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Original Research

Effect of *Ruta chalepensis* on Zinc, Lipid Profile and Antioxidant Levels in the Blood and Tissue of Streptozotocin-Induced Diabetes in Rats Fed Zinc-Deficient Diets

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Key Messages

1) Despite the continuing introduction of hypoglycemic drugs, diabetes and related complications is still a major global medical problem.
2) The modern hypoglycemic drugs including insulin and other oral hypoglycemic agents such as thiazolidinediones, biguanides, sulphonylure as, control the glucose blood level as long as they are regularly administered, but they also produced lots of undesirable effects.
3) The search for appropriate hypoglycemic agents has been focused on plants used in traditional medicine. In other words, finding effective compounds with fewer side effects to treat diabetes and its complications.

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ABSTRACT

In diabetes, oxidative stress and lipid abnormalities are common and pronounced and represent important factors that are involved in the development of complications of diabetes. Zinc deficiency generally induces oxidative stress, but it is well known that the antioxidant *Ruta chalepensis* has an effective modulator role in oxidative stress in metabolic diseases. The aim of this study was to investigate the effect of *R. chalepensis* extract on blood biochemical parameters, tissue zinc status and antioxidant systems in rats with diabetes that were fed zinc-deficient diets.

We divided 28 male albino Wistar rats into 4 groups: 2 groups, 1 group with diabetes, 1 group without diabetes, were fed zinc-sufficient diets, while the other 2 groups of rats with diabetes were fed zinc-deficient diets. One group was not treated, and the other was treated with the extract of *R. chalepensis*. After 3 weeks of dietary manipulation, the fasting animals were killed.

The body-weight gains of the zinc-deficient animals with diabetes were lower than those of the zinc-adequate animals with diabetes. It was noticed also that inadequate dietary zinc intake increased the glucose, cholesterol, triglyceride, urea, uric acid, creatinine and lipid peroxidation levels. In addition, the zinc-deficient diet led to a decrease in zinc tissues (femur, liver, kidney), glutathione concentration and both glutathione peroxidase and glutathione-S-transferase activities. However, *R. chalepensis* treatment ameliorated all the previous parameters approximately to their normal levels.

It seems that *R. chalepensis* supplementation is a potent factor in reducing the oxidative severity of zinc deficiency in experimental diabetes through its hypoglycemic and antioxidant actions.

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RÉSUMÉ

Lors de diabète, le stress oxydatif et les anomalies lipidiques sont fréquents et prononcés, et représentent les facteurs importants qui participent à l'apparition des complications du diabète. La carence en zinc induit généralement un stress oxydatif. Lors de maladies métaboliques, l'antioxydant *Ruta chalepensis* estbien connu pour son rôle efficace de modulateur du stress oxydatif. L'objectif de la présente étude était d'examiner les effets de l'extrait de *R. chalepensis* sur les paramètres biochimiques du sang, le statut en zinc tissulaire et les systèmes antioxydants chez les rats diabétiques qui suivent un régime carencé en zinc.

Nous avons réparti 28 rats Wistar albinos mâles en 4 groupes : 2 groupes, dont 1 groupe de rats diabétiques et 1 groupe de rats non diabétiques, qui ont suivi un régime suffisant en zinc, alors que les 2 autres groupes de rats diabétiques ont suivi un régime carencé en zinc. Un groupe n'a pas reçu de traitement, et l'autre groupe a reçu un traitement à l'extrait de *R. chalepensis*. Après 3 semaines de manipulation alimentaire, les animaux à jeun ont été abattus.

La prise de poids des animaux diabétiques carencés en zinc étaient plus faible que ceux des animaux diabétiques dont l'apport en zinc était adéquat. On a également remarqué qu'un apport alimentaire inadéquat en zinc augmentait le glucose, le cholestérol, les triglycérides, l'urée, l'acide urique, la créatinine et les taux de peroxydation lipidiques. De plus, le régime carencé en zinc menait à une diminution du zinc dans les tissus (fémur, foie, reins), de la concentration en glutathion et des activités de la glutathion-peroxydase et de la glutathion-S-transférase. Toutefois, le traitement par *R. chalepensis* rendait approximativement à tous les paramètres précédents leur taux normal.

Il semble que la supplémentation en *R. chalepensis* soit un facteur puissant de réduction de la gravité oxydative de la carence en zinc lors de diabète expérimental du fait de son action hypoglycémique et antioxydante.

