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The Impact of Vitamin E Supplementation on Urinary Bladder Contractility in Streptozotocin-Induced Diabetic Rats

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Abstract

Background: To determine whether vitamin E protects streptozotocin-induced diabetic rats from diabetic urinary bladder dysfunction and discover its possible mechanism.

Materials and Methods: A total of 40 rats were randomly divided into four groups: a control group (A), a diabetic group (B), a group given vitamin Eonly (C), and a diabetic group given vitamin E therapy for 8 weeks (D). Diabetes was induced in the rats by 65 mg/kg streptozosin (STZ) via an intraperitoneal (i.p.) injection. Vitamin E was given in a dose of 50 mg/kg/day i.p. Under urethane anaesthesia (1.2 g/kg) subcutaneously and decapitation, contractile responses to carbachol of detrusor strips in all groups were studied *in vitro*. The levels of nitrite, nitrate, malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were detected in bladder tissues homogenates. Apoptosis studies were performed by detection of the levels of caspase 3 and cell death detection.

Results: The bladder weights were significantly increased ($p<0.001$) in diabetic groups compared to the other studied groups. Contractile responses to carbachol increased in the diabetic group more than in the other groups ($p<0.001$). Vitamin E improved the contractile responses of group D and improved them but still significantly higher than those of control group ($p < 0.05$). Vitamin E treatment decreased the tissue MDA, nitrite, nitrate and GSH levels of group D which were significantly higher in group B than A and C groups ($p<0.001$). All enzyme activities of group B were significantly lower than those of the other groups, although they increased significantly in group D but still lower than those of A and C groups. However, no significant differences were detected between the levels of GPx and SOD of group D and those of A and C groups.

Conclusions: These data suggest that vitamin E supplementation may be beneficial in delaying the progression of diabetic dysfunction in experimental animal model.

Keywords: Vitamin E, Bladder contractitliy, Diabetes, Rats

Introduction

Bladder dysfunction is one of the most common complications of diabetes mellitus (DM) affecting about 40–80% of diabetic patients. It is characterized by impaired bladder sensation, increased bladder capacity, decreased bladder contractility and increased residual urine (1). Detrusor overactivity is the presence of inappropriate spontaneous detrusor contractions whilst the bladder is filling, which can lead to urgency, frequency and urinary incontinence (2). Detrusor overactivity affects a large percentage of the population, but the pathophysiological mechanisms involved remain unclear. In humans, overactive bladder, as an increase in detrusor smooth muscle sensitivity to muscarinic agonists, has been reported by several groups (3,4). Diabetic bladder dysfunction or diabetic cystopathy, which presents with urodynamically abnormal bladder function, is a frequently recognized complication of diabetes mellitus (5). Apoptosis may be important in the pathophysiology of DM cystopathy. However, the mechanism that induces bladder dysfunction remains unclear (6).

Animal models of diabetes exhibit similar dysfunction of detrusor muscle to that seen in humans. Thus, in rats with diabetes induced by streptozotocin, the detrusor can exhibit overactivity and muscarinic receptor supersensitivity similar to that observed in the human overactive bladder (7). Diabetes-induced cell death has been observed in multiple organs in vivo (8). Oxidative stress induced by diabetes may play an important role in apoptosis under hyperglycemic conditions. Oxidative stress occurs as a result of the imbalance between reactive oxygen species (ROS) production and their neutralization by antioxidants (9).

Experimental studies showed that high-dose thiamine and benfotiamine new therapy could prevent incipient diabetic nephropathy in rats. Benfotiamine prevents experimental diabetic retinopathy as a complication of diabetes (10,11). Vitamin E is a well-known dietary antioxidant that can eliminate free-radical damage. It may be beneficial for the maintenance of good health and disease prevention (12).

The aim of this study is to test for potential protective effects of vitamin E on streptozotocin (STZ)-induced diabetic rats regarding diabetic bladder dysfunction, and search for the possible mechanism by which vitamin E can exert such protective effects.

Materials and Methods

Experimental Animals

Forty adult male white albino rats weighing 200-250 gm were included in the experiment. The animals were provided by the Institutional Animal Care, Faculty of Medicine, Assiut University. All rats were housed in a room with controlled temperature (22°C ± 2°C), humidity (50% ± 5%), and a 12-hour light/dark cycle and were fed on chow and water ad libitum. The experiment was performed after a stabilization period of seven days. The Ethics Committee of Faculty of Medicine, Assiut University, Egypt, has approved the experimental protocol.

Grouping and Diabetes Induction

The rats were randomly divided into 4 groups containing 10 animals each: Group A: non-diabetic group of rats injected with saline as a control group. Group B: Diabetic group. Group C: Non diabetic group given vitamin E only "Sigma Chemical Co., St. Louis, MO" (13) being injected intraperitonealy (i.p.) in a dose of 40 mg/kg/d for 8 weeks. Group D: diabetic rats given vitamin E (40 mg/kg/d i.p.) for 8 weeks.

Diabetes was induced by a single intraperitoneal injection of streptozotocin STZ "Sigma Chemical Co., St. Louis, MO" (14) in a dose of 65 mg/kg body weight; dissolved in 0.01 M citrate buffer, pH 4.5. Plasma glucose levels were determined from tail vein blood samples (Gluco Dr, All Medicus, Gyeonggi-do, Korea) 2 days after STZ administration; Rats with a serum postprandial glucose level of 180–300 mg/dl were considered as mildly diabetic and were included in the experiment. All rats were kept under identical conditions for 8 weeks with free access to food and water. Eight weeks after the initial treatment, rats were fasted overnight and sacrificed; blood samples were then collected for measurement of plasma glucose levels.

Solutions and drugs

Krebs solution, Streptozotocin, and carbachol were purchased from Sigma Chemical Company (St Louis, MO, USA). All reagents in this study for analytical grade were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

In vitro organ bath experiment

After urethane anesthesia (1.2 g/kg subcutaneously) