the drugs of a natural origin with fewer side effects. Flavonoids, a class of natural drugs with high biological activity are abundant in plants. They are reported to have protective effects against the development of diabetes as well as a mitigation effect of diabetes consequences [14]. Therefore, the present investigation was aimed at evaluating the modulator effects of *Beta vulgaris var cicla* (chard) administration for prevention the development of diabetic pathology observed in zinc deficiency by evaluating body weight gain, zinc status, glucose level and antioxidant system in streptozotocine diabetic rats.

MATERIALS AND METHODS

Preparation of extract

Beta vulgaris var cicla (chard) aerial parts were collected in December 2012 from Guelma region (East of Algeria 36 ° 27' 58" North, 7 ° 26' 2" East). The collected aerial part of the plant material was washed thoroughly and dried in shade. The powdered plant material (250 g) was soaked in 80 % methanol and kept for three

days. The extract was concentrated to dryness by rotary evaporator in low pressure yielding the dried extract (21.45 g).

Determination of total polyphenols content

Total polyphenols in chard extract were determined with Folin-Ciocalteu reagent, according to the method of Li *et al.* [15]. Briefly, 200 μ l of the chard extract was mixed with 1 ml of Folin-Ciocalteu phenol reagent (10 x dilutions) and allowed to react for 4 min. Then 800 μ l of saturated $\text{Na}_2\text{-CO}_3$ solution was added and allowed to stand for 2 h before the absorbance of the reaction mixture was read at 765 nm. The total polyphenol contents of the extract were expressed as mg gallic acid equivalents per gram of extract.

Determination of total flavonoids content

Total flavonoids contents were estimated according to the Dowd method which was modified by Bahorun *et al.* [16]. Briefly, a diluted solution (1 ml) of each extract was mixed with an equal volume of aluminum trichloride (AlCl_3) in methanol (2%). The absorbance was read at 430 nm after 10 min. Quercetin was used as reference compound to produce the standard curve and the results were expressed as g of quercetin equivalents (QE)/kg of dry mass

Determination of DPPH radical scavenging activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of the chard extract was measured according to the procedure described by Mansouri *et al.* [17]. Appropriate dilution series of extract (0–2 mg/ml) were prepared in methanol 25 μ l of each dilution was added to 975 μ l of a 6x10⁻⁵M methanolic solution of DPPH followed by vortexing. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. The decrease in absorbance of the resulting solution was then measured spectrophotometrically at 517 nm against methanol. All measurements were made in triplicate and averaged. The DPPH radical scavenging activity was calculated using the following equation:

$$\% \text{ scavenging activity} = [(A_0 - A_1)/A_0] * 100$$

Where A_0 is the absorbance of control and A_1 is the absorbance of test or standard.

Animals

Male albino Wistar rats weighing 200–250g with age 10–12 w, were obtained from Pasteur institute; Algiers, Algeria. Prior to experiments, the animals were allowed to acclimate to their surroundings for two weeks. Rats were housed in individual plastic cages with bedding. Standard rat food and tap water were available *ad-libitum* for the duration of the experiments. The temperature was maintained at 22 \pm 2 $^\circ\text{C}$. A 12/12-h light/dark cycle was maintained, with lights on at 06.00 AM, unless otherwise noted.

Induction of experimental diabetes and diet preparation

Diabetes was induced with fresh streptozotocine (STZ) solution using a previously described method [18]. STZ was intraperitoneally administered at a dose of 50 mg/kg body weight dissolved in citrate buffer (0.01 M, pH 4.5). Blood glucose was measured seven days after induction of diabetes on samples taken from tail vein. The diabetic state was confirmed by a glucose-meter (ACCU-CHEK, Roche Diagnostics, Paris, France) when the glucose concentration exceeded 14 mmol/l. The diet for rats consisted of (in grams per kilogram diet): cornstarch 326, sucrose 326, protein 168 (egg white solids), lipids 80 (corn oil), fiber 40 (cellulose), vitamin mix (sigma) and mineral mix 40. The latter was formulated to contain either adequate (54 mg/kg) [19] or inadequate (1.2 mg/kg) quantities of Zn, as determined by atomic absorption spectroscopy. The mineral mix supplied (in grams per kilogram diet) calcium hydrogen orthophosphate 13; disodium hydrogen orthophosphate 7.4; calcium carbonate 8.2; potassium chloride 7.03; magnesium sulphate 4; ferrous sulphate 0.144; copper sulphate 0.023; potassium iodide 0.001, manganese sulphate 0.180 and zinc carbonate 0.1. The zinc deficiency diet contained no additional zinc carbonate.

