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Amelioration of Altered Serum, Liver, and **Kidney Antioxidant Enzymes Activities by** Sodium Selenite in Alloxan-Induced Diabetic Rats

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Abstract

Background: The aim of this study was to evaluate the possible protective effect of sodium selenite on serum, liver, and kidney antioxidant enzymes activities in alloxan-induced type 1 diabetic rats.

Methods: Forty Sprague-Dawley male rats were randomly divided into four groups; Group one as control, Group two as sham-treated with sodium selenite by 1 mg/kg intraperitoneal (i.p.) injections daily, Group three as diabetic untreated, and Group four as diabetic treated with sodium selenite by 1 mg/kg i.p. injections daily. Diabetes was induced in the third and fourth groups by subcutaneous alloxan injections. After eight weeks the animals were euthanized and livers and kidneys were immediately removed and used fresh or kept frozen until analysis. Before the rats were killed blood samples were also collected to measure glutathione peroxidase (GPX) and catalase (CAT) activities in sera.

Results: Glutathione peroxidase and CAT activities serum, liver, and kidney were all significantly less in the diabetic rats than in the controls. Sodium selenite treatment of the diabetic rats resulted in significant increases in GPX activity in the kidneys and livers, and CAT activity in the sera and livers.

Conclusions: Our results indicate that sodium selenite might be a potent antioxidant that exerts beneficial effects on both GPX and CAT activities in alloxan-induced type 1 diabetic rats.

Keywords: Antioxidant enzymes activity, Diabetes, Rat, Sodium selenite

Introduction

Chronic diseases such as diabetes and cardiovascular disease increase oxidants and decrease antioxidants in patients (1, 2). Conditions that increase oxidants and reduce antioxidants also exacerbate these diseases (1-5). In diabetic patients, hyperglycemia causes glycation of macromolecules such as proteins, lipids, nucleic acids (6). The glycation macromolecules can alter their functions, increase lipid peroxidation, and reduce antioxidant enzyme activity, causing damage to the cells (7). Clinical and experimental studies have shown that disturbing the

balance of the oxidant-antioxidant system can contribute to the pathogenesis of chronic diseases such as cancer, cardiovascular disease, diabetes, and many diabetic complications (8).

Selenium is an antioxidant. It can act as an antioxidant directly or as a cofactor in antioxidant enzymes such as glutathione peroxidase (GPX) in a number of biological processes in humans and other species (9). Selenium deficiencies can induce cancer, coronary heart disease, and liver necrosis (10-14). The possible protective effects of sodium selenite on

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serum, kidney, and liver antioxidant enzyme activities in alloxan-induced type 1 diabetic rats have not yet been reported. Therefore, this study was designed to investigate the effect of sodium selenite on the antioxidant enzymes GPX and catalase (CAT) activities in serum, kidney, and liver from alloxan-induced type 1 diabetic rats.

Materials and Methods

Animals

Thirty mature male Sprague—Dawley rats (180-200 g) were obtained from the Pasteur Institute of Tehran and allowed to adapt to the new location for one week. This study was approved by the Animal Ethics Committee of the Medical University of Lorestan according to the National Health and Medical Research Council guidelines. The rats were divided into four groups of 10. The groups were as follows: Group 1 as control, Group 2 as non-diabetic treated with 1 mg/kg intraperitoneal (i.p.) daily sodium selenite, Group 3 as diabetic without sodium selenite treatment, and Group 4 as diabetic treated with 1 mg/kg i.p. daily sodium selenite.