Introduction

Diabetes mellitus is a serious and complicated metabolic disorder. Worldwide, 415 million adults were living with diabetes in 2015 (global prevalence, 8.8%), and the estimate is projected to rise to more than 642 million by 2040 (1). Diabetes is widely reported to be accompanied by increased levels of oxidative damage, which are associated with hyperglycemia, hypoinsulinemia, hypercholesterolemia and insulin resistance (2,3). The vast majority of cases of diabetes fall into 2 broad etiopathogenic categories: patients with type 1 diabetes have a deficiency of insulin secretion, whereas those with type 2 diabetes have a combination of resistance to insulin action and inadequate compensatory insulin secretory response (4). Patients with diabetes have increased oxidative stress and impaired antioxidant defense systems, which appear to contribute to the initiation and progression of diabetes-induced complications (5). Zinc is the most abundant trace intracellular element required for a number of cellular processes, including cell proliferation, reproduction, immune function and defense against free radicals (6). It is a component of more than 1000 zinc-associated transcription factors, including DNA-binding proteins with zinc fingers, and is required for more than 300 zinc-containing metalloenzymes (7). Zinc is required for normal insulin metabolism. Zinc is concentrated in the islet cells that are related to the synthesis, storage and secretion of insulin (8). It seems to be reasonable, therefore, that changes in body zinc status could affect the production, storage and secretion of insulin (9). Zinc is an important cofactor of many antioxidant enzymes. In other words, it is an essential component of Cu/Zn superoxide dismutase (SOD) and also helps to minimize the effects of inflammatory substances, thereby preserving cell health and insulin sensitivity (10). Another mechanism that explains the antioxidant role of zinc in diabetes refers to its ability to compete with iron and copper for binding sites on cell membranes. The iron and copper ions can catalyze the production of lipid peroxides, and the replacement of these metals by zinc in the plasma membrane could prevent lipid peroxidation in patients with diabetes (11). Therefore, zinc deficiency increases the risk for several chronic disease states, such as diabetes (12), and this risk may be associated with an increased vulnerability to oxidative stress (13). Indeed, increasing evidence suggests that zinc plays an important role as an antioxidant and protects cellular components from oxidation (14). Several studies have demonstrated increases in oxidative stress in cases of low cellular zinc content (15). The plant *Ruta chalepensis* has been used in many treatments, such as against obesity (16), as an antibacterial (17) and as a stimulatory for reproduction (18). So this study was carried out to examine the modulator effects of *R. chalepensis* administration for prevention of the development of diabetic pathology observed in zinc deficiency by evaluating body weight gain, zinc status, carbohydrate metabolism and the antioxidant system in rats with streptozotocine-induced diabetes.

Methods

Animals

Male albino Wistar rats weighing 200 to 250 g, 10 to 12 weeks of age, were obtained from the Pasteur Institute in Algiers. Prior to experiments, the animals were allowed to acclimate to their surroundings for 2 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 unless otherwise noted. The temperature was maintained at 22°C±2°C. A 12/12-hour light/dark cycle was maintained, with lights on at 6 AM, unless otherwise noted. The Ethical Committee of Annaba University, Algeria, approved the study protocol. The animals were maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory animals prepared by Annaba University, Algeria.

Preparation of extract

R. chalepensis aerial parts were collected in December 2012 from the Guelma region (east of Algeria). The samples were dried in a ventilated place at room temperature. The dry plant was then powdered in an electric grinder, and 250 grams of powder were extracted 3 times with 80% methanol. The extract was filtered, concentrated in a vacuum evaporator (Bûchi, New Castle, Delaware, United States) at 40°C and then lyophilized. The yield of the lyophilized extract was 23.45% of the initial crude material. This extract was stored in the dark at room temperature.

Phytochemical screening

Preliminary screening of secondary metabolites, such as alkaloids, flavonoids, saponins, tannins and terpenoids, were carried out according to the common phytochemical methods described by Trease and Evans (19). All tests were realized in a solution of the extract of *R. chalepensis* dissolved in methanol with a concentration of 10%, and the methods were as follows.

Test for flavonoids

The methanol extract (5 mL) was added to a concentrated of HCl (1 mL) and 0.5 g of Mg. A pink or red coloration that disappear on standing (3 min) indicated the presence of flavonoids.

Test for saponins

Five mL of the extract was shaken vigorously with 10 mL of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

Test for tannins

About 2 mL of the extract was stirred with 2 mL of distilled water, and few drops of ferric chloride (FeCl3) solution were added. Formation of green precipitate was an indication of the presence of tannins.

Test for alkaloids

An evaporate of 20 mL of methanol extract, the dry residue, was dissolved in 5 mL of HCl (2N) and filtered. A few drops of Mayer reagent and Wagner were added. The presence of precipitate indicated the alkaloids.

Test for terpenoids

Then 2 mL of the organic extract were dissolved in 2 mL of $CHCl_3$ and evaporated to dryness, and 2 mL of H_2SO_4 were then added and heated for about 2 minutes. Development of a grayish color indicated the presence of terpenoids.

Acute toxicity study

Animals were randomly allotted to control and test groups. The acute toxicity of each extract (lethal dose, 50%) (LD50) was estimated by the oral route using the procedure reported by Lorke (20). The method estimates the dose of the extract that will kill 50% of the population by a given route. The investigation was carried out with a minimum number of experimental animals. Extract was given at doses of 100, 250, 500, 1000, 2000 and 5000 mg/kg. Rats were kept under observation for 72 hours. Body weights, symptoms of toxicity, behaviour changes and mortality were controlled.