Experimental design

Twenty eight rats were divided into four groups seven each. The first group was non-diabetic rats fed with sufficient zinc diet containing 54 mg Zn/kg diet (ND). The second group diabetic rats received sufficient zinc diet (DSZ). The third group diabetic zinc deficient rats given a diet containing 1.2 mg Zn/kg diet (DZD). The fourth group diabetic zinc deficient rats treated orally with methanolic extract of *Beta vulgaris* at dose of 500 mg/kg (DZD-B). The treatment of animals was carried out for 21 days. Body weight was recorded regularly and blood was collected after overnight-fasting from the tail vein at 0, 7, 14 and 21 days for glucose determination using the glucose-meter.

Tissue samples preparation

On day 21 rats were sacrificed under ether anesthesia. Heart, kidneys and liver were excised, washed with isotonic saline, and blotted to dry. The right femur was taken, and the connective tissues and muscle were removed. After that, one kidney of each animal, femur, and one fragment of liver were weighed and dried at 80 $^\circ\text{C}$ for 16 h and zinc concentration in each tissue was determined. Heart, the second kidney and the second fragment of liver were processed immediately for assaying reduced glutathione (GSH), Malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and glutathione S-transferase (GST).

Tissues zinc analyses

The dried kidneys, livers and femurs were heated in silica crucibles at 480 $^\circ\text{C}$ for 48 h and the ash dissolved in hot 12 M hydrochloric acid for zinc analysis utilizing a flame atomic absorption spectrophotometer (Pye Unicam SP 9000, Hitchin, UK). 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 mg/ml zinc nitrate standard solution using 5% glycerol to approximate the viscosity characteristics [20]. 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.

Lipid peroxidation and antioxidant analyses

Tissue preparation

About 1 g of liver, heart and kidney was homogenized in 2 ml ice cold TBS (50 mmol Tris, 150 mmol NaCl, pH 7.4). Then the homogenates were centrifuged at 10000 \times g for 15 min at 4 $^\circ\text{C}$, and the resultant supernatant was used for the determination of MDA, GSH, proteins, GSH-Px and GST.

Lipid peroxidation estimation

Malondialdehyde (MDA), a terminal product of lipid peroxidation, was measured to estimate the extent of lipid peroxidation in liver, heart and kidney homogenate by using the method described by Ohkawa *et al.* [21], which was based on TBA reactivity. Briefly, 0.5 ml of 20% trichloroacetic acid, 0.5 ml of homogenate and 1 ml of 0.675% TBA were mixed into tubes. Then the mixture was warmed for 15 min at 100 $^\circ\text{C}$. Tubes were cooled in room temperature and centrifuged at 3000 rpm for 10 min. 4 ml ml of n-butanol was added. The optical density of supernatant was measured at 532 nm and the malondialdehyde (MDA) was expressed as nmol/mg protein

Estimation of reduced glutathione

The reduced glutathione was estimated utilizing a colorimetric technique as mentioned by Ellman [22] and modified by Jollow *et al.* [23] based on the development of a yellow color when DTNB is added to compounds containing sulfhydryl groups. The GSH concentration (nmol GSH/mg protein) was obtained from the absorbance at 412 nm.

Assay of glutathione-S-transferase

GST activity was estimated according to the method of Habig *et al.* [24], using p-nitrobenzyl chloride as substrate. The absorbance was measured

at 340 nm at 30-second intervals for 3 min. The enzyme activity was expressed as $\mu\text{mol CDNB-GSH conjugate formed}/\text{min}/\text{mg protein}$.

