RESEARCH PAPER

Protective effect of selenium supplementation on antioxidant defence and cardiovascular diseases in alloxan diabetic rats

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Abstract

To investigate the protective effect of selenium supplementation in the cardiovascular diseases associated with diabetes mellitus caused by lipid peroxidation and oxidative stress. Thirty five male albino (wistar) rats of 06 weeks of age were randomly divided into five groups of seven each. Two groups were pre-treated for 10 days by sodium selenite (2 mg/kg.bw/d) via orogastric route. The first group was served as the control, the second as the normal treated intra peritoneally by selenium (1.89 mg/kg.bw/d). Three groups were intra peritoneally injected with alloxan (150 mg/kg.bw) to induce diabetes. One diabetic group treated intra peritoneally by insulin (3UI/100g .bw), another diabetic group (pre-treated by selenium 2 mg/kg.bw/d for 10 days) treated intra peritoneally by selenium (1.89 mg/kg.bw/d) for further three weeks. The administration of alloxan significantly increased blood glucose, total cholesterol, LDL-C, triglycerides, lipids, heart MDA content, GST activity. In contrast blood HDL-C, insulin levels, G6PDH activity, heart GSH, GPx, Catalase activities were significantly decreased. Supplementation of sodium selenite restores the parameters measured compared to untreated diabetic rats and diabetic treated by insulin. To conclude the present study shows that treatment of diabetic rats with sodium selenite prevented cardiovascular diseases induced by hyperlipemia, lipid peroxidation and free radicals generated during diabetes.

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Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, dyslipidemia, and disturb protein metabolism that results from defects in both insulin secretion and/or insulin action. The disease is associated with reduced quality of life and increased risk factors for mortality and morbidity. The long-term hyperglycemia is an important factor in the development and progression of micro- and macrovascular complications which include neuropathy, nephropathy, cardiovascular (CVD) and cerebrovascular diseases (Altan, 2003; Strojek, 2003). The underlying goal of all diabetes treatment and management is to maintain an adequate blood glucose concentration (Charpentier, 2002).

Cardiovascular disease (CVD) is the major cause of death in patients with diabetes (Giugliano et al., 1996). It is estimated that incidence of CVD is three to seven-fold higher for individuals with diabetes than in the non-diabetic population (Rosen et al., 1998). Lipoprotein abnormalities have been identified among the several risk factors that could account for this increase in CVD incidence in diabetes (Assmann and Schulte, 1988). Antioxidant micronutrients are being widely studied for their alleged beneficial properties in the prevention of human diseases, including cancer, arthritis and cardiovascular diseases (Bidlack et al., 1996). Previous studies have shown their efficacy in experimental diabetes mellitus by repressing hepatic glucose production and increasing muscle insulin activity (Guerrero and Rodriguez, 2005). In STZ-induced diabetic rats, daily sodium selenite treatment (15–20 µmol/kg body weight, intraperitoneally) reduced or normalized high blood glucose levels and restored left ventricular pressure parameters without any positive effect on low insulin level (McNeill et al., 1991; Battel et al., 1998). Recently, in our previous studies, we have demonstrated that treatment of STZ-induced diabetic rats with a low amount of sodium selenite (5 µmol/kg, intraperitoneally) compared with the others (McNeill et al., 1991; Battel et al., 1998) could protect the ultra structure of heart against diabetes induced alterations, reverse the increase in platelet aggregation and thromboxane B2, and restore the altered cardiac mechanical and electrical activities (Ayez et al., 2002; Ayez et al., 2004). In vitro studies had shown also that when rat adipocytes were incubated with sodium selenite, glucose transport was stimulated markedly (Ezaki, 1990). Whether the insulinlike effects of selenium compounds in cultured adipocytes can explain the published beneficial effects in diabetic animals is not well understood yet. Even recently, it has been demonstrated that selenium exerts both insulinlike and non-insulin-like actions in adipocytes (Heart and Sung, 2003).

As summarized earlier, there is ample evidence that selenium compounds can restore some metabolic parameters and, to some extent, diminished cardiac function in experimental diabetes. Selenium is an essential dietary component for mammals, including humans. One of its well-understood functions is that it is present in the active center of glutathione peroxidase, which scavenges various peroxidases and protects macromolecules from oxidative damage (Rotruck et al., 1973). Low concentrations of selenium are essential for the synthesis of selenocysteine-containing enzymes (Koller and Exon, 1986).

Although selenium compounds are commonly used as a dietary supplement for the treatment of selenium-deficiency diseases (Neve, 1991), the effects of these compounds on the altered antioxidant system of diabetic heart have been controversial (Mak et al., 1996; Doi et al., 2001). Thus, we aimed to investigate whether beneficial effects extend to the alterations in the blood lipids parameters, heart lipid peroxidation TBARS, glutathione content, anti oxitative enzyme activities in heart such: glutathione peroxydase GPX, glutathione –S- transferase GST, Catalase (CAT) which have important roles in the antioxidant defense mechanisms.

Materials and methods

Chemicals

Animals and experimental design

Thirty five male albino wistar rats of 06 weeks of age with a body weight ranging from 180-220 g were

obtained from the Pasteur institute (Algeria). Animals were acclimated for 2 weeks under the same laboratory conditions of photoperiod (12 h light: 12 h dark) with a minimum relative humidity of 40 % and room temperature of 23± 2°C. Food (standard diet, supplied by the "ONAB, el harrouch", Algeria), and water were available ad libitum. Rats were randomly divided into five groups of seven (7) males each. Two groups were pre-treated for 10 days by sodium selenite (2 mg/kg.bw/d) via orogastric route. The first group was served as the control (con), the second as the normal treated intra peritoneally by selenium (1.89 mg/kg.bw/d) (normal-Se) (Berg et *al.*, 1995). Three groups were intra peritoneally injected with alloxan (150 mg/kg.bw) to induce diabetes (Rotruck and *al.*, 1973). After 72 h alloxan injection; the blood glucose level of the whole blood obtained from the tail vein of the over night fasted animal was tested and those with a blood glucose level above 3 g/l were deemed diabetic. One diabetic group was treated intra peritoneally by insulin (3UI/100g .bw) (DM-Insulin) (Suthagar et *al.*, 2009). Another diabetic group (pre-treated by selenium 2 mg/kg.bw/d for 10 days) was treated intra peritoneally by selenium (1.89 mg/kg.bw/d) (DM- Se) for further three weeks. At the end of experimental period, rats were fasted for 12 hours, and then sacrificed by decapitation and fasting blood sample were collected from the sacrificed animals in heparinised vial and plain vial for hemolysate preparation and for serum preparation respectively. The remaining blood was centrifuged at 2200xg for 15 min at 04°C, and the serum stored at -20°C for biochemical analysis of: glucose, insulin, total cholesterol, HDL-cholesterol, triglycerides and total lipids. The total blood for glucose 6 phosphate dehydrogenase (G6PDH) activity measured.

The heart was rapidly washed with saline and stored at -20°C.

Tissue preparation
1 g of heart was homogenized in 2 ml of buffer solution of phosphate buffered Saline 1:2 (w/v: 1g tissue with 2ml PBS, pH 7.4). Homogenates were centrifuged at 10000xg for 15 min at 4°C and the resultant supernatant was used for the determination of glutathione (GSH), malondialdehyde (MDA) content, and glutathione peroxydase (GPx), glutathione-S- transferase (GST) and catalase activities.

Determination of blood glucose, G6PDH activity, insulin, total cholesterol, HDL cholesterol, triglycerides, lipids
Plasma glucose level was assayed with a commercial kit (spin react, Spain ref 41011) and determined by enzymatic colorimetric method using glucose oxidase enzyme (Kaplan, 1984). The Glucose-6-Phosphate Dehydrogenase (G6PDH) activity in erythrocytes was determined by monitoring the NADPH production at 340 nm and at 37° using the Rx monza analyser (Loher and Waller, 1974). Total insulin as determined by electrochimiluminescence method (Sapin et *al.*, 2001). However plasma total cholesterol, HDL cholesterol, triglycerides and total lipids were determined by methods using kits from Spinreact (ref: 1001091, 1001097, 1001311, 1001270) respectively (Naito and Kaplan, 1984; Buccolo, 1973; Kaplan, 1984). LDL determined by Friedewald formula (Friedwald et *al.*, 1972).

Determination of reduced glutathione
Liver glutathione (GSH) concentration was measured utilizing the method described by weckberker and Cory (1988).

Determination of Glutathione peroxidase (GPx)
Glutathione peroxidase (GPx) (E.C.1.11.1.9) activity was measured by the procedure of Flohe and Gunzler (1984). Supernatant obtained after centrifuging 5% heart homogenate at 1500×g during 10 min followed by 10000×g for 30 min at 4 ◦C was used for GPx assay. 1ml of reaction mixture was prepared which contained 0.3 ml of phosphate buffer (0.1M, pH7.4), 0.2ml of GSH (2mM), 0.1 ml of sodium azide (10mM), 0.1ml of H_2O_2 (1mM) and 0.3ml of heart supernatant. The reaction was terminated by addition of 0.5ml 5% TCA after 15 min of incubation at 37°C. Tubes were centrifuged at 1500×g for 5 min and the supernatant was collected. 0.2 ml of phosphate buffer (0.1MpH7.4) and 0.7 ml of DTNB (0.4 mg /ml) were

added to 0.1ml of reaction supernatant. After mixing, absorbance was recorded at 420 nm.

Determination of thiobarbituric acid reactive substances (TBARS)

The lipid peroxidation level in heart homogenate was measured as malondialdehyde (MDA) which is the end product of lipid peroxidation, and reacts with thiobarbituric acid (TBA) as a TBA reactive substance (TBARS) to produce a red coloured complex with a peak absorbance at 532 nm (Buege and Aust, 1984).

Thus, 125μl of supernatant were homogenized by sonication with 50 μl of PBS,125 μl of TCA–BHT (trichloroacetic acid–butylhydroxytoluene) in order to precipitate proteins, and then centrifuged (1000×g, 10 min, and 4 °C). After wards, 200 μl of supernatant were mixed with 40 μl of HCl (0.6M) and 160 ml of TBA dissolved in Tris, and then the mixture was heated at 80 °C for 10 min. The absorbance of the resultant supernatant was obtained at 530 nm. The amount of TBARS was calculated using a molar extinction coefficient f1.56×105 M/cm.

Determination of glutathione-S-transferase

Glutathione-S-transferase (GST) (EC2.5.1.18) catalyzes the conjugation reaction with glutathione in the first step of mercapturic acid synthesis. The activity of GST was measured according to the method of Habig *et al.* (1974). The P-nitro benzyl chloride was used as substrate. The absorbance was measured at 340 nm at 30 s intervals for 3min.

Determination of catalase activity

The catalase (CAT) activity was determined according to the method of Aebi (1984). The H_2O_2 decomposition rate was followed by monitoring absorption at 240 nm. One unit of CAT activity is defined as the amount of enzymes required to decompose 1μmol of hydrogen peroxide in 1 min. the enzyme activity was expressed as μmol H_2O_2 consumed /mn/mg protein.

Protein estimation

The protein contents of various samples were determined according to the method of Bradford (1976) by using bovine serum albumin as a standard.

Statistical analysis

Analysis of variance (ANOVA) was used to compare multiple group means, followed by student's t- test to determine statistical significance ($p < 0.05$) among the different groups. All statistical analysis were performed using MINITAB software (version 13.31). Results are expressed as means ± SEM.

Results

Effects of treatments on plasma biochemical parameters

a) Blood glucose, insulin levels and G6PDH activity:

alloxan-induced diabetes in rats produced a significant decrease in blood insulin level (73%), G6PDH activity (23%) and increase in glucose level (75%) compared to the control group. Insulin treatment significantly increase blood insulin level, G6PDH activity respectively by (69%, 15%), and decrease glucose level by 67% compared to no treated diabetic rats. Also selenium treatment significantly increase insulin level and G6PDH activity respectively by (66%, 20%), and decrease significantly the level of glucose by 42% compared to no treated diabetic rats (table 01).

b) Blood total cholesterol, HDL-C, LDL-C, Triglycerides and total lipids

Diabetic tats produced a significant increase in total cholesterol (47%), LDL-C (60%), triglycerides (57%), lipids (33%) and decrease in HDL-C by 4% compared to the control group. However in insulin treatment significantly decrease the T cholesterol, LDL-C, triglycerides and lipids levels respectively by (26% ,39%, 23% ,15%), and increase HDL-C level by 7% compared to no treated diabetic rats. Selenium treatment decrease significantly the levels of T cholesterol, LDL-C, triglycerides and lipids levels respectively by (48% ,63%, 53% ,21%), and increase HDL-C level by 12% compared to no treated diabetic rats (table 01).

Effects of treatments on heart tissue parameters

Alloxan-induced diabetes in rats produced a significant decrease in GSH content by 20% (fig 01), GPx (fig 02), catalase (fig 04) activities respectively by (26%,33%), and increase in GST (fig03)activity (18%),

TBARS (fig 05) content by 47% in heart tissue compared to control group. Insulin treatment

significantly increase GSH content (11%), GPx and catalase activities (11%, 25%) respectively and decrease GST activity by 8% and MDA content by 24% compared to no treated diabetic rats. Sodium selenite treatment significantly increase GSH level by 17%, GPx and catalase activities respectively by (20%, 28%), and increase GST activity by 15% and MDA content by38% compared to no treated diabetic rats.

Table 1. Blood glucose, insulin, total cholesterol, HDL-C, LDL-C, triglycerides, lipids levels and G6PDH activity in control, normal treated with selenium (Normal +Se), diabetic (DM), diabetic treated with insulin (DM+ Insulin) and diabetic rats treated with selenium (DM+ Se).

Parameters &treatments	Experimental groups				
	Cont (n=7)	Normal +Se (n=7)	DM (n=7)	DM+ Insulin (n=7)	DM+ Se (n=7)
Glucose (mmol/l)	3.5±0.11	3.44±0.11	13.87 ± 0.62^{abd},	4.49 ± 0.24^{abc}	8.04 ± 0.4^{abcd}
Insulin (µ UI/ml)	2.34 ± 0.048	2.41 ± 0.04	0.64 ± 0.06^{abd}	2.06 ± 0.06^{abc}	1.90 ± 0.038^{abcd}
G-6-PDH(m UI/10GR)	141.9±2	143.5±1	109.8 ± 3^{abd}	130.4 ± 2.2^{abc}	136 ± 1.3^{abcd}
Cholestérol (mmol/l)	T2.48 ±0.1	2.48 ± 0.21	4.67 ± 0.13^{abd}	3.45 ± 0.05^{abc}	2.41 ± 0.08^{cd}
HDL-C (mmol/l)	0.92 ±0.04	0.97 ± 0.04	0.88 ± 0.015^{b}	0.90 ± 0.0025^{b}	1.00 ± 0.038^{cd}
LDL-C	1.21 ±0.02	1.17 ±0.04	3.01 ± 0.08^{abd}	1.84 ± 0.07^{abc}	1.10 ± 0.08^{cd}
Triglycérides (mmol/l)	0.67 ± 0.045	0.65± 0.022	1.55 ± 0.14^{abd}	1.20 ± 0.031^{abc}	0.733 ± 0.051^{cd}
Lipides T (mg/dl)	512± 59	497 ± 19	763 ± 33^{abd}	652 ± 49^{abc}	605 ± 24^{abcd}

Values are given as mean ± SEM for group of 7 animals each. p≤0.05, (a: comparison with cont, b: comparison with normal+Se, c: comparison with DM, d: comparison with DM+ Insulin).

Discussion

It is generally considered that hyperglycaemia is the major factor in the pathogenesis of diabetic complication (Odetti et *al.*, 1996). The persistent hyperglycaemia and the development of diabetic complications results in exaggerated synthesis of free radicals and defective scavenging systems. Results of the present study showed that diabetic rats exhibited a significant increase in plasma glucose level. The beneficial effect of selenium administration on plasma glucose level has several systemic consequences. Firstly, selenium includes stimulation of glucose uptake and regulation of metabolic process such as glycolysis, gluconeogenesis, fatty acid synthesis, and pentose phosphate pathway (Stapleton et *al.*, 1997; Hei et *al.*, 1998). Secondly selenium does activate a key proteins involved in the insulin signal cascade (Stapleton, 2000). Also selenium cause partial restoration of mRNA levels and activities of two key glycolytic enzymes (glucokinase and pyruvate

kinase). It also decreases the elevated mRNA concentration and the activity of a major gluconeogenic enzyme phospho enol pyruvate carboxy kinase (Becker et *al.*, 1996). Also it has ability to restore the expression of both the lipogenic enzymes, glucose 6 phosphate dehydrogenase (G6PDH) and fatty acid synthase (FAS). Increase both G6PDH and FAS, mRNA suggesting that the regulation of expression by the mimetic occur pretranslationally (Berg et *al.*, 1995).
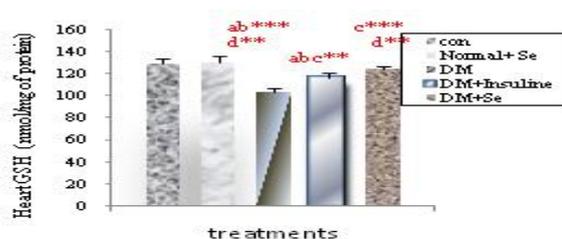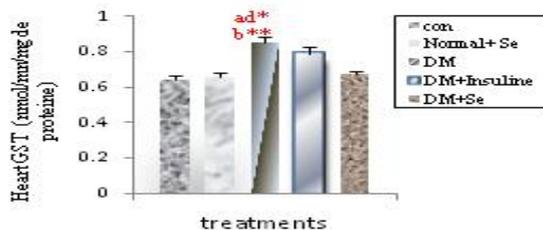

Fig. 1. Reduced glutathione GSH level (nmol/mg protein) in heart of control and treated groups a: comparison with control, b: comparison with normal+Se, c: comparison with DM, d: comparison

with DM+Insulin. Values are means±SEM for seven rats in each group. * p≤0.05, ** p≤0.01, *** p≤0.001.
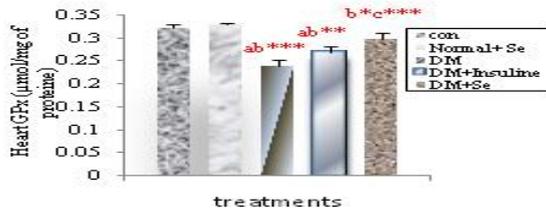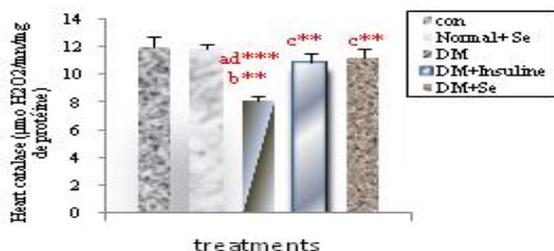

Fig. 2. Glutathione peroxydase GPx activity (μmol/mg protein) in heart of control and treated groups a: comparison with control, b: comparison with normal+Se, c: comparison with DM, d: comparison with DM+Insulin. Values are means±SEM for seven rats in each group. * p≤0.05, ** p≤0.01, *** p≤0.001.

Other study with a medium oral dose of 2 mg/kg/d (11.6 μmol/kg) selenite was able to lower the blood glucose of alloxan-induced kun-min mice (Ding et al., 1997).

Fig. 3. Glutathione-S-transferase activity (nmol/mg protein) in heart of control and treated groups a: comparison with control, b: comparison with normal+Se, c: comparison with DM, d: comparison with DM+Insulin. Values are means±SEM for seven rats in each group. * p≤0.05, ** p≤0.01, *** p≤0.001.
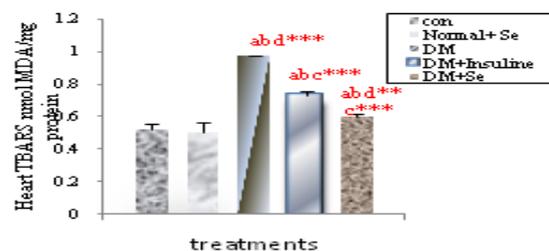

Fig. 4. Catalase (CAT) activity (μmol H2O2)/mg protein) in heart of control and treated groups a: comparison with control, b: comparison with normal+Se, d: comparison

with DM+Insulin. Values are means±SEM for seven rats in each group. * p≤0.05, ** p≤0.01, *** p≤0.001.

G6PDH activity was decreased in diabetic rats compared with normal control and normal selenium treated rats (table 01). Insulin treatment and selenium treatment normalized the decrease in G6PDH activity in diabetic rats. Other study (Zhang et al., 2000) has shown that high glucose led to increased PKA activity and phosphorylation of G6PDH in cultured bovine aortic endothelial cells. That PKA can directly phosphorylate G6PDH and inhibit G6PDH activity (Zhang et al., 2000).

The present results demonstrated that diabetes significantly increased the levels of total cholesterol, LDL-C, triglycerides, Total lipids. And decreased of HDL-C which did have a direct detrimental effect on heart function, these results are in accordance with those of other investigations (Opie, 1970; Rodrigues and McNeill, 1986; Rodrigues et al., 1986). Administration of sodium selenite significantly ameliorated the adverse influence of alloxan. Thus selenium treatment normalizes the alteration in cholesterol metabolism that occurs in diabetes (Veki et al., 1993). Selenium dependant peroxidase can detoxifiy a wide variety of peroxides including lipid derived-species (LOOH) present in LDL ox, thus selenium treatment can ameliorate the modification occurring in LDL-cholesterol by oxidative injury (Ness et al., 1994).

Fig. 5. TBARS (nmol MDA/mg protein) in heart of control and treated groups a: comparison with control, b: comparison with normal+Se, c: comparison with DM, d: comparison with DM+Insulin. Values are means±SEM for seven rats in each group. * p≤0.05, ** p≤0.01, *** p≤0.001.

Oxidative stress markers: TBARS levels were measured as an index of malondialdehyde production

and hence lipid peroxidation. Results indicated a significant increase of TBARS in diabetic animals compared with control animals (fig 05). Insulin and selenium treatment normalized or ameliorated the increase of TBARS level. These results show, as demonstrated by other investigations, that diabetes leads to increased oxidative stress. Thus furthers evaluations of enzymes and chemicals associated with the antioxidant system were done.

As shown in fig 01, the normal function of GSH coupling relies on a sufficient supply of NADPH. This a decrease in NADPH should lead to decreased GSH levels. Figure 01 shows that GSH levels were significantly decreased in untreated diabetic rats compared with normal control and normal treated by selenium. Insulin treatment ameliorated GSH level however selenium treatment normalized the significant decrease in GSH level in diabetic rats. Examination of other enzymes associated with oxidant stress (glutathione peroxidase, catalase and glutathione-s-transferase) show a significant differences between control and treated rats. There was a decrease in GPx and catalase activities and decrease in GST activity in the diabetic group (fig 2, 3, 4). Although there are clearly other factors responsible for the increased lipid peroxidation such as increased production of reactive oxygen species (Maritim et *al.*, 2003).

Selenium treatment increase significantly GPx, catalase activities and decrease GST activity compared to untreated diabetic rats and insulin treated diabetic rats.

There are a few studies which suggest that selenium levels per se may be important in heart function (Zhong et *al.*, 1990; Koehler et *al.*, 1988).

In conclusion, this study demonstrates that Selenium is known to act to prevent free radical damage to cells. Selenium has been administered to humans to reduce the incidence and severity of Keshan disease, a cardiomyopathy which occurs in selenium-poor areas of China. Theoretically, diabetics who have an increased incidence of heart disease, may benefit both from an improvement in glucose homeostasis due to selenite treatment as well as from a direct cardioprotective effect of the element, which play an important role in oxidative stress, to near normal values.

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