Induction of experimental diabetes

Diabetes was induced by a fresh streptozotocine solution, which was intraperitoneally administered at a dose of 50 mg/kg body weight after being dissolved in citrate buffer (0.01 M, pH 4.5). Blood glucose levels were measured 7 days after induction of diabetes in samples taken from the 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 (in grams per kg of diet) of cornstarch 326, sucrose 326, protein 168 (egg-white solids), lipids 80 (corn oil), fibre 40 (cellulose), vitamin mix (sigma) and mineral mix 40. The latter was formulated to contain either adequate (54 mg/kg) (21) or inadequate (1.2 mg/kg) quantities of zinc, as determined by atomic absorption spectroscopy. The mineral mix was supplied (in grams per kilogram of diet) by 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-deficient diet contained no additional zinc carbonate.

Experiment design

The 28 rats were divided into 4 groups of 7 each. The first group included rats *without* diabetes that were fed a zinc-sufficient diet containing 54 mg zinc/kg (ND). The second group included rats *with* diabetes that received a zinc-sufficient diet (DSZ). The third group included rats *with* diabetes that were fed a zinc-deficient diet containing 1.2 mg zinc/kg diet (DZD). The fourth group included zinc-deficient rats *with* diabetes that were treated orally with methanolic extract of *R. chalepensis* at doses of 500 mg/kg (DZD-R). The treatment of the animals was carried out for 3 weeks.

Blood collection and preparation of blood and tissue samples

Rats were killed by cervical cuts under ether anesthesia. Then 2 mL of blood were drawn and used for determination of serum glucose, triglyceride, urea, uric acid, creatinine and cholesterol levels. The heart, kidneys and liver were excised, washed with isotonic saline and blotted to dry. The right femur was taken, and the connective tissues and muscles were removed. After that, the kidneys, femurs and 1 fragment of the livers were weighed and dried at 80°C for 16 hours, and the zinc concentration in each tissue was determined. Hearts, kidneys and the second fragment of livers were processed immediately for assaying reduced glutathione (GSH), malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and glutathione-S-transferase (GST).

Measurement of biochemical parameters

Urea, uric acid, creatinine, cholesterol and triglyceride concentrations were measured using commercial kits (Spinreact, Girona, Spain).

Tissues zinc analyses

The dried kidneys, livers and femurs were heated in silica crucibles at 480°C for 48 hours, and the ash was dissolved in hot 12 M HCl acid for zinc analysis utilizing a flame atomic absorption spectrophotometer (Pye Unicam SP 9000, Hitchin, United Kingdom). 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. All tubes were soaked in HCl (10% v/v) for 16 hours and rinsed with doubly distilled water to avoid zinc contamination from exogenous sources.

Lipid peroxidation and antioxidant analyses

Tissue preparation

About 1 gram of liver, heart and kidney was homogenized in 2 mL ice-cold TBS (50 mM Tris, 150 mM NaCl; pH 7.4). Then the homogenates were centrifuged at 10 000×*g* for 15 minutes at 4°C, and the resultant supernatant was used for the determination of MDA, GSH, proteins, GST and GSH-Px.

Lipid peroxidation estimation

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 (22), which was based on thiobarbituric acid (TBA) reactivity. Briefly, 0.5 mL of 20% trichloracetic acid, 0.5 mL of homogenate and 1 mL of 0.675% TBA were mixed in tubes. Then the mixture was warmed

for 15 minutes at 100°C. The tubes were cooled at room temperature and centrifuged at 3000 rpm for 10 minutes; 4 mL of n-butanol were then added. The optical density of the supernatant was measured at 532 nm, and the MDA was expressed as nmol/mg protein.

Estimation of reduced glutathione

The reduced glutathione was estimated utilizing the colorimetric technique of Jollow et al (23) based on the development of a yellow color when Ellman reagent (5, 5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) was added to compounds containing sulfhydryl groups. The GSH concentration (nmol GSH/mg protein) was obtained from the absorbance at 412 nm.

Determination of antioxidants enzymes

The enzymatic activity of GSH-Px was measured by the method of Flohe and Günzler (24), and the GST activity of tissues was measured spectrophotometrically by the method of Habig et al (25).

Protein determination

The protein concentration in the tissues' homogenates was determined by the Bradford method, using bovine serum albumin as a standard (26).

Histologic studies

Pancreas obtained by dissection was washed with isotonic saline (0.9%). Then it was immediately fixed in Bouin solution for 24 hours, processed by using a graded ethanol series and embedded in paraffin. The paraffin sections were cut into 5 µm slices and stained with hematoxylin and eosin (27). All pictures were taken using optic microscopy; the magnification was 400×.

Statistical analysis

Data were reported as mean ± SEM. Results comparisons were carried out by using 1-way analysis of variance followed by the Student t test to compare means among the groups. Differences were considered statically significant at $p<0.05$.

Results

Phytochemical screening

The phytochemical analysis of the methanol extract of *R. chalepensis* showed the presence of various groups of secondary metabolites, such as alkaloids, flavonoids, tannins, saponins, terpenoids, steroid and sterols, which are of medicinal importance.