Assay of glutathione peroxidase

The enzymatic activity of glutathione peroxidase (GSH-Px) was measured by the method of Flohe and Günzler [25]. This method was based on the reduction of hydrogen peroxide (H_2O_2) in the presence of reduced glutathione (GSH), the latter is transformed into (GSSG) under the influence of GSH-Px and the reading absorbance was at 412 nm. The enzyme activity was expressed as $\mu\text{moles of reduced GSH}/\text{min}/\text{mg protein}$.

Protein determination

Protein concentration in the tissue homogenates was determined by Bradford method [26], using bovine serum albumin as a standard.

Statistical analysis

Results were expressed as $\text{mean} \pm \text{SEM}$. Statistical significance of the results obtained for various comparisons was estimated by applying one-way analysis of variance (ANOVA) followed by Student's *t*-test to compare means between the different groups and the level of significance was set at $p < 0.05$.

RESULTS

Total polyphenol and flavonoid

Total polyphenol content (mg/g) determined by Folin-Ciocalteu colorimetric method was 31.231 ± 0.0066 (GAE mg/g of extract) for methanol extract of chard. Polyphenol content was determined by a linear regression equation of gallic acid and expressed as GAE of extract ($y = 0.0069x + 0.0105$, $r^2 = 0.999$). The flavonoid content determined by aluminum chloride method was found to be 6.85 ± 0.087 (QE mg/g of extract). Flavonoids content was determined by a linear regression equation of quercetin and expressed as QE of extract ($y = 0.0049x + 0.0034$, $r^2 = 0.999$).

DPPH radical scavenging activity

The extract was capable of scavenging DPPH radicals in a concentration dependent manner. Ascorbic acid was used as reference for radical scavenger. The scavenging activity of chard extract and ascorbic acid on DPPH radicals increased between 0–2 mg/ml and were 58.68% and

95.72% at a concentration of 2 mg/ml , respectively. DPPH scavenging activity is best presented by EC50 value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution. A higher DPPH radical scavenging activity was associated with a lower EC50 value. EC50 values for chard extract on DPPH radical scavenging activity was found as 1.74 mg/ml (fig. 1).

Body weight gain

Body weight gain is shown in table 1. The results indicated a very significant reduction in body weight of diabetic rats compared to non-diabetic rats. Meanwhile, body weight of diabetic animals fed zinc deficiency (DZD) was lower ($p < 0.001$) than diabetic rats given adequate zinc diet (DSZ). However, the treatment with the extract was ameliorated the growth rate in DZD (table 1).

Femur, kidney and liver zinc concentrations

Zinc level in the three organs (kidney, liver; femur) of diabetic rats (DSZ) was generally lower ($p < 0.05$, $p < 0.001$) than non-diabetic rats. On the other hand zinc concentration in liver ($p < 0.01$), femur ($p < 0.001$) and kidney ($p < 0.05$) were significantly lower in DZD group than in DSZ group. However, zinc tissues contents were significantly restored in DZD-B group (table 1).

Fasting blood glucose level

The determination of fasting blood glucose level (FBG) on 0, 7th, 14th and 21th day, showed that STZ injection in rats led to a significant increase in FBG level. It was also noticed that a significant increase in FBG concentration in Zn-deficient diabetic animals. Meanwhile, a clear reduction in FBG levels was observed from the 7th day after the treatment of Zn-deficient diabetic animals with the methanol extract (fig. 2).

MDA, GSH, GSH-Px and GST values

MDA, GSH, GSH-Px, and GST values are shown in fig. 3, 4, 5 and 6 respectively. The control diabetic rats had high MDA level and low GSH content, GSH-Px activity and GST activity in all studied organs as compared to non-diabetic rats. Moreover, it was observed that zinc deficiency caused high MDA in liver, heart and kidney of diabetic animals with a decrease in GSH, GST and GSH-Px. Whereas, *Beta vulgaris var cicla* administration resulted an improvements of the previous hepatic, cardiac and renal oxidative stress markers.

