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Research Article

Potential Ameliorative Effects of Selenium and Chromium Supplementation Against Toxicity and Oxidative Stress in Streptozotocin Diabetic Rats

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Abstract

Background and Objective: Diabetes mellitus is a persistent syndrome affecting carbohydrate, fat and protein metabolism. The current study was undertaken to assess and evaluate the ameliorator property of selenium and chromium each alone or in combination as antioxidants and as a biological membrane stabilizer in the caring against diabetes mellitus oxidative stress. **Methodology:** The rats were divided into seven groups (10 per group). The 1st group was served as a control group, while, the 2nd group was treated with STZ and considered as (Diabetic untreated group). The 3rd and 4th groups were normal and treated with Se or Cr each alone. The last three groups were diabetic and treated with either Se and/or Cr each alone or in combination and all animals were treated for 30 days. **Results:** Administration of STZ (50 mg kg^{-1} i.p.) to rats resulted in hyperlipidemia, an increase in renal parameters as well as the pancreatic and hepatic MDA level and decreasing glutathione (GSH and CAT) contents of the liver and pancreas. While, inducing an increment in (TNF-α and CRP) as compared to control group. In contrast, the administration of Se (0.5 mg kg^{-1}) or/and Cr (600 μg day^{-1}) to diabetic rats attenuates the toxicity of diabetes, objectified by pancreatic tissues improvement as in TEM sections. But, this alleviation is more pronounced in the diabetic group treated with the both antioxidants. Thus, the synergistic effect of Se and Cr is most powerful in reducing the toxicity induced by STZ and improving the liver and the pancreas antioxidant status. **Conclusion:** Diabetes, induced harmful variations in metabolism by elevation of oxidative stress markers and thus Se and Cr are struggling against the oxidative stress via their elaboration in metabolism in addition to their antioxidant properties which may reduce the suffering of diabetic patients.

Key words: Diabetes, selenium, chromium, oxidative stress

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus is a chronic disease characterized by elevated blood sugar levels resulting from either a lack of insulin production or resistance to insulin. Approximately 230 million people worldwide had diabetes in 2010. The worldwide number of people with diabetes is projected to increase to 333 million in 2025 and 430 million in 2030. As the prevalence of diabetes has risen to epidemic proportions worldwide, the vascular complications of diabetes have now become one of the most challenging health problems[1].

A comparatively minor proportion (10%) of patients suffering from diabetes mellitus has type 1 or insulin-dependent diabetes. However, the majority of diabetes patients are not insulin-dependent and able at least initially, to produce the hormone. This type of Diabetes Mellitus (DM) is termed type 2 diabetes. Insulin Resistance (IR) is a fundamental aspect of the etiology of type 2 diabetes. Irrespective of the etiology, subjects with diabetes have an increased risk of ischemic heart disease, atherosclerosis and nephropathy[2].

Currently-available drug regimens for management of diabetes mellitus have certain drawbacks such as vascular complications and hepatotoxicity[3]. Therefore, there is a need for safer and more effective antidiabetic drugs[4]. Antioxidants have been used extensively in experimental diabetes to reduce or reverse the effects of free radicals.

Diabetes mellitus is a heterogeneous disease characterized by microvascular pathology leading to long-term complications clinically manifested. It is a common metabolic disorder characterized by relative or absolute lack of insulin. The insulin-sensitizing action of chromium has been indicated as the mechanism of its antidiabetic activity in experimental models of type 1 and 2 diabetes mellitus (T2DM). Chromium is required for optimal insulin activity and normal carbohydrate and lipid metabolism[5].

Selenium (Se), one of the essential trace elements for human beings, has a major metabolic significance. It has been well recognized that Se is incorporated as the amino acid selenocystein during translation of primary structures[6]. The Se also plays an important role as an essential constituent of selenoproteins. Approximately 100 kinds of selenoproteins are speculated to exist in mammalian systems. To date, glutathione peroxidases (GPxs), thioredoxin reductases, iodothyronine deiodinases and selenoprotein P have been identified as major selenoproteins[7].

A selenium deficiency in humans causes decreased gluthatione peroxidase activity and cardiomyopathy. In Se-deficient rats, the insulin secretory reserve was significantly reduced and glucose intolerance developed in rats maintained on Se and vitamin E deficient diets. Free radical induced damage may play a role in the development of complications in diabetes mellitus. There are several potential sources of increased free radical production in diabetes, including autoxidation of glucose, activation of leukocytes and increased transition metal availability[8].

The present study was carried out in order to estimate the effects of diabetes on the oxidative stress and antioxidant status of the STZ diabetic rats. Also, this investigation has been undertaken to determine the mechanism of action of selenium and chromium in combination to delay or prevent the onset or the progression of diabetes and their complications as well as its effect on the antioxidant status.

MATERIALS AND METHODS

Chemicals: Streptozotocin (STZ), selenium (Se) and chromium (Cr) were purchased from Sigma Chemical Co. USA. Other commercial kits used for the determination of glucose, cholesterol, triglycerides, total protein, creatinine, urea and uric acid were purchased from Stanbio Laboratory, USA. Urea and calcium kits were purchased from Quimica Clinica Aplicada S.A. Spain. Total lipids kit was purchased from Diamond diagnostics, Egypt. Tumor Necrosis Factor-α (TNFα) kit (purchased from CytImmune Sciences, USA). All reagents were of the highest purity commercially available.