Acute toxicity study

Under the present experimental conditions, *R. chalepensis* methanol extract up to 5000 mg/kg did not produce mortality, macroscopic tissue injury or weight loss during the observation period. Given that lethal effects were not observed at any of the administered doses, the oral LD50 of the methanol extract estimated in rats must be above 5000 mg/kg.

Body weight gain

Body weight gain is shown in Table 1. The results indicated a very significant reduction in the body weights of rats with diabetes compared to those without diabetes. Meanwhile, the body weights of the rats with diabetes that were fed zinc-deficient diets (DZD) were lower ($p<0.01$) than those of rats with diabetes that were given adequate-zinc diets (DSZ), but body weights were significantly lower in the rats treated with zinc (DZD-R).

Femur, kidney and liver zinc concentrations

The zinc concentrations in femur, kidney and liver are also indicated in Table 1. The zinc levels in the 3 organs of rats with diabetes (DSZ) were generally lower ($p<0.05$, $p<0.001$) than those in rats without diabetes. On the other hand, zinc concentrations in liver ($p<0.01$), femur ($p<0.001$) and kidney ($p<0.05$) were significantly lower in the DZD group than in the DSZ group. However, tissue contents of zinc were significantly higher in the DZD-R group ($p<0.001$) as compared to the DZD group.

Blood biochemical values

The findings are illustrated in Table 2. As expected, the diabetes state affected the biochemical parameters most significantly. The results obtained also indicated that zinc deficiency led to an increase in glucose ($p<0.001$), cholesterol ($p<0.001$), urea ($p<0.001$) and creatinine ($p<0.001$), uric acid ($p<0.05$), triglyceride ($p<0.05$), aspartate aminotransferase (AST, or GOT) ($p<0.001$) and alanine aminotransferase (ALT, or GPT) ($p<0.001$) levels, whereas the methanol extract of *R. chalepensis* restored the altered variables.

MDA, GSH and GSH-Px values

MDA, GSH and GSH-Px values are shown in Figures 1, 2, 3 and 4, respectively. The rats with diabetes had high MDA levels and low GSH content and GSH-Px activity in all studied organs than did the rats without diabetes. Moreover, it was noticed that zinc deficiency caused a slight but obvious augmentation of MDA in liver, heart and

Table 1
Body weight gain, tissue zinc levels in non-diabetic rats (ND), diabetic zinc-sufficient rats (DZS), diabetic zinc-deficient rats (DZD) and diabetic zinc-deficient rats given methanol extract of *Ruta chalepensis* (DZD-R)

Parameters	Experiment groups			
	ND	DSZ	DZD	DZD-R
Initial body weight (g)	134.00±6.40	173.20±9.76	170.00±8.40	179.40±13.12
Final body weight (g)	159.20±3.36	154.40*±8.72	133.60§±14.48	193.66**±10.53
Femur zinc (µg/g dry weight)	73.00±3.00	60.34†±4.11	44.34¶±2.64	67.50**±4.24
Kidney zinc (µg/g dry weight)	68.13±5.85	56.00*±6.33	53.00‡±7.25	71.00**±2.62
Liver zinc (µg/g dry weight)	66.00±4.37	43.00†±6.65	39.00§±2.49	67.00**±1.67

Note: Values are mean ± SEM; number of samples = 7.
* $p<0.05$.
† $p<0.001$: comparison of DSZ with ND.
‡ $p<0.05$.
§ $p<0.01$.
¶ $p<0.001$: comparison of DZD with DZS.
** $p<0.001$: comparison of DZD-R with DZD.

Table 2
Mean blood glucose, serum creatinine, serum cholesterol, serum uric acid, serum urea, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and serum triglycerides concentrations in nondiabetic rats (ND), diabetic zinc-sufficient rats (DSZ), diabetic zinc-deficient rats (DZD) and diabetic zinc-deficient rats given methanol extract of *Ruta chalepensis* (DZD-R)

Parameters	Experimental groups			
	ND	DSZ	DZD	DZD-R
Glucose (mg/dL)	98.20±2.56	381.40†±23.92	484.80§±54.56	125.60¶±11.52
Creatinine (mg/dL)	0.66±0.20	0.65*±0.12	1.25§±0.22	1.24±0.37
Cholesterol (mg/dL)	80.00±4.80	60.60*±4.72	127.00§±10.00	90¶±9.60
Uric acid (mg/dL)	2.40±0.24	3.18*±0.30	3.90‡±0.24	2.12¶±0.89
Urea (mg/dL)	25.40±4.80	60.00†±4.72	127.00§±10.00	42.00¶±13.60
Triglycerides(mg/dL)	56.00±13.60	73.20*±8.64	96.40‡±4.88	71.80±14.16
GOT (IU/L)	77.50±9.00	94.56†±5.34	108.75§±10.57	98.56±5.44
GPT (IU/L)	74.56±7.62	89.14†±8.79	103.88§±11.71	91.56±5.62

Note: Values are mean ± SEM; number of samples = 7.
* p<0.05.
† p<0.001: comparison of DZS with ND.
‡ p<0.05.
§ p<0.001 comparison of DZD with DSZ.
¶ p<0.001: comparison of DZD-R with DZD.

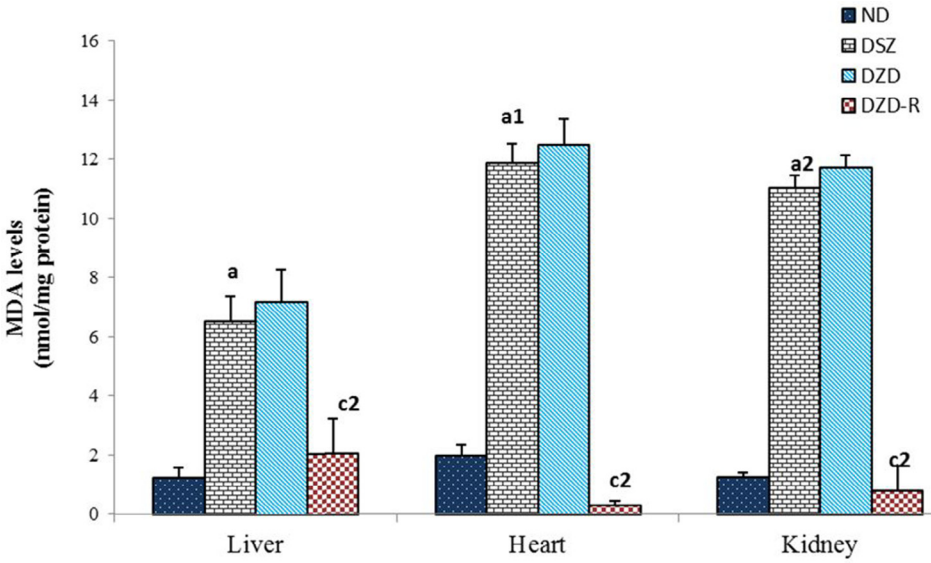

Figure 1. MDA level in nondiabetic rats (ND), diabetic zinc-sufficient rats (DZS), diabetic zinc-deficient rats (DZD) and diabetic zinc-deficient rats given methanol extract of *Ruta chalepensis* (DZD-R), malondialdehyde (MDA), standard error of the mean (SEM). a, a^1, a^2, c^2: alphabetical for statistical differences. $^{a}p<0.05$. $^{a1}p<0.01$. $^{a2}p<0.001$: comparison of DZS with ND. $^{c2}p<0.001$: comparison of DZD-R with DZD. *Note:* Values are mean ± SEM; number of samples = 7.

kidney of diabetic animals as well as a significant decrease in GSH content and the activities of both GSH-Px and GST. However, *R. chalepensis* administration resulted in improvements in the hepatic, cardiac and renal oxidative stress parameters.

Pancreatic histopathologic results

The histology of the pancreas (Figure 5) revealed that the rats without diabetes had intact pancreatic islets. However, both the zinc-sufficient rats with diabetes (DSZ) and the zinc-deficient rats with diabetes (DZD) showed depleted islet cells. Rats with diabetes that had been treated with the extract of *R. chalepensis* (DZD-R) showed preserved islet cells, which is an improvement over the state of the untreated rats with diabetes.

Discussion

The use of medicinal and dietetic plants today is the most widespread medicine worldwide. In other words, the use of herbal treatment and finding new substances with biological activity is 1 of the largest scientific concerns. Therefore, several studies have been conducted to evaluate the secrets of plants. Among them this study, which was devoted to searching for the potential antidiabetic effects of the aerial methanol extract part of *R. chalepensis* in animals fed low-zinc diets. According to the findings obtained, *R. chalepensis* extract up to 5000 mg/kg did not induce any tissue damage, weight loss or mortality in the rats during the period of the experiment, and lethal effects were not observed at any of the administered dosages. Therefore, the oral LD50 of the extract must be greater than 5000 mg/kg to cause harm. In the current study, rats with diabetes weighed less than rats without diabetes; this is consistent with a previously published report (28). It raises the possibility of disturbance of the metabolic state of the animals, suggesting that diabetes had reduced the ability of those rats to utilize food intake as normal subjects do. On the other hand, rats fed a zinc-deficient diet had less body weight gain than did rats fed a zinc-adequate diet, which is in agreement with a previously published investigation (29). It has been well documented that rats fed low-zinc diets voluntarily decrease consumption (reduce appetite) and maintain a very

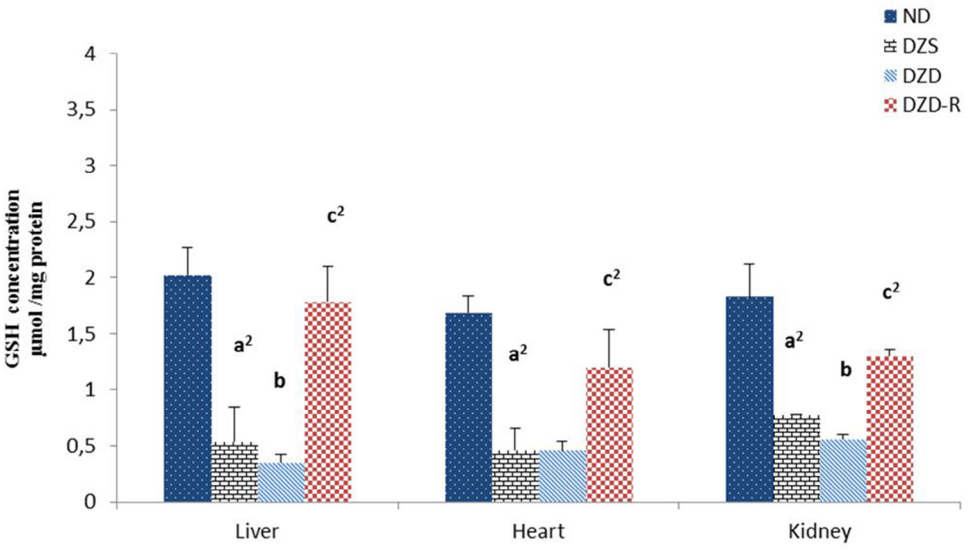

Figure 2. GSH level in nondiabetic rats (ND), diabetic zinc-sufficient rats (DSZ), diabetic zinc-deficient rats (DZD) and diabetic zinc-deficient rats given methanol extract of *Ruta chalepensis* (DZD-R), reduced glutathione (GSH), standard error of the mean (SEM). a^2, b, c^2: alphabetical for statistical differences. $^{a2}p<0.001$: comparison of DSZ with ND. $^{b}p<0.05$: comparison of DZD with DSZ. $^{c2}p<0.001$: comparison of DZD-R with DZD. *Note:* Values are mean ± SEM; number of samples = 7.

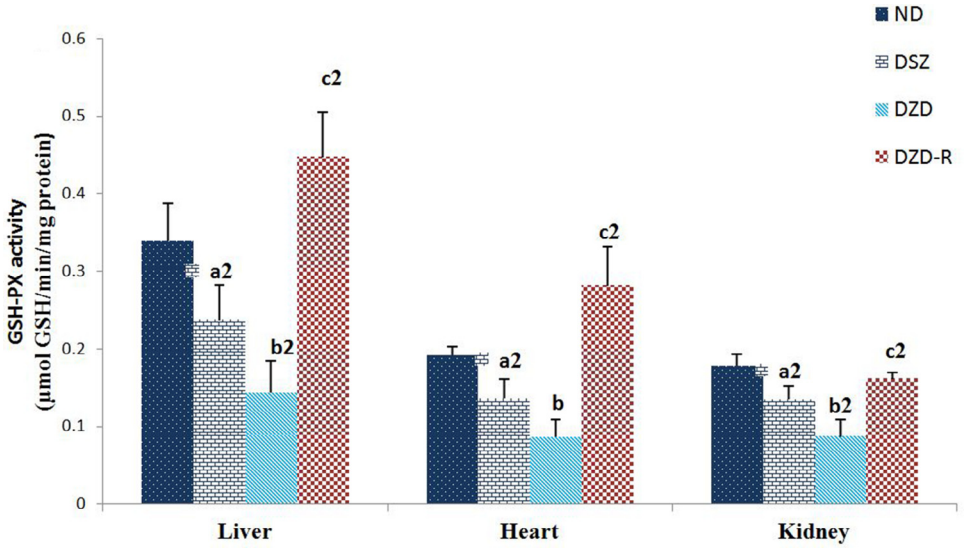

Figure 3. GSH-Px activity in nondiabetic rats (ND), diabetic zinc-sufficient rats (DSZ), diabetic zinc-deficient rats (DZD) and diabetic zinc-deficient rats given methanol extract of *Ruta chalepensis* (DZD-R), glutathione peroxidase (GSH-Px), standard error of the mean (SEM). a^2, b, b^2, c^2: alphabetical for statistical differences. $^{a2}p<0.001$: comparison of DSZ with ND. $^{b}p<0.05$. $^{b2}p<0.001$: comparison of DZD with DSZ. $^{c2}p<0.001$: comparison of DZD-R with DZD. *Note:* Values are mean ± SEM; number of samples = 7.

low growth rate (30). However, the body weight gains of rats with diabetes fed a low-zinc diet and administrated *R. chalepensis* extract were higher than those of zinc-deficient rats with diabetes. The greater gain in body weight of these animals probably resulted from an increase in daily food consumption and promotion of protein synthesis (31). Moreover, *R. chalepensis* extract might have the ability (as do *Artemisia herba-alba* and *Nymphaea stellata*) to reverse gluconeogenesis and control protein loss (32). Tissue concentrations of zinc in the livers, femurs and kidneys of rats with diabetes were lower than those of rats without diabetes. These findings indicate the effect of diabetes on a body's zinc status. It has been postulated that low levels of zinc in patients with diabetes may be due to excessive urinary output and gastrointestinal malabsorption (33). The results of this investigation show also that there was a significant decrease in zinc content in the previously mentioned organs of animals with diabetes that were fed zinc-inadequate diets compared to the rats with diabetes that were fed zinc-adequate diets; this finding coincides with the findings of previous investigations (34). On the other hand, zinc concentrations in various tissues of zinc-deficient rats with diabetes that were treated with methanol extract were restored. This was undoubtedly due to the antioxidant effect of this plant extract against the development of the diabetic state, resulting in a decline of zinc loss through urine (35). The mean fasting blood glucose concentrations in animals fed the low-zinc diet were found to be higher than those of the animals receiving adequate dietary zinc. This may be related to altered glucose utilization by tissues or to an increased rate of endogenous glucose production (36). Moreover, blood glucose levels were reduced in zinc-deficient animals with diabetes that were treated with *R. chalepensis* as compared to zinc-deficient animals that were untreated. This finding correlated with the histologic studies of the pancreas, where the extract preserved islet cells. In that case, the

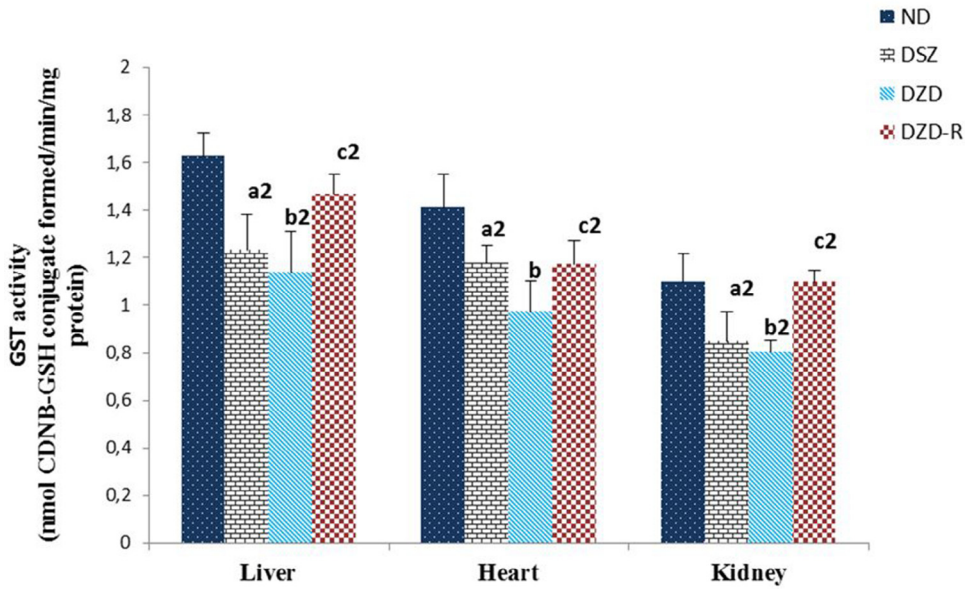

Figure 4. GST activity in nondiabetic rats (ND), diabetic zinc-sufficient rats (DSZ), diabetic zinc-deficient rats (DZD) and diabetic zinc-deficient rats given methanol extract of *Ruta chalepensis* (DZD-R), glutathione transferase (GSH-Px), standard error of the mean (SEM). a^2, b, b^2, c^2: alphabetical for statistical differences. $^{a2}p<0.001$: comparison of DSZ with ND. $^{b}p<0.05$. $^{b2}p<0.001$: comparison of DZD with DSZ. $^{c2}p<0.001$: comparison of DZD-R with DZD. *Note:* Values are mean ± SEM; number of samples = 7.

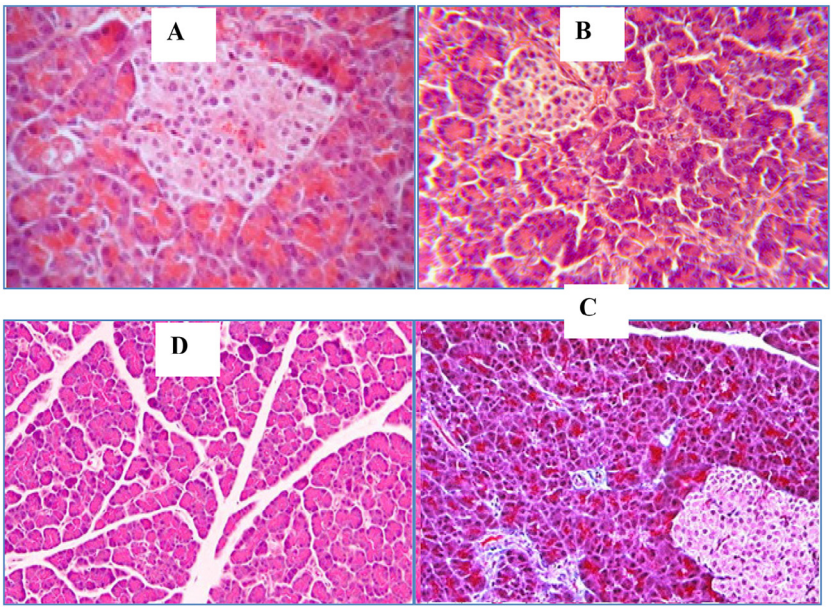

Figure 5. Effect of *Ruta chalepensis* extract on histopathologic damage in the pancreas after 3 weeks of treatment in experiment groups: A, Section of pancreas tissue from nondiabetic rats (ND) showing normal architecture. B, Section of pancreas tissue from diabetic zinc-sufficient rats (DSZ) showing degenerative vascular changes in the islets. C, Section of pancreas tissue from diabetic zinc-deficient rats (DZD) showing more degenerative vascular changes in the islets. D, Section of pancreas tissue from diabetic zinc-deficient rats given methanol extract of *Ruta chalepensis* (DZD-R) showing apparently normal population of pancreatic islets. Optic microscopy: sections were stained using the hematoxylin-eosin method (400×).

hypoglycemic effect of *R. chalepensis* might be due to the presence of flavonoids and other substances, such as glycosides, alkaloids, saponin, tannins and triterpenes. These compounds have been reported to be responsible for the hypoglycemic action (37) or a result of ameliorative insulin secretion (38), and the phytochemical screening confirmed the richness of this plant in these active compounds. The cholesterol, triglyceride, creatinine, urea and uric acid levels of zinc-deficient rats were higher than in those with sufficient zinc intake. This might be due to the catabolism of lipids and proteins as a result of increased demand for energy (39). The treatment of zinc-deficient rats with diabetes with *R. chalepensis* extract produced potential improvement of these altered serum lipid parameters. These results are in agreement with the work of Nuraliev and Avezov (40), who demonstrated the effects of rutin (a metabolite of *R. chalepensis*) on the diminishing of both cholesterol and low-density lipoprotein-cholesterol. In other words, the rutin might have an effect on insulin release through quercetin, which is a metabolite of rutin. Moreover, the hypolipidemic effect of *R. chalepensis* extract might also be mediated via inactivation of 5-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which is a key

enzyme for cholesterol synthesis. This coincides with the work of Jung et al (41), who noticed that flavonoids decrease liver HMG-CoA reductase activity in mice with type 2 diabetes, and the work of Nazıroğlu et al (42), who noticed that apple cider vinegar modulated lipids profiles in ovariectomized mice fed high-cholesterol diets. Meanwhile, the normalized concentrations of urea, creatinine and uric acid were possibly due to the improvement of protein synthesis and/or metabolism. In this experiment, significant rises in serum GOT and GPT activities in rats with diabetes were also found, which could relate to excessive accumulation of amino acids (glutamic and alanine) in the serum of animals with diabetes as a result of amino acid mobilization from protein stores (43). These excessive amino acids are then converted to ketonic bodies (alpha keto-glutaric and pyruvate), for which the enzymes GOT and GPT are needed. In other words, the gluconeogenetic action of GOT and GPT plays the role of providing new supplies of glucose from other sources such as amino acids. It is interesting to note that Grefley and Sandstead (44) found evidence of decreased oxidation of the carbon chain of alanine when zinc was restricted and led to alanine accumulation in blood.

In general, the present investigation indicated that some symptoms associated with zinc deficiency in rats with diabetes can be prevented by treatment with *R. chalepensis*, which normalized some biochemical parameters and brought them closer to those of the zinc-sufficient group with diabetes. This finding is in agreement with the results of Osama Mohamed (45) who reported that *Ruta graveolens* treatment had antihyperglycemic and antihyperlipidemic efficacy, which may be mediated via pancreatic and extrapancreatic effects. The reduced antioxidant capability in diabetes was the result of increased production of oxygen metabolites, which curbs the activity of the antioxidant defense system (46). In addition, several studies demonstrated increased free-radical production or increased oxidative damage in response to zinc deficiency in vitro and in vivo (47). According to the results obtained, there was an increase in MDA, which confirms the deleterious effect of zinc deficiency by increasing lipid peroxidation. The reason for the depletion of glutathione might be a result of the higher consumption of glutathione and the higher oxidative damage in zinc deficiency (48). The observed decline in GSH-Px and GST activities might be due to modification of the sulfhydryl groups in these enzymes by oxygen free radicals (49). Elevated glucose and hydrogen peroxide levels have also been found to inactivate GSH-Px and GST (50). This study confirmed a beneficial effect of *R. chalepensis* in attenuating oxidative stress and oxidative damage. In other words, the findings showed a significant reduction in the formation of thiobarbituric acid reactive substances and an augmentation of GSH concentrations plus an improvement in GSH-Px and GST activities in animals given *R. chalepensis*. Thus, it has been reported that several polyphenol compounds isolated from *R. chalepensis*, such as rutin, possess strong antioxidant properties, which reduce the formation of reactive oxygen species by directly inhibiting the reactive oxygen-generating enzymes. In addition, it has been mentioned that *R. chalepensis* can induce expression of several antioxidant enzyme genes, including GSH-Px and GST (51).

Conclusions

This study revealed that the combination of zinc deficiency and diabetes affected growth rate, zinc status, carbohydrate metabolism and the antioxidant system. The administration of methanol extract of *R. chalepensis* reduced the severity of these complications via its antioxidant benefits. Further clinical studies are required to assess the efficacy of this plant in protecting against the development of diabetes associated with increased free radical activity as a result of zinc deficiency.

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