Table 1: Body weight gain, tissue zinc levels in non-diabetic rats (ND), diabetic zinc sufficient rats (DSZ), diabetic zinc-deficient rats (DZD) and diabetic zinc-deficient rats given methanol extract of *Beta vulgaris* (DZD-B)

Groups	Initial body weight (g) mean \pm SEM	Final body weight(g) mean \pm SEM	Femur zinc ($\mu\text{g}/\text{g}$ dry wt) mean \pm SEM	Kidney zinc ($\mu\text{g}/\text{g}$ dry wt) mean \pm SEM	Liver zinc ($\mu\text{g}/\text{g}$ dry wt) mean \pm SEM
ND	134 \pm 6.4	159.2 \pm 3.36	73 \pm 3	68.13 \pm 5.85	66 \pm 4.37
DSZ	173.2 \pm 9.76	154.4 \pm 8.72	60.34 \pm 4.11	56 \pm 6.33	43 \pm 6.65
DZD	170 \pm 8.4	133.6 \pm 14.48	44.34 \pm 2.64	53 \pm 7.25	39 \pm 2.49
DZD-B	133.8 \pm 22.64	159.3 \pm 10.53	53.4 \pm 3.54	63.23 \pm 4.52	51 \pm 3.97

^a $p < 0.05$, ^{a2} $p < 0.001$: comparison of DSZ with ND; ^b $p < 0.05$, ^{b1} $p < 0.01$, ^{b2} $p < 0.001$ comparison of DZD with DSZ; ^c $p < 0.05$, ^{c1} $p < 0.001$ ^{c2} $p < 0.001$: comparison of DZD-B with DZD., number of samples=7.

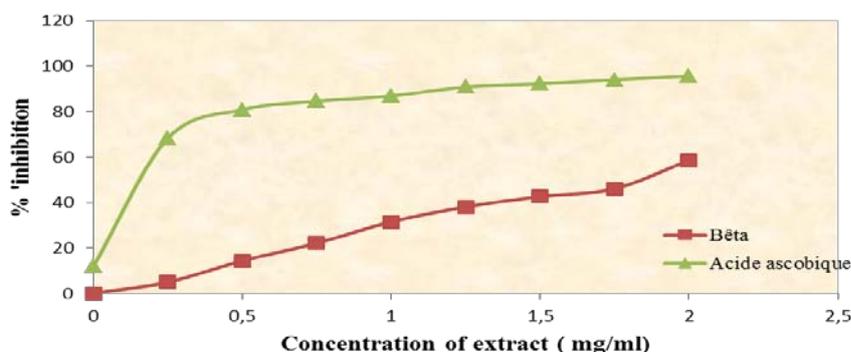


Fig. 1: Antioxidant activity of *Beta vulgaris* extract by DPPH free radical method

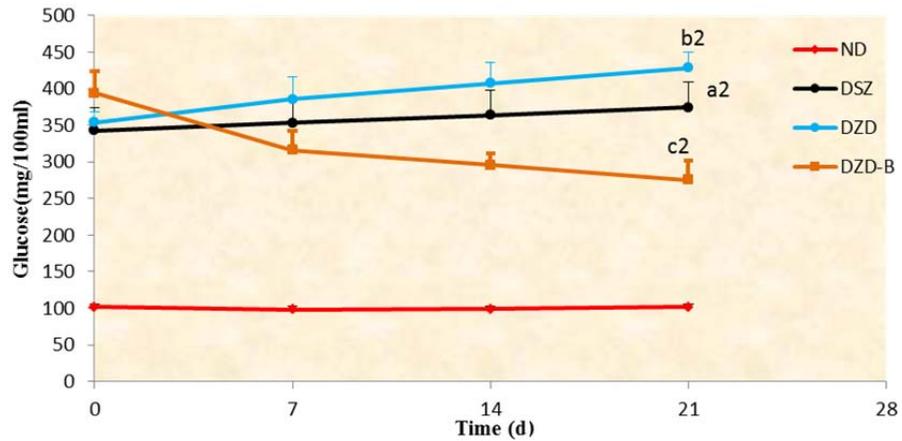


Fig. 2: Blood glucose levels in non-diabetic rats (ND), diabetic zinc sufficient rats(DSZ), diabetic zinc deficient rats (DZD)and diabetic zinc deficient rats were given methanol extract of *Beta vulgaris var cicla* (DZD-B)

^{a2}p<0.001: comparison of DSZ with ND; ^{b2}p<0.001 comparisons of DZD with DSZ; ^{c2}p<0.001: comparison of DZD-B with DZD. Values are mean±SEM, a number of samples = 7.