Diabetes induction

Diabetes was induced after overnight fasting in the third and fourth groups by injection of alloxan monohydrate (120 mg/kg) subcutaneously (5). Beta cell degradation by alloxan leads to increased insulin release. Because of acute hypoglycemia, the rats received 10% sucrose solution for 48 hr in place of drinking water. Five days after induction of diabetes, blood samples were collected from tails. Blood glucose was measured by glucometer and the rats with blood glucose levels ≥300 mg/dl (16.7 mmol/l) were defined as diabetic (15, 16). During the first five days after diabetes induction, 1-3 rats per group died from alloxan toxicity. The rats were kept at 12/12 dark-light periods in 21 ± 3 °C. All animals were allowed free access to food and water during the experiment. The sodium selenite treatment of Group 4 began on the first day of diabetes induction. After eight weeks, animals were euthanized with Nesdonal (50 mg/kg, i.p.). Blood samples were obtained from hearts and allowed to clot for 20 minutes at room temperature and then centrifuged at 3000 rpm for 10 minutes in a bench top centrifuge and serum was collected (5). Livers and kidneys were removed immediately and used fresh or kept frozen for further analyses.

CAT Activity

Catalase activity was estimated using the method of Sinha. The reaction was started by the addition of 20 µl of sample in 2 ml of 30 mmol/l hydrogen peroxide (H₂O₂) in 50 mmol/l potassium phosphate buffer pH 7.0. Enzyme units are expressed as mmol/l of consumed H₂O₂ per min g or ml (18).

GPX Activity

GPX activity was determined using a GPX assay kit (Randox Lab., Ltd., UK) according to the manufacturer's protocol.

Statistical analysis

All values are expressed as means \pm standard deviations (SD). The data were compared between groups by the Mann-Whitney U test. Statistical analyses were performed using the SPSS 13 for Windows software. p values < 0.05 were considered statistically significant.

Results

Effect of sodium selenite on serum, kidney, and liver GPX activity in diabetic rats

Th GPX activity in serum, kidney, and liver are shown in Table 1. Serum GPX activity in the untreated diabetic rats was significantly less than that of the controls $(44.86 \pm 10.89 \text{ vs. } 69.19 \pm 20.11 \text{ U/mg})$ protein). Serum GPX activity of the selenium-treated diabetic rats was not significantly different from that of the untreated diabetic rats (52.24 \pm 13.76 vs. 44.86 ± 10.89 U/mg protein). Renal GPX activity in the untreated diabetic rats was significantly less (1.81fold) than that of the controls $(37.06 \pm 16.49 \text{ vs. } 66.92 \text{ m})$ ± 8.25 U/mg protein). Renal GPX activity in the selenium-treated diabetic rats was significantly greater than that of the untreated diabetic rats (65.04 \pm 7.73 vs. 37.06 ± 16.49 U/mg protein). Liver GPX activity in the untreated diabetic rats was significantly less (1.48-fold) than that of the controls (42.77 \pm 6.78 vs. 63.52 ± 13.30 U/mg protein). Liver GPX activity in the sodium selenite-treated diabetic rats was significantly greater (22.63%) than that of the untreated diabetic rats (52.45 \pm 15.99 vs. 42.77 \pm 6.78 U/mg protein). The GPX activities in the kidneys and

livers of the sodium selenite-treated non-diabetic rats were relatively high and similar to the levels found in the control animals.

Effect of sodium selenite on serum, kidney, and liver CAT activity in diabetic rats

Serum, kidney, and liver CAT activities are shown in Table 2. Serum CAT activity in the untreated diabetic

rats was significantly less (1.79-fold) than that of the controls (46.00 \pm 7.27 vs. 83.49 \pm 29.71 U/mg protein). Serum CAT activity of sodium selenite-treated diabetic rats was significantly greater than that of the diabetic untreated rats (71.63 \pm 19.68 vs. 46.00 \pm 7.27 U/mg protein).

Table 1. The effect of sodium selenite on serum, liver, and kidney GPX activity in alloxan-induced diabetic rats (Values represented as mean \pm S.D).