Experimental animals: Seventy adult male albino rats weighing 180-200 g were obtained from the animal house of Faculty of Pharmacy, Zagazig University and were housed in metal cages and bedded with wood shavings and kept under standard laboratory conditions of aeration and at room temperature of about 25°C with 12/12 light and dark cycle. The animals were allowed to free access of standard diet (Valley Group Co., Egypt) and water *ad libitum* throughout the experimental period. We have followed the European Community Directive (86/609/EEC) and national rules on animal care that were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals 8th edition. The animals were accommodated to the laboratory conditions for 2 weeks before being experimented, one group served as control and six as treated groups.

Animals and experimental design: After 2 weeks of acclimation, male albino rats weighing 180-200 g were randomly divided into 7 groups (10 rats per group) as follows: Group A was served as a control group and injected i.p. with citrate buffer (pH = 4.5). Group B (STZ group) was injected i.p. by a single dose of STZ (50 mg kg^{-1})[9]. Group C in which rats were administered Se by i.p. injection (0.5 mg kg^{-1} b.wt.)[10]. Group D was administered Cr (600 μg day^{-1})[11] by i.p. injection. Group E was treated with STZ for induction of diabetes and then was injected i.p. daily with Se (0.5 mg kg^{-1} b.wt). Group F was injected with STZ (50 mg kg^{-1}) and then injected i.p. daily with Cr (600 μg day^{-1}), respectively. Group G was injected i.p. with STZ (mg kg^{-1}) and then followed by treatment with Se (mg kg^{-1}) and then followed by i.p. injection of Cr (600 μg day^{-1}) concomitantly.

To induce experimental diabetes, STZ was dissolved in an acidified solution of citrate buffer (pH 4.5) to obtain the required freshly prepared concentration and diabetes mellitus was induced in the fasted animals by i.p. injection of STZ within a few minutes of preparations at a dose level of (50 mg kg^{-1} b.wt.). The treatments were continued daily for 30 days and were started after 48 h of STZ injection. The diabetic state was assessed by measuring the plasma glucose concentration after 48 h of STZ injection. Rats exhibiting plasma glucose levels above 300 mg dL^{-1} were selected for the experiment and included in the study.

Chromium chloride ($CrCl_3.6H_2O$) and Se were dissolved in citrate buffer (pH 4.5). Chromium administrated at a concentration of 600 μg kg^{-1} i.p. for 30 days, which is an equivalent dose of chromium for a 70 kg adult human.

Blood sample and tissues collection: Blood samples of the fasted rats were collected from the medial retro-orbital venous plexus immediately with heparinized capillary tubes (Heparinized Micro Hematocrit Capillaries, Mucaps) under light ether anesthesia[12]. Then the blood was centrifuged at 3000 rpm for 15 min to serum and plasma for different biochemical analyses. The animals were then decapitated under ether anesthesia and tissue samples (liver and pancreas) were collected and preserved in -20°C for subsequent biochemical analysis and pancreatic tissues were used for transmission electron microscope.

Determination of blood glucose level: Plasma blood glucose levels were assessed using the oxidase method described by Trinder[13].

Hepatorenal biomarkers determination: The serum Total Cholesterol (TC) and triglycerides (TG) were determined by the method of Carr *et al.*[14]. Total lipids were determined according to the methods of Warnick *et al.*[15]. The protein content was determined by the method described using bovine serum albumin as the standard. The levels of urea and creatinine in serum were estimated spectrophotometrically using diagnostic kits according to the manufacturer's instructions. The data were expressed as milligram per deciliter.

Inflammation marker: The CRP was determined according to the method reported by Wener *et al.*[16] using SEA821-Enzyme-linked Immunosorbent Assay Kit for C Reactive Protein (CRP).

Determination of tumor marker TNF-α: Tumor Necrosis Factor-α (TNF-α) was assayed by ELISA using a kit (Cat# 88-7340-88) from CytImmune Sciences, USA. Serum was diluted appropriately to make sure that TNF-α value was within the range of the standard curve according to the manufacturer's instruction. The TNF-α levels were expressed as picogram per milliliter.

Preparation of tissue homogenates for measurement of antioxidant enzymes: The remainder tissues of liver and pancreas (about 0.30 g) were used for the analysis of oxidative stress and antioxidant parameters. They were washed with physiological saline (0.9%) and 0.1 m ethylenediaminetetra acetic acid (EDTA) to remove any red blood cells and clots for the removal of blood and later the fatty parts were removed as not to record a reading on spectrophotometer for blood antioxidant enzymes, so to get a more accurate reading for antioxidant enzymes in tissue homogenates only and then we blotted the tissues over a piece of filter paper. Tissue was perfused with a 50 mM (sodium phosphate buffer saline (100 mM Na_2HPO_4/NaH_2PO_4) (pH 7.4). Then, tissues were homogenized in 5 mL cold buffer per gram tissue and centrifuged at 5000 rpm for 1/2 h. The resulting supernatant was transferred into eppendorf tubes and preserved in a deep freeze until used for various antioxidant assays.

Determination of oxidative stress markers: Liver and pancreas homogenates were used for the estimation of reduced glutathione according to the method of Sedlak and Lindsay[17]. The GSH in free supernatant was determined at 412 nm and expressed milligram per gram tissue. Liver and pancreas lipid peroxide were measured by a colorimetric

reaction with thiobarbituric acid-positive reactant substances (TBARS) and was expressed in terms of the malondialdehyde (MDA) concentration by using 1,1,3,3-tetramethoxy propane as a standard at 535 nm according to the method described by Ohkawa *et al.*[18]. Catalase (CAT) activity in homogenate liver tissue was determined according to the methods of Beers Jr. and Sizer[19] in which the disappearance of hydrogen peroxide is followed spectrophotometrically at 240 nm.

Transmission Electron Microscopic (TEM) study: For an ultrastructural examination portion of the pancreas was fixed in 2.5% glutaraldehyde for 24-48 h. The specimens were then washed in phosphate buffer (pH 7.2-7.4) 3-4 times for 20 min every time and post-fixed in a buffered solution of 1% osmium tetraoxide for 2 h, after that washed in the same buffer 4 times for 20 min each. Fixed specimens were dehydrated in ascending grades of ethyl alcohol (30, 50, 70, 90 and 100%), cleared in two changes of propylene oxide and embedded in Epon resin[20].

Semi-thin sections (1 μm thick) were stained with toluidine blue for 2 min and examined by the use of a light microscope. The resin blocks were retrimmed to get rid of the undesired tissue. Ultrathin sections (60-90 nm thick) and representative fields of semithin sections were selected and were cut with a diamond knife using a Reichert OMVs ultramicrotome, mounted on copper grids and double stained with uranyl acetate and lead citrate[21]. The grids were examined and photographed using a transmission electron microscope (JEOL JEM-1200 EX II, Japan) operated at 60-70 kV, Faculty of Agriculture (Electron microscope unit), Mansoura University.

Statistical analysis: Data were collected, arranged and reported as Mean±SEM of seven groups (each group was considered as one experimental unit), summarized and then analyzed using the computer program SPSS version 15.0. The statistical method was one way ANOVA test and if significant differences between means were found, Duncan's multiple range test according to Snedecor and Cochran to estimate the effect of different treated groups[22].

RESULTS

Lipid profile: Induction of diabetes in male rats by a single dose of STZ produced a significant increase ($p \leq 0.005$) in plasma cholesterol, triglycerides and total lipid levels of rats (Table 1). Meanwhile, administration of either Se or Cr each alone to normal rats induced non-significant changes in cholesterol, triglycerides and total lipid levels as compared to a normal control group. The diabetic group treated with either Se or Cr each alone afforded significant decrease in cholesterol, triglycerides and total lipid levels as compared to STZ group (Diabetic untreated group). Administration of a mixture of antioxidant elements (Se+Cr) succeeded in restoring all the parameters cited above and afforded significant decrease in cholesterol, triglycerides and total lipids as compared to diabetic untreated group (STZ) by 68.25, 59.95 and 206.78 fold.

Table 1: Effect of of Se (0.5 mg kg^{-1}) and/or Cr (600 μg kg^{-1}) and their combinations on plasma metabolic markers

	Lipid profile		
Groups	Cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})	Total lipids (mg dL^{-1})
Control group	71.25±2.36^{ef}	62.36±4.25^{ef}	211.64±7.25^{ef}
STZ group	163.29±6.98^{a}	149.67±3.69^{a}	465.25±8.96^{a}
Selenium	69.25±1.89^{f}	60.98±4.25^{f}	210.69±5.69^{f}
Chromium	69.25±5.25^{f}	61.25±3.69^{f}	208.25±9.25^{f}
STZ+selenium	146.36±5.36^{cd}	128.36±4.36^{b}	274.58±9.26^{bc}
STZ+chromium	140.36±5.69^{d}	120.95±1.74^{c}	266.78±9.25^{c}
STZ+selenium+chromium	128.69±4.36^{b}	117.18±3.69^{d}	248.96±7.87^{d}

Values are expressed as Mean±SE, n = 10 in each treatment group

Table 2: Effect of of Se (0.5 mg kg^{-1}) and/or Cr (600 μg kg^{-1}) and their combinations on plasma blood glucose

Groups	Blood glucose level (mg dL^{-1})
Control group	90.68±7.42^{ef}
STZ group	320.65±9.62^{a}
Selenium	90.15±5.36^{ef}
Chromium	89.65±6.58^{f}
STZ+selenium	146.69±7.58^{bc}
STZ+chromium	137.64±4.36^{c}
STZ+selenium+chromium	129.36±3.98^{d}

Values are expressed as Mean±SE, n = 10 in each treatment group

Blood glucose level: By the end of the period of treatment, STZ treatment induced a highly significant elevation in plasma glucose of the diabetic untreated group as compared to control group (Table 2). Serum blood glucose of normal rats treated with either Se or Cr afforded non-significant changes in blood glucose level as compared to normal control group. The treatment of diabetic rats with either Se or Cr each antioxidant alone elicited a significant decrease in blood glucose level as compared to diabetic untreated group (STZ), it was obvious that Cr diabetic group induced more decrement in blood glucose level than Se diabetic treated group alone as it was 137.64 mg dL^{-1} in Cr group but it was 146.69 mg dL^{-1} in diabetic group treated with Se. Treatment of diabetic rats with combination of antioxidant mixture of Se and Cr achieved a significant reduction by 129.36 fold in the plasma glucose level as compared to diabetic untreated group.

Table 3: Effect of Se (0.5 mg kg^{-1}) and/or Cr (600 µg kg^{-1}) and their combinations on protein metabolites

Groups	Total protein (mg dL^{-1})	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})	Uric acid (mg dL^{-1})
Control group	5.74±0.89^{c}	21.03±2.31^{e}	0.75±0.03^{cd}	3.12±0.03^{e}
STZ group	3.65±0.67^{f}	46.35±3.11^{a}	1.91±0.04^{a}	4.01±0.06^{a}
Selenium	5.84±0.98^{c}	22.35±3.21^{d}	0.74±0.03^{d}	3.22±0.25^{d}
Chromium	5.98±0.58^{bc}	23.55±3.25^{d}	0.73±0.06^{d}	3.27±0.63^{c}
STZ+selenium	5.20±0.32^{e}	25.78±2.25^{cd}	0.98±0.08^{c}	3.34±0.07^{c}
STZ+chromium	5.09±0.79^{de}	27.68±1.68^{b}	1.02±0.023^{b}	3.57±0.05^{b}
STZ+selenium+chromium	6.01±1.03^{a}	21.34±2.61^{e}	0.71±0.05^{d}	3.20±0.01^{de}

Values are expressed as Mean±SE, n = 10 in each treatment group

Table 4: Effect of Se (0.5 mg kg^{-1}) and/or Cr (600 µg kg^{-1}) and their combinations on Tumor Necrosis Factor-α (TNF-α) and C-Reactive Protein (CRP)

Groups	TNF-α (ng mL^{-1})	CRP (mg L^{-1})
Control group	126.52±4.62^{e}	7.25±1.02^{de}
STZ group	205.36±5.36^{a}	22.02±2.36^{a}
Selenium	126.25±3.63^{e}	6.89±1.36^{e}
Chromium	125.35±4.25^{e}	6.66±2.25^{e}
STZ+selenium	151.24±2.10^{b}	15.36±2.41^{b}
STZ+chromium	139.54±6.25^{cd}	10.38±1.59^{c}
STZ+selenium+chromium	128.41±3.32^{de}	6.54±1.33^{e}

Values are expressed as Mean±SE, n =10 in each treatment group

Protein metabolite markers: To investigate the total protein content and renal functions, total protein level and blood biochemical parameters (Urea, creatinine and uric acid) were measured. Results obtained revealed that the total protein level decreased significantly in diabetic untreated rats (STZ group) as compared to a normal control group (Table 3). Meanwhile, the group treated with either Se or Cr alone elicited non-significant increase in total protein level as compared to control group by 5.84 and 5.98 fold. The same observation has been noticed by the treatment of diabetic groups each antioxidant separately as they afforded a significant increase in total protein levels as compared to diabetic untreated group. The diabetic group treated with a combination of antioxidants Se and Cr elicited a significant increase in total protein level as compared to a normal control group and thus recorded significant elevation in total protein level as compared to the diabetic untreated group.

Assessment all the parameters of renal functions (Urea, creatinine and uric acid) were increased when the mice exposed to STZ for induction of diabetes. Administration of Se or/and Cr to diabetic groups, restored all parameters cited above to their normal levels (Table 3).

Serum Urea was decreased significantly after treatment of diabetic rats with either Se or Cr each alone or their combination by 25.78, 27.68 and 21.34 respectively as compared to the diabetic untreated group. Serum creatinine activity of Se and/or Cr or their combinations treated diabetic groups was decreased by 0.98, 1.02 and 0.71 fold when compared with STZ group. The same observations were noticed in serum uric acid in diabetic groups treated with either Se, Cr or their combination group which recorded the best ameliorative results obtained as diabetic group treated with combination of Se and Cr restored uric acid level to its normal level and showed non-significant changes as compared to normal control group as the same in urea and creatinine levels (Table 3).

Marker of tumor and inflammation: The treatment of normal rats with either Se and Cr afforded non-significant decrease in TNF-α and CRP levels when compared with the normal control group (Table 4). Treatment of diabetic rats with either Se or Cr each alone afforded significant decrease in TNF-α and CRP levels as compared to STZ treated group and elicited increase in TNF-α and CRP levels as compared to a normal control group. While, the diabetic rats treated with combination of Se and Cr elicited non significant increase in TNF-α level and afforded non-significant decrease in CRP levels as compared to a normal control group.

Oxidative stress markers: Table 5 shows that treatment of normal rats with either Se or Cr each alone induced non-significant changes in GSH, CAT and MDA levels as compared to a normal control group. The GSH and CAT were decreased significantly in the diabetic untreated group (STZ group) as compared to a normal control group in both liver and pancreas. Meanwhile, STZ afforded a significant increase in MDA level in both liver and pancreas. These changes were ameliorated significantly after treatment of diabetic rats with either Se or Cr each alone or combined as diabetic group treated with Se or Cr induced significant increase in both GSH and CAT levels as compared to the diabetic untreated group. While, decreased MDA level in both liver and pancreas tissues. Treatment of diabetic rats with a combination of Se and Cr retrieved the normal levels of GSH, CAT and MDA levels as this group recorded non-significant increase in both GSH and CAT levels of both liver and pancreas and afforded a non-significant decrease in MDA level respectively in liver and pancreas as compared to control group.

Table 5: Effect of Se (0.5 mg kg^{-1}) and/or Cr (600 µg kg^{-1}) and their combinations on oxidative stress markers in both liver and pancreas homogenates

	GSH (U g^{-1})		CAT (U g^{-1})		MDA (µmol g^{-1})	
Groups	Liver (U g^{-1})	Pancreas (U g^{-1})	Liver (U g^{-1})	Pancreas (U g^{-1})	Liver (U g^{-1})	Pancreas (U g^{-1})
Control group	16.25±2.32^{c}	20.35±2.20^{b}	40.36±3.65^{b}	50.36±2.22^{b}	10.36±1.02^{cd}	13.12±1.12^{cd}
STZ group	8.25±2.10^{d}	7.36±1.52^{d}	23.25±4.11^{d}	20.35±4.36^{d}	59.68±0.95^{a}	77.25±1.35^{a}
Selenium	16.75±2.25^{bc}	20.56±1.25^{ab}	41.26±3.68^{ab}	50.89±4.22^{b}	9.83±0.36^{d}	10.41±2.01^{f}
Chromium	16.22±1.25^{c}	20.36±2.25^{b}	40.48±3.65^{b}	50.22±1.36^{b}	10.65±1.01^{cd}	12.02±1.69^{e}
STZ+selenium	15.36±1.58^{d}	17.65±1.75^{c}	30.51±3.32^{c}	40.64±3.63^{c}	22.36±1.20^{b}	26.64±1.02^{b}
STZ+chromium	14.42±2.36^{e}	17.25±2.36^{c}	33.52±4.25^{c}	41.36±4.25^{c}	24.78±1.69^{b}	27.25±2.69^{b}
STZ+selenium+chromium	16.98±1.63^{ab}	20.44±2.32^{b}	40.55±1.25^{b}	51.98±5.32^{ab}	10.25±1.11^{cd}	13.06±0.98^{cd}

Values are expressed as Mean±SE, n = 10 in each treatment group

TEM examination

Acinar cells: Ultrastructure of control pancreas showed acinar cells with euchromatic nuclei, well-developed cisternae of rough endoplasmic reticulum, mitochondria and numerous electron dense secretory granules of variable sizes in the apical part (Fig. 1a). Electron microscopic examination of the diabetic untreated group showed electron micrograph of STZ (Diabetic untreated group) pancreas showing marked changes in pancreatic acini represented by appearing of irregular contours Nuclei (N) and damaged Mitochondria (M), dilated rough Endoplasmic Reticulum (ER), autophagic vacuole (Yellow arrow) and decrease of secretory granules (Red arrow) and cytoplasmic vacuolation. Scale bar = 5 µm (Fig. 1b). Electron microscopic examination of the selenium treated group showing normal euchromatic Nuclei (N), well developed cisternae of rough Endoplasmic Reticulum (ER) and numerous electron dense secretory zymogen granules (Blue arrows) of variable size in the apical part. Scale bar = 5 µm (Fig. 1c). Electron microscopic examination of the chromium treated group showing normal Nuclei (N), numerous and well developed rough Endoplasmic Reticulum (ER) and numerous electron dense secretory zymogen granules (Orange arrows) of variable size in the apical part. Scale bar = 5 µm (Fig. 1d). Electron micrograph of diabetic group treated with Se treated group showing normal and regular boundaries of Nucleus (N) and appearance of a well-developed Endoplasmic Reticulum (ER) and appearance of more secretory granules (Yellow arrow) and increase in zymogen granules (red arrow) and Mitochondria (M). Scale bar = 5 µm (Fig. 1e). Electron micrograph of the diabetic group treated with Cr showing normal and regular nucleus with normal boundaries (N), some degenerated Endoplasmic Reticulum (ER), some enlarged Mitochondria (M), large amount of secretory granules (green arrow). Scale bar = 5 µm (Fig. 1f). Pancreatic acini after supplementation with selenium and chromium showed marked improvement represented by an increase in zymogen granules, regular contours of nuclei and mitochondria with an ameliorative developing cisternae of Mitochondria (M) and well developed flattened Endoplasmic Reticulum (ER) and appearance of more secretory granules (Orange arrow). Scale bar = 5 µm (Fig. 1g).

DISCUSSION

Diabetes mellitus is widely recognized as one of the leading causes of death in the world[23]. It is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated, resulting in elevated blood glucose levels[3]. The presence of high glucose levels *in vivo* can modify a number of biochemical processes[24]. This in turn may inspire the function of diverse organs such as liver and pancreas[3].

Abundant studies have shown that there is an elevation in manufacturing and release of free radicals with diabetes and reported that they perform a role in diabetic complications through glucose autoxidation and protein glycation[25] as well as decreasing the effectiveness of enzymic and nonenzymic antioxidant. High levels of free radicals cause injury to cellular proteins, membrane lipids and nucleic acids resulting in eventually cell death[26].

The liver is the target organ of diabetic toxicity[27] and the leakage of hepatic enzymes such as ALT, AST and ALP is commonly used as an indirect biochemical index of hepatocellular damage[28]. In the present finding, diabetic state caused a significant increase in the activities of ALT, AST and ALP, probably resulting from hepatocyte membrane damage. If the liver is injured, its cells spill out the enzymes into the blood. These results are consistent with the previous findings released by some research groups who had found an association between diabetes toxicity and the increased oxidative stress in rats[29].

Oxidative stress is one of the most dangerous effects on the cellular activities and thus, according to our results combination between Se and Cr greatly scavenged free radical molecules and thus decreased MDA level as it is the final end product of lipid peroxidation and also increased the enzymatic

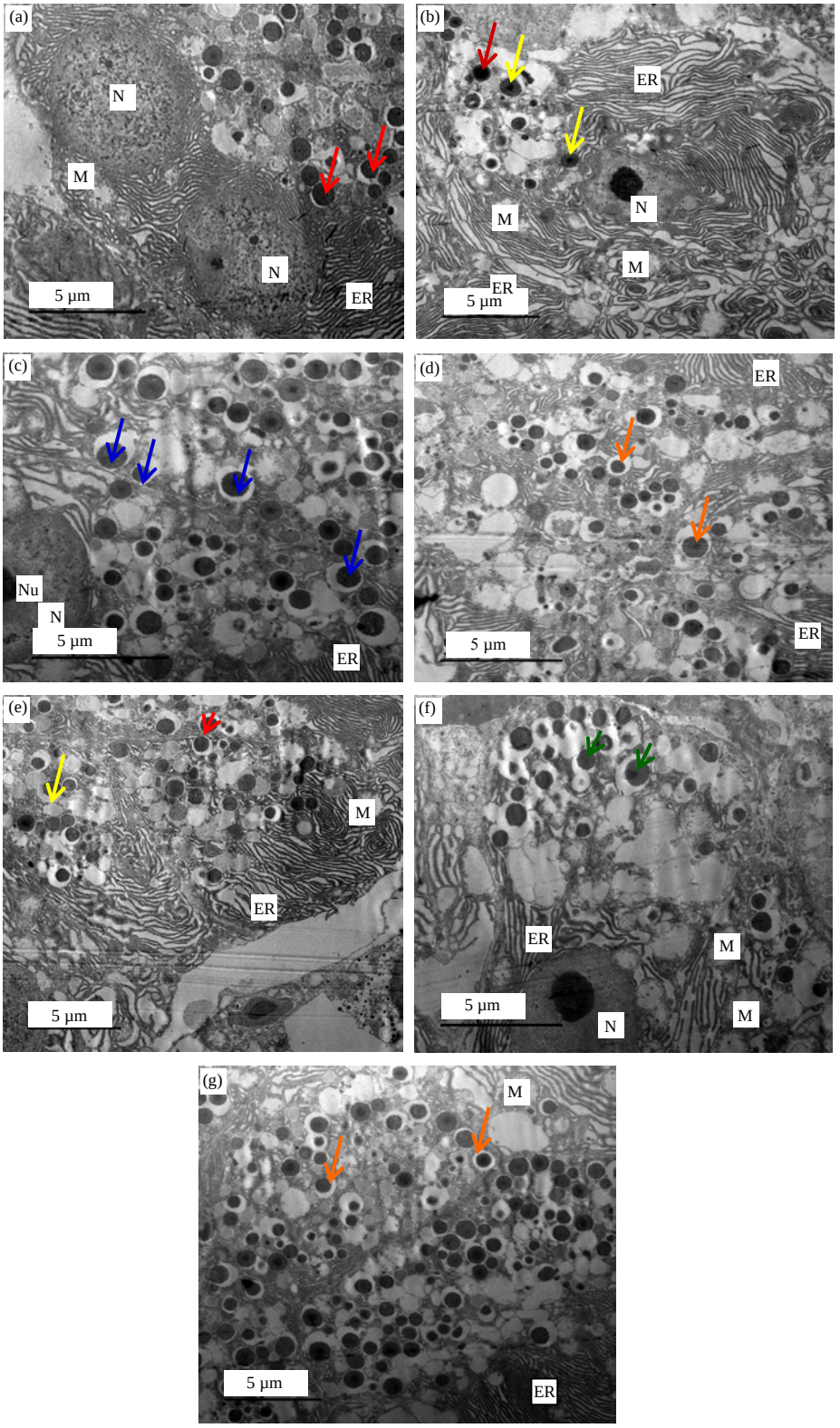

Fig. 1(a-g): Exocrine part, transmission electron micrographs of pancreas sections, (a) Control, (b) STZ, (c) Selenium, (d) Chromium, (e) Diabetic group treated with Se, (f) Diabetic group treated with Cr and (g) STZ+Cr+Se group. Scale bar = 5 µm

capacities of CAT and GSH and thus improving liver function activities and thus enhancing the conversion of blood glucose into glycogen and thus decreasing blood glucose level which reflect the solution for diabetes mellitus complications.

Oxidative stress, cellular redox potential and their link to inflammatory cytokines appear to be the key elements mediating the cytotoxic effects of STZ as well as the protective effects of the combined mixture of Se and Cr.

The GSH acts as a multifunctional intracellular non-enzymatic antioxidant and protects cells against several toxic oxygen-derived chemical species. It is considered to be an important scavenger of free radicals and a cofactor of several detoxifying enzymes against oxidative stress[30].

In this study, GSH level decreased in the liver extracts of the diabetic untreated group compared to control rats, which reflects its consumption through the oxidative stress. The scavenging property and the ability of Se and Cr to inhibit the radical generation could further reduce the oxidative threat caused by STZ[31].

Converted tissue GSH and TBARS levels and the decrement in liver catalase activity clearly reflect oxidative stress in STZ-diabetic rats. The obvious feature of diabetes is the impairment of energy metabolism, which is evident in STZ-treated rats by the elevated levels of creatinine and urea levels[32].

Antioxidant enzymes are considered to be the first line of cellular defense against oxidative damage. The inhibition of GSH involved in the free radical removal has led to the accumulation of H_2O_2, which promoted lipid peroxidation and modulation of DNA, altered gene expression and cell death[33]. The administration of Se has increased antioxidant enzyme activities in diabetic treated rats, is probably related to its ability to reduce the accumulation of free radicals. However, Se supplementation has increased the activities of selenoproteins, perhaps by increased incorporation of selenocysteine in selenoproteins[34].

Though, the beneficial role of Se in reducing oxidative stress parameters in the present study might be related to its mild antioxidant potential. It is known that STZ induces free radical formation either through direct promotion of free radical generation[35] or the inhibition of antioxidant enzymes[36]. However, lipid peroxidation is a basic cellular deteriorating process induced by oxidative stress and occurs readily in the tissues rich in highly oxidizable polyunsaturated fatty acids[37].

The increased lipid peroxidation observed in this study after induction of experimental diabetes could implicate the oxidative stress[36]. It was found that the co-administration of Se with Cr has protected the liver from lipid peroxidation and from any changes in GSH and antioxidant enzymes. This finding could be explained according to Ognjanovic *et al.*[38] by the important role of Se in preventing hydroxyl radical formation and in protecting the integrity and the functions of tissues[39].

The TNFα is known to cause cell injury by the generation of oxidative stress. Meanwhile, oxidative stress is documented to be a major contributor to insulin resistance in diabetes, aging and STZ toxicity[40].

There are diverse lines of proof to support the assumption that a strong link exists between TNF-α and the GSH status which should force a strong impact on glucose metabolism. It has been reported that permanent TNF-α secretion could induce oxidative stress through alteration of GSH metabolism[41]. The present results would clearly support the presence of a fundamental link between the GSH status and TNF-α. The defense compensatory mechanism reflected by the reduced GSH levels (hepatic) in STZ treated rats was accompanied by highly elevated levels of their plasma TNF-α emphasizing the inverse relationship between both elements.

Considering the fact that TNF-α is overproduced in diabetes[42] and based on the solid inverse relation between GSH and TNF-α. It may be concluded that TNF-α was overproduced during early phases of STZ treatment. This may support the hypothesized link between oxidative stress status and TNF-α.

The C-Reactive Protein (CRP) is a protein found in blood plasma, whose levels rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T cells. Its physiological role is to bind to the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via the C1Q complex[43]. CRP is synthesized by the liver in response to factors released by macrophages and fat cells (adipocytes). It is not related to C-peptide (insulin) or protein C (blood coagulation)[44].

Numerous studies have demonstrated an essential role of oxidative stress in the etiology of diabetes and its elaborations. Meanwhile, the join between inflammatory cytokines and oxidative stress and their role in the pathogenesis of several diseases, including diabetes is well established[45] and this appeared clearly in C-reactive protein results as CRP was higher in the diabetic untreated group while it is reduced after treatment of diabetic rats with combination of selenium and chromium.

It is important to assure the importance of the idea that lipid aggregation in skeletal muscle and liver leads to the development of insulin opposition. In experimental models, consolidated cholesterol levels and its subsequent deposition in liver mitochondria was found to induce selective decrease

in the mitochondrial GSH stores. That was adequate by itself to sensitize hepatocytes to TNF-α mediated cell death[46]. It's worthy to refer to the study of Yu *et al.*[47] reporting that hyperlipidemia leads to elevated uptake of fatty acids by muscle cells and production of fatty acid metabolites that stimulate inflammatory cascades and inhibit insulin signaling and this finding support our obtained data that explain the increasing values of total lipids and total cholesterol in diabetic untreated group.

Moreover, Jakus[48] reported that elevated plasma levels of fatty acids suppress glucose uptake by interfering with the insulin receptor substrate proteins signaling pathway. Therefore, the current data showed elevated levels of plasma and liver lipid parameters; which correlated with the well documented diabetes-associated hyperlipidemia[49] may reflect a TNF-α mediated mechanism contributing to insulin resistance and hyperglycemia in these rats.

Meanwhile, the corrected levels of lipid parameters in diabetic rats treated with selenium and chromium support the assumption that this effect could be secondary to their essential role of TNF-α and GSH status mitigating oxidative stress and sensitizing cells to insulin action[50]. Indeed, the effects of selenium and chromium in upgrading lipid profile in diabetic animals are well established[51,29].

The present untreated diabetic rats showed a significant increase in blood glucose concentration compared with the control group. This result is in agreement with Dahech *et al.*[52] and Ramar *et al.*[53].

The STZ action in β-cells is accompanied by characteristic alterations in blood insulin and glucose concentrations. Two hours after injection, the hyperglycemia is observed with a concomitant drop in blood insulin. About 6 h later, hypoglycemia occurs with high levels of blood insulin. Finally, hyperglycemia develops and blood insulin levels decrease[54]. These changes in blood glucose and insulin concentrations reflect abnormalities in β-cell function.

The STZ impairs glucose oxidation[55] and decreases insulin biosynthesis and secretion[56] and these effects confirmed our obtained results as the blood glucose level was elevated significantly in the diabetic untreated group, but this elevation was significantly reduced by treatment of diabetic rats with a combination of Se and Cr.

It has been found that oxidative stress is associated with the molecular mechanism of the decreased insulin biosynthesis and secretion, which is the main etiology of glucose toxicity. Indeed, it was suggested that the pancreas may be more susceptible to oxidative stress than other tissues and organs, because pancreatic islet cells show extremely weak manifestation of antioxidative enzymes[45].

The obtained results revealed that chromium in combination with selenium to diabetic rats improved lipid profile, reduced blood glucose level, reduced the inflammation marker and tumor necrosis factor-α and improve liver and kidney functions and our obtained results are explained and confirmed on the basis that Cr showed multiple beneficial effects in T2DM, including attenuating body weight gain, improving lipid profiles[57] and enhancing endothelial function. Chromium improves insulin sensitivity and may reduce other associated complications[58]. The mechanisms of Cr action have remained obscure, despite multiple pathways of action being proposed, including a decrease in hepatic glucose production, an increase in peripheral glucose disposal and a reduction of intestinal glucose absorption[59].

Thus, it can be concluded that enhanced oxidative stress caused by variations in GSH and TNF-α status could start a broad spectrum of impaired cell signaling pathways including insulin signaling. Apparently, this effect may account for impaired glucose metabolism in STZ-treated rats. Since hyperglycemia was authenticated to be a main mechanism mediating the development of diabetes complications[40]. Actually, the present study revealed that the combination of Se supplementation with Cr synergistically afflicted hyperglycemia in STZ-treated rats and ameliorated the impaired energy metabolism of the same rats. This was indicated by the corrected levels of biochemical parameters after antioxidant mixture treatment. The mechanisms mediating the protective effects of Se and Cr are mainly based on their characteristic feature being antioxidants.

This comprises possible effects of Se and Cr of the following items which in fact are interrelated. Because of the antioxidant properties of Se[60] it is particularly suitable for the prevention and/or treatment of diabetic complications that emerge from an overproduction of reactive oxygen and nitrogen species.

The major antidiabetic effects of Se and Cr may lie in their ability to affect one or more of the effector molecules regulating glucose metabolism. Most of the components affecting glucose transport and metabolism are protein molecules. These implicate insulin receptors, glucose transporters and enzymes among others protein molecules. Indeed, Guilliams[61] reported that protein is the fundamental mediator of glucose transport into cells. Glucose transport is the chief step in glucose metabolism. The membrane-related insulin receptors are the initial elements mediating glucose transport and the possible protection approaching to them by the interactive effects of both Se and Cr is of special concern. This is because glucose transport is the rate limiting step for overall glucose metabolism[62].

It is worthy to point out the study of Moustafa *et al.*[63] who showing deteriorated, glucose transport in adipocytes of aged hyperglycemic rats. Oxidative stress and reinforced lipid peroxidation in these cells were proposed to mediate this damage. Therefore, it seems logical to suggest that STZ-induced oxidative stress may result in a modification in the average of glucose transport.

In fact, the two antioxidants used in the study (Se and Cr) were carefully chosen to serve this aim. Both Se and Cr are lipid soluble, therefore, both can act to protect the cell membrane-associated receptors initiating the first and the most serious step in glucose transport. Being water soluble, Se may have the eventuality to protect cellular components other than those correlating with cellular membrane[64].

The role of oxidative stress in interposing the cyto-subversive effects of STZ on insulin-secreting β-cells are well determined[40]. The β-cells are highly vulnerable to free radical damage which has been attributed, at least in part, to the low activities of oxygen free radical scavenging enzymes in islet cells[65]. Inconveniences, in calcium homeostasis have been reported to an essential role in STZ induced β-cell destruction[66].

Summing up, the current findings favor the concept that oxidative stress may be a final common pathway in the development of diabetes. Deteriorative changes have been provoked in diabetic rats in the metabolism of carbohydrates, lipids, proteins and nucleic acids.

In general, treatment with a Se-Cr mixture may be valuable in reducing the risks of diabetes and its complications.

The present ultrastructural study of the diabetic group showed marked changes in pancreatic acini represented by dilated rough endoplasmic reticulum, a decrease of secretory granules, cytoplasmic vacuolation, damaged mitochondria. Moreover, β-cells and α-cells showed obvious vacuolation and decrease of secretory granules and pyknotic nuclei. The STZ, like glucose, affects α and β-cells directly, stimulating the β-cell and inhibiting the α-cell. So, STZ might have direct toxic actions on both cell types, permanently blocking the β-cells and irreversibly inhibiting the α-cells[67].

Degirmenci *et al.*[68] reported a decrease in secretory granules of β-cells, vacuolization and swelling of mitochondria. The semiquantitative evaluation of islet ultrastructure after STZ exposure *in vitro* demonstrated that mouse β-cells showed signs of both necrotic and apoptotic cell death, disturbances in the insulin secretory pattern during and after an STZ perfusion. The STZ induces damage to β-cell[69].

These results come parallel with early reports suggested that chromium enhances insulin binding, insulin receptor number and beta-cell sensitivity[70]. Chromium enhances tyrosine phosphorylation of the insulin receptor in response to low doses of insulin. More recent work showed that chromium enhances glucose uptake.

Bogolepov[71] stated that vacuolation was one of the structural indications of permeability disorders of the membranes, which results in an enhanced transport of water and electrolytes into the cell. The permeability disorder could be attributed to many cellular membrane insults caused by reactive oxygen species mediated formation of lipid peroxides, which ultimately generate self-sustaining lipid peroxidation[72] and this vacuolation was reported clearly in our obtained results in the diabetic untreated group but it becomes less after treatment of rats with a combination of Se and Cr and this confirm our obtained results.

CONCLUSION

In conclusion, this study investigated the effect of STZ on a cell and threw light on the potential of both selenium and chromium in the prevention or treatment of diabetes due to the importance of human diabetes mellitus as a world health problem as well as the increasing demand by patients to use safer products and elements with antidiabetic activity, because insulin and oral hypoglycemic drugs possess undesirable side effects. Therefore, the study is designed to evaluate the effects of Se and Cr supplementation on the metabolic risk factors and to determine the effects of their supplementation on the transmission electron microscope status of pancreatic tissues in diabetic rats.

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