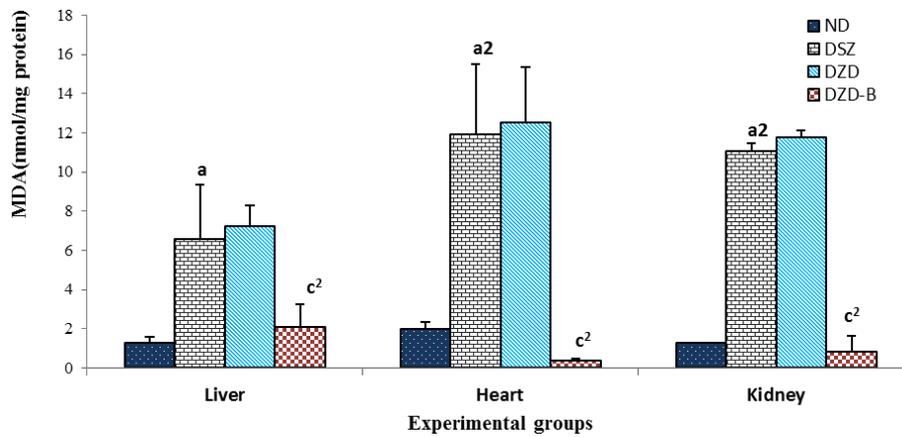


Fig. 3: MDA level in non-diabetic rats (ND), diabetic zinc sufficient rats(DSZ), diabetic zinc deficient rats (DZD)and diabetic zinc deficient rats were given methanol extract of *Beta vulgaris var cicla* (DZD-B)

^ap<0.05, ^{a2}p<0.001: comparison of DSZ with ND; ^{c2}p<0.001: comparison of DZD-B with DZD. Values are mean±SEM, number of samples = 7.

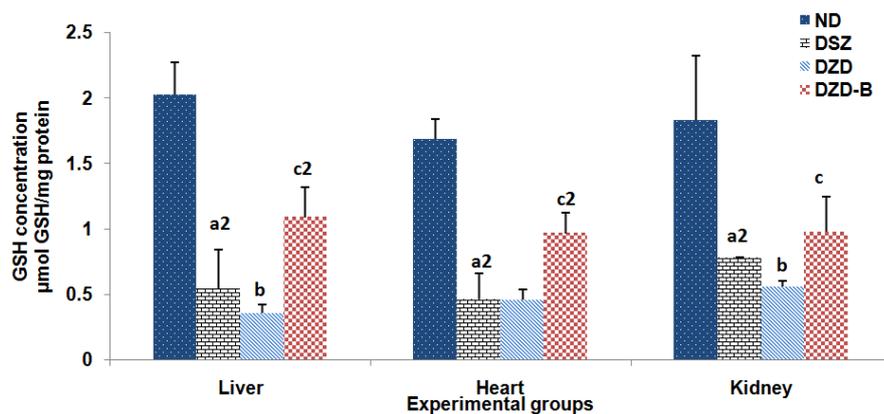


Fig. 4: GSH level in non-diabetic rats (ND), diabetic zinc sufficient rats(DSZ), diabetic zinc deficient rats (DZD)and diabetic zinc deficient rats given methanol extract of *Beta vulgaris var cicla* (DZD-B)

^{a2}p<0.001: comparison of DSZ with ND; ^bp<0.05 comparison of DZD with DSZ; ^cp<0.05 ^{c2}p<0.001: comparison of DZD-B with DZD. Values are mean±SEM, number of samples = 7.

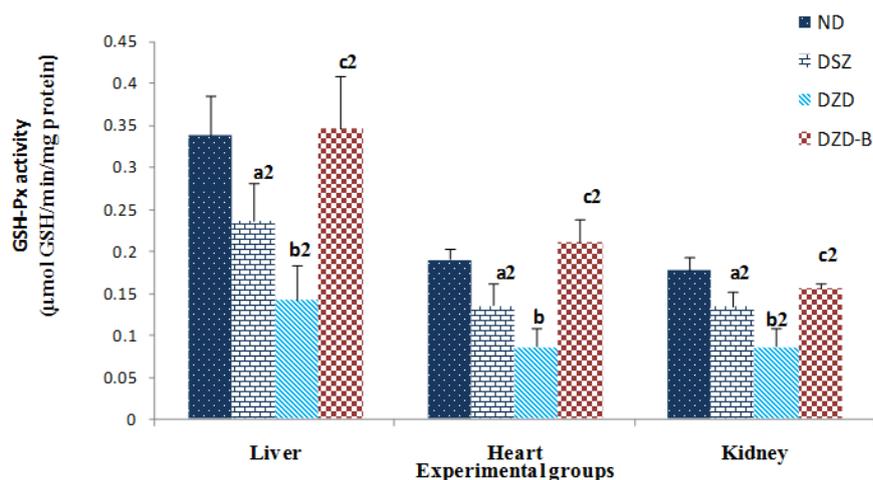


Fig. 5: GSH-Px activity in non-diabetic rats (ND), diabetic zinc sufficient rats (DSZ), diabetic zinc deficient rats (DZD) and diabetic zinc deficient rats given methanol extract of *Beta vulgaris var cicla* (DZD-B)

^{a2}p<0.001: comparison of DSZ with ND; ^bp<0.05, ^{b2}p<0.001 comparison of DZD with DSZ; ^{c2}p<0.001: comparison of DZD-B with DZD. Values are mean±SEM, a number of samples = 7.

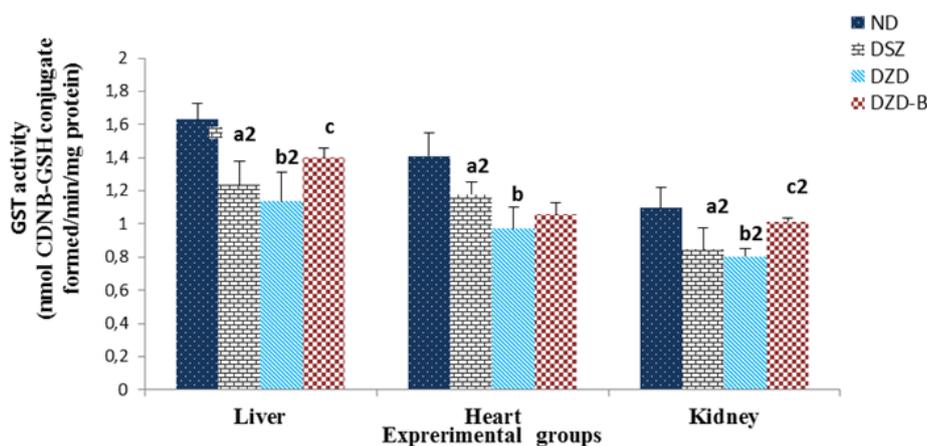


Fig. 6: GST activity in non-diabetic rats (ND), diabetic zinc sufficient rats (DSZ), diabetic zinc-deficient rats (DZD) and diabetic zinc-deficient rats given methanol extract of *Beta vulgaris var cicla* (DZD-B)

^{a2}p<0.001: comparison of DSZ with ND; ^bp<0.05, ^{b2}p<0.001 comparison of DZD with DSZ; ^cp<0.05, ^{c2}p<0.001: comparison of DZD-B with DZD. Values are mean±SEM, number of samples = 7.

DISCUSSION

Plants have been used for many years as a source of traditional medicine to treat various diseases and conditions. Many of these medicinal plants are also excellent sources for phytochemicals, many of which contain potent antioxidant and antidiabetic factors. Therefore, several studies were conducted to evaluate the secrets of plants among them this study, which is devoted to search for the potential antioxidant and antidiabetic effects of methanol extract aerial part of *Beta vulgaris var cicla* in animals fed a low zinc diet. According to the findings obtained *Beta vulgaris var cicla* is rich in phenolic compounds and flavonoids. Phenolic compounds such as quercetin, rutin, narigin, catechin, caffeic acid, gallic acid and chlorogenic acid, which are very important plant constituents [27]. Medicinal plants are known to produce diverse substances possessing antioxidant properties having ability to protect the human body against cellular oxidation. Anti-oxidation are vital substances which possess the ability to protect the body from damage caused by free radicals inducing oxidative stress [28]. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide of lipid hydroxyl and halt the oxidative mechanism that leads to degenerative diseases [29]. Flavonoids are proactive

polyphenolic compounds found in most plants and cannot be synthesized or produced by the humans [30]. They were found to be effective in controlling various biological activities. In other words, they are anti-inflammatory, anti-angiogenic, antimicrobial, antioxidant, hypertension reduction and have anti-cholesterol properties [31]. In recent times, research interest has been paid to polyphenols and flavonoids as a result of their antioxidant capacity which is principally based on the redox properties of their hydroxyl groups and the structural relationship between different functional groups in their structure which enable them to actively serve as free radical scavengers, reducing agents, singlet oxygen quenchers metal chelators, and hydrogen donors [32]. *B. vulgaris var cicla* is rich in minerals, vitamins and phytochemicals and is one of the healthiest vegetables. Main identified secondary metabolites are flavonoids, flavonoid glycosides and saponins. Antioxidative activity of different Swiss chard extracts and their components is proven in several tests, as well as anti-mitotic activity on MCF-7 human breast cancer cells, antiproliferative activity on human colon cancer and hypoglycemic activity [33]. Phenolic content and antioxidant activities have been most frequently studied. In comparison with other largely consumed vegetables in the Mediterranean diet, Swiss chard displays high antioxidant activity [34]. In the current study, diabetic rats weighed

less than non-diabetic rats, this is consistent with some previously published reports [35]. This raises the possibility of the metabolic state disturbance of animal, suggesting that the diabetic condition had exacerbated reduced the ability of the diabetic rats to utilize food intake as normal subjects. On the other hand, rats fed a zinc deficiency diet had less body weight gain compared with rats fed a zinc adequate diet, which is concomitant with previously published investigations [36]. It has been well documented that rats fed low zinc diet voluntarily decrease diet consumption (reduce appetite) and maintain a very low growth rate [37]. Body weight gain of diabetic rats fed a low zinc diet and administrated with *Beta vulgaris* extract was higher than that of zinc deficient diabetic rats. The ameliorative in body weight of these animals might be as a result of an increase in daily food consumption and promotion of protein synthesis. Moreover *Beta vulgaris* extract has the ability to reverse gluconeogenesis and control protein loss [38]. Zinc tissues concentrations including liver, femur and kidney in diabetic rats were lower than that of non-diabetic rats. These findings, indicating the effect of diabetes on body zinc status. It has been postulated that low level of zinc in diabetic patients may be due to excessive urinary output and gastro intestinal malabsorption [39, 40]. The results of this investigation showed also that there was a significant decrease of zinc content in the previous organs of diabetic animals fed inadequate zinc diet compared to their diabetic fed adequate zinc diet, which was coincide with previous investigations [37, 41]. On the other hand zinc concentration in various tissues of zinc deficient diabetic rats treated with methanol extract was restored. This is probably due to the antioxidant effect of this plant extract against the development of diabetic state resulting in a decline of urine zinc loss. The mean fasting blood glucose concentration in animals fed low zinc diet was found to be higher than that of those receiving adequate dietary zinc during the experiment period. This may be related to altered glucose utilization by tissues or to increased rate of endogenous glucose production [42]. Moreover, blood glucose was reduced in Zn-deficient diabetic animals that were treated with chard as compared to Zn-deficient animals. Experiments performed by Yanardag *et al.* [43] demonstrated the hypoglycemic effect of *Beta vulgaris var cicla* extract in diabetic rats. This hypothesis was substantiated by further studies of the same group, who demonstrated a 40% reduction of glycaemia, without any loss of weight or impairment of liver functions [34, 44]. The mechanism for the hypoglycemic action of the extract has been tentatively attributed to saponins that inhibit gluconeogenesis and glycogenolysis [45]. However, other molecular pathways potentially involved in hypoglycemic effects remain to be deeply investigated. In fact, some evidences suggested that the hypoglycemic activity of chard extract might be due to flavonoids through the inhibition of glucose transporters. For instance, quercetin which is present in chard, showed evidence of anti-diabetic effects via inhibition of the intestinal glucose transporter GLUT2 [46]. Another complementary hypoglycemic mechanism could be the flavonoid induced inhibition of the α -amylase and α -glucosidase activities [47]. The inhibition of this enzyme could delay the digestion and absorption of carbohydrates and consequently suppress post-prandial hyperglycemia [48]. Some C-glycosylflavones, i.e. vitexin, vitexin-2-O-glycoside and VOR contained in *Beta vulgaris* leaves and seeds, were found to be strongly inhibit α -glucosidase and could be the most probable cause of the hypoglycemic effect [34]. In general, the reduced antioxidant capability in diabetes was the result of increased production of oxygen metabolites, which curbs the activity of the antioxidant defense system [49]. Moreover, several studies demonstrated increased free radical production or increased oxidative damage in response to zinc deficiency *in vitro* or *in vivo* [50]. Zinc status has been shown to affect glutathione concentrations in tissues. It was found an increase in MDA and a reduction of GSH, GSH-Px and GST in liver, heart and kidney of diabetic rats fed a low zinc diet. The increase of MDA confirms the deleterious effect of zinc deficiency in increasing lipid peroxidation as already reported in animals [51]. Glutathione acts synergistically with zinc in protecting sulfhydryl groups, the reason for depletion of glutathione might have contributed to the higher consumption of glutathione and higher oxidative damage in zinc deficient rats [52]. The observed decline in GSH-Px and GST activities probably due to modification of the sulfhydryl groups in these enzymes by oxygen free radicals [53]. Elevated glucose and hydrogen peroxide levels have also been found to inactivate GST and GSH-Px [54]. This study confirmed a beneficial effect of *Beta vulgaris var cicla* in attenuating oxidative stress and

oxidative damage. In other words, the findings showed a significant reduction in the formation of TBARS level and an augmentation of GSH concentration with an improvement of both GSH-Px and GST activities. Thus, it has been reported that several polyphenol compounds isolated from *Beta vulgaris* possess a strong antioxidant properties, which reduce the formation of ROS by directly inhibiting the reactive oxygen generating enzymes [31]. El-Gamal *et al.* [55] indicated that chard treatment decreased the renal MDA levels and increased the CAT activity in gentamicin induced nephrotoxicity in rats. Jain and Singhai [56] reported that chard administration significantly decreased the MDA level and increased the GSH concentration in rats and suggested that the antioxidant effect of chard plays an important protective role against ethanol-mediated toxicity. In addition, Ozsoy-Sacan *et al.* [57] mentioned significantly decreased liver MDA and increased GSH levels in chard administered diabetic rats. Chakole *et al.* [58] reported also that chard administration reduced the LPO and prevented necrosis in the paw of rats. It has been suggested that the antioxidant and free radical-scavenging activities might be also due to the presence of terpenoids in chard. Pyo *et al.* [59] showed a linear correlation between radical-scavenging effect and polyphenolic concentration of chard. Phenolic acids and flavonoids containing multiple hydroxyl groups have higher antioxidant activities against free radical species.

CONCLUSION

The present study indicated that the combination of zinc deficiency and diabetes affected the growth rate, zinc status, carbohydrate metabolism and antioxidant system. Whereas, the administration of methanol extract of *Beta vulgaris var cicla* reduced these severity complications, suggesting that *Beta vulgaris var cicla* has an important effect as a most potent agent in protecting against the clinical disease associated with increased free radical activity as a result of zinc deficiency.

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AUTHORS CONTRIBUTION

Zine Kechrid formulated the present hypothesis. Zine Kechrid with Malika Hamdiken were responsible for writing the report. Malika Hamdiken was also responsible for the analysis of the data.

CONFLICT OF INTERESTS

Declared none

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