Experimental groups		Serum	Kidney	Liver
GPX activity (U/mg protein)	Control (non-diabetic untreated)	69.19 ± 20.11*	66.92 ± 8.25*	63.52 ± 13.30*
	Non-diabetic treated	70.86 ± 13.76 *	70.16 ± 5.11 *	68.67 ± 13.33*
	Diabetic untreated	44.86 ± 10.89	37.06 ± 16.49	42.77 ± 6.78
	Diabetic treated	52.241 ± 13.76#	65.04 ± 7.73 *	52.45 ± 15.99* #√

^{*}P<.05 compared with diabetic untreated group.

Table 2. The effect of sodium selenite on serum, liver, and kidney CAT activity in alloxan-induced diabetic rats (Values represented as mean \pm S.D).

Experimental groups		Serum	Kidney	Liver
CAT activity (U/mg protein)	Control (non-diabetic untreated)	83.49 ± 29.71*	89.88 ± 19.01*	84.94 ± 23.55*
	Non-diabetic treated	$76.45 \pm 21.43*$	53.84 ± 20.02	61.26 ± 11.77 *
'AT ac J/mg p	Diabetic untreated	46.00 ± 7.27	46.75 ± 10.39	26.13 ± 7.94
	Diabetic treated	71.63 ± 19.68*	45.71 ± 23.75#	58.84 ± 23.91*#

^{*}P < .05 compared with diabetic untreated group.

Renal CAT activity in the untreated diabetic rats was significantly less (1.92-fold) than that of the controls (46.75 \pm 10.39 vs. 89.88 \pm 19.01 U/mg protein). Renal CAT activity in the sodium selenium-treated diabetic rats was not significantly different from that of the untreated diabetic rats (45.71 \pm 23.75 vs 46.75 \pm 10.39 U/mg protein).

Liver CAT activity in the untreated diabetic rats was significantly less (3.25-fold) than that of controls (26.13 ± 7.94 vs 84.94 ± 23.55 U/mg protein), while liver CAT activity in the sodium selenite-treated diabetic rats was significantly greater than that of the untreated diabetic rats (58.84 ± 23.91 vs 26.13 ± 7.94 U/mg protein). The CAT activities in the sera, kidneys, and livers of the sodium selenite-treated diabetic rats were all somewhat less than those of the controls, but greater than those of the diabetic untreated rats in sera and livers.

Discussion

This study showed that sodium selenite can increase the reduced serum, renal, and liver GPX and CAT in alloxan-induced diabetic rats. There is much evidence that free radicals play a key role in the most pathogenic pathway of diabetic injuries (8). Free radicals such as superoxide can increase lipid peroxidation, carcinogenesis, inflammation, early aging, cardiovascular diseases, and tissue damage in diabetes (19-24). Antioxidants, such as vitamin E, coenzyme Q10, and rosmarinic acid, and antioxidant enzymes, such as CAT, SOD, and GPX decrease the increased lipid peroxidation that occurs carcinogenesis, inflammation. early aging. cardiovascular diseases, and diabetes (24). Much evidence indicates that oxidative stress plays a key role in the pathogenesis of diabetes (24).

[#]P < .05 compared with controls.

 $[\]sqrt{P}$ < .05 compared with the non diabetic treated group.

[#]P < .05 compared with controls.

These antioxidants and antioxidant enzymes protect the cells against oxidative stress-mediated injuries by converting the toxic free radicals to non-toxic products (19, 20). Therefore the use of antioxidants as complementary therapies can be useful for the treatment of diseases related to oxidative stress. Glutathione peroxidase and CAT activities, as markers of antioxidant enzymes status, were significantly less in the untreated diabetic rats than in the controls. Sodium selenite treatment significantly increased serum, renal, and liver GPX and CAT activities compared with the untreated animals. Sodium selenite treatment also resulted in similar serum GPX and CAT activities of the treated animals similar to as those of the control group. The antioxidant enzymes GPX and CAT are considered to be indicators of antioxidant status (25). Previous studies showed that antioxidants such glibenclamide and metformin (26), vitamins E and C, melatonin (27), zinc (28), vanadium and Trigonella (29, 30), salidroside (31), flavonoids (32), taurine (26), N-acetyl cysteine, L-arginine (33), lycopene (34), succinic acid monoethyl ester (35), rutin (36), aminoguanidine and natural (37),compounds (38) increased antioxidant enzymes and antioxidant status in diabetics. Research also showed that vitamins E and C together reduced lipid peroxidation and increased the antioxidants growth stimulating hormone (GSH), SOD, and GPX in chlorpyrifos-ethyl-induced lung toxicity in rats (39). Also, one previous study showed that sodium selenite can increase GSH, SOD, GPX, and CAT, and decrease lipid peroxidation in liver and kidneys from Cd-induced oxidative damage (11).

The results of our study agree with others showing

References

- 1. Ríos-Silva M, Trujillo X, Trujillo-Hernández B, Sánchez-Pastor E, Urzúa Z, Mancilla E, Huerta M. Effect of chronic administration of forskolin on glycemia and oxidative stress in rats with and without experimental diabetes. Int J Med Sci. 2014 Mar;11(5):448-52.
- 2. Csányi G, Jr Miller FJ. Oxidative stress in cardiovascular disease. Int J Mol Sci. 2014 Apr;15(4):6002-8.
- 3. Ahmadvand H, Tavafi M, Khosrowbeygi A. Amelioration of altered antioxidant enzymes activity and glomerulosclerosis by coenzyme Q10 in alloxan

that sodium selenite can increase GPX and CAT activities. Therefore, sodium selenite as an antioxidant with beneficial effects on antioxidant enzymes might be useful in reducing the complications of various types of tissue damage seen in diabetics. Antioxidant therapy is one of the most important treatment strategies for the prevention and slowing of the progression of diabetic complications such as hyperglycemia, hyperlipidemia, hepatic damage, and nephropathy. Although the detailed mechanisms of sodium selenite antioxidant function cannot be fully explained by our results, several studies have explained some mechanisms of oleuropein antioxidant function. Sodium selenite may directly eliminate free radicals such as lipid peroxyl, peroxyl, and/or alkoxyl radicals in vitro and in vivo. Also, micronutrients such as selenium, manganese, and zinc are involved in the structure or catalytic activity of antioxidant enzymes. If the supply of these minerals is inadequate, enzymatic defenses may be impaired. Therefore, sodium selenite as a beneficial antioxidant could be proposed as a supplement in diabetics to prevent of diabetic nephropathy.

This study showed that sodium selenite possesses antioxidant activity and has beneficial effects in increasing the reduced serum, renal, and liver antioxidant enzymes in alloxan-induced diabetic rats. The elevation of antioxidant enzymes activities may decrease diabetic complication such as nephropathy in diabetic patients.

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induced diabetic rats. J Diabetes Complications. 2012 Nov-Dec;26(6): 476-82.

- 4. Ahmadvand H, Mabuchi H, Nohara A, Kobayahi J, Kawashiri MA. Effects of coenzyme Q (10) on LDL oxidation in vitro. Acta Med Iran. 2013;51(1): 12-8.
- 5. Zatalia SR, Sanusi H. The role of antioxidants in the pathophysiology, complications, and management of diabetes mellitus. Acta Med Indones. 2013 Apr;45(2):141-7.
- 6. Arasteh A, Farahi S, Habibi-Rezaei M, Moosavi-Movahedi AA. Glycated albumin: an overview of the

- In Vitro models of an In Vivo potential disease marker. J Diabetes Metab Disord. 2014 Apr;13:49.
- 7. Katta AV, Katkam RV, Geetha H. Lipid peroxidation and the total antioxidant status in the pathogenesis of age related and diabetic cataracts: a study on the lens and blood. J Clin Diagn Res. 2013 Jun;7(6):978-81.
- 8. Stadler K. Oxidative stress in diabetes. Adv Exp Med Biol. 2012;771:272-87.
- 9. Fraga CG. Relevance, essentiality and toxicity of trace elements in human health. Mol Aspects Med 2005 Aug-Oct; 26(4-5): 235-44.
- 10. Alexanian I, Parissis J, Farmakis D, Pantziou C, Ikonomidis I, Paraskevaidis I, Ioannidou S, Sideris A, Kremastinos D, Lekakis J, Filippatos G. Selenium contributes to myocardial injury and cardiac remodeling in heart failure. Int J Cardiol. 2014; 176(1): 272-3.
- 11. Ognjanović BI, Marković SD, Pavlović SZ, Zikić RV, Stajn AS, Saicić ZS. Effect of chronic cadmium exposure on antioxidant defense system in some tissues of rats:protective effect of selenium. Physiol Res. 2008;57(3):403-11.
- 12. Soudani N, Troudi A, Bouaziz H, Ben Amara I, Boudawara T, Zeghal N. Cardioprotective effects of selenium on chromium (VI)-induced toxicity in female rats. Ecotoxicol Environ Saf. 2011 Mar;74(3): 513-20.
- 13. Suryo Rahmanto A, Pattison DI, Davies MJ. Photo-oxidation-induced inactivation of the selenium containing protective enzymes thioredoxin reductase and glutathione peroxidase. Free Radic Biol Med. 2012 Sep;53(6):1308-16.
- 14. Roy G, Sarma B, Phadnis P, Mugesh G. Selenium-containing enzymes in mammals: Chemical perspectives. J Chem Sci. 2005 Jul;117(4):287-303.
- 15. Haidara MA, Mikhailidis DP, Rateb MA, Ahmed ZA, Yassin HZ, Ibrahim IM, et al. Evaluation of the effect of oxidative stress and vitamin E supplementation on renal function in rat with streptozotocin-induced type 1 diabetes. J Diabet Complications 2009; 23 (2): 130–6.
- 16. Tavafi M, Ahmadvand H, Tamjidipoor A, Delfan B, Khalatbari AR. Satureja khozestanica essential oil ameliorates progression of diabetic nephropathy in uninephrectomized diabetic rats. Tissue and Cell. 2011 Mar-Apr;43(1):45-51.

- 17. Chakrabarti S, Brodeur J. Influence of selenium on the metabolism of bromobenzene and a possible relationship to its hepatotoxicity. Environ Res. 1985 Aug;37(2):327-39.
- 18. Sinha, AK. Colorimetric assay of catalase. Anal Biochem. 1972 Jun;47:389-94.
- 19. Kim HY, Sin SM, Lee S, Cho KM, Cho EJ. The butanol fraction of bitter melon (Momordica charantia) Scavenges Free Radicals and Attenuates Oxidative Stress. Prev Nutr Food Sci. 2013 Mar;18(1):18-22.
- 20. Tekiner-Gulbas B, Westwell AD, Suzen S. Oxidative stress in carcinogenesis: new synthetic compounds with dual effects upon free radicalsand cancer. Curr Med Chem. 2013;20(36):4451-9.
- 21. Salama SA, Omar HA, Maghrabi IA, AlSaeed MS, EL-Tarras AE. Iron supplementation at high altitudes induces inflammation and oxidative injury to lung tissues in rats. Toxicol Appl Pharmacol. 2014 Jan;274(1):1-6.
- 22. Ivanova DG, Yankova TM. The free radical theory of aging in search of a strategy for increasing life span. Folia Med (Plovdiv). 2013 Jan-Mar;55(1): 33-41.
- 23. Tullio F, Angotti C, Perrelli MG, Penna C, Pagliaro P. Redox balance and cardioprotection. Basic Res Cardiol. 2013;108(6):392.
- 24. Sarmento RA, Silva FM, Sbruzzi G, Schaan BD, Almeida JC. Antioxidant micronutrients and cardiovascular risk in patients with diabetes: a systematic review. Arq Bras Cardiol. 2013; 101(3): 240-248.
- 25. Flores-Mateo G, Carrillo-Santisteve P, Elosua R, Guallar E, Marrugat J, Bleys J, et al. Antioxidant enzyme activity and coronary heart disease: meta-analyses of observational studies. Am J Epidemiol. 2009 Jul;170(2):135-47.
- 26. Erejuwa OO, Sulaiman SA, Wahab MS, Salam SK, Salleh MS, Gurtu S. Antioxidant protective effect of glibenclamide and metformin in combination with honey in pancreas of streptozotocin-induced diabetic rats. Int J Mol Sci. 2010 May;11(5):2056-66.
- 27. El-Batch M, Hassan AM, Mahmoud HA. Taurine is more effective than melatonin on cytochrome P450 2E1 and some oxidative stress markers in streptozotocin-induced diabetic rats. J Agric Food Chem. 2011 May;59(9):4995-5000.
- 28. Lima VB, Sampaio Fde A, Bezerra DL, Moita

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- Neto JM, Marreiro Ddo N. Parameters of glycemic control and their relationship with zinc concentrations in blood and with superoxide dismutase enzyme activity in type 2 diabetes patients. Arq Bras Endocrinol Metabol. 2011 Dec;55(9):701-7.
- 29. Siddiqui MR, Taha A, Moorthy K, Hussain ME, Basir SF, Baquer NZ. Amelioration of altered antioxidant status and membrane linked functions by vanadium and Trigonella in alloxan diabetic rat brains. J Biosci. 2005 Sep;30(4):483-90.
- 30. Mohammad S, Taha A, Bamezai RN, Basir SF, Baquer NZ. Lower doses of vanadate in combination with trigonella restore altered carbohydrate metabolism and antioxidant status in alloxan-diabetic rats. Clin Chim Acta. 2004 Apr; 342(1-2):105-14.
- 31. Li F, Tang H, Xiao F, Gong J, Peng Y, Meng X. Protective effect of salidroside from Rhodiolae Radix on diabetes-induced oxidative stress in mice. Molecules. 2011 Dec;16(12):9912-24.
- 32. Stefek M. Natural flavonoids as potential multifunctional agents in prevention of diabetic cataract. Interdiscip Toxicol. 2011 Jun; 4(2): 69-77.
- 33. Granstam SO, Granstam E. Endothelin-induced changes in blood flow in STZ-diabetic and non-diabetic rats: relation to nitric oxide synthase and cyclooxygenase inhibition. J Physiol Sci. 2011 Nov;61(6):497-505.

- 34. Ali MM, Agha FG. Amelioration of streptozotocin-induced diabetes mellitus, oxidative stress and dyslipidemia in rats by tomato extract lycopene. Scand J Clin Lab Invest. 2009;69(3): 371-9. 35. Saravanan R, Pari L. Succinic acid monoethyl ester prevents oxidative stress in streptozotocinnicotinamide-induced type2 diabetic rats. J Basic Clin Physiol Pharmacol. 2006;17(2):115-32.
- 36. Kamalakkannan N, Stanely Mainzen Prince P. Rutin improves the antioxidant status in streptozotocin-induced diabetic rat tissues. Mol Cell Biochem. 2006 Dec;293(1-2):211-9.
- 37. Stoppa GR, Cesquini M, Roman EA, Ogo SH, Torsoni MA. Aminoguanidine prevented impairment of blood antioxidant system in insulin-dependent diabetic rats. Life Sci. 2006 Feb;78(12):1352-61.
- 38. Ranilla LG, Kwon YI, Apostolidis E, Shetty K. Phenolic compounds, antioxidant activity and in vitro inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. Bioresour Technol. 2010 Jun;101(12):4676-89.
- 39. Karaoz E, Gultekin F, Akdogan M, Oncu M, Gokcimen A. Protective role of melatonin and a combination of vitamin C and vitamin E on lung toxicity induced by chlorpyrifos-ethyl in rats. Exp Toxicol Pathol. 2002 Aug;54(2):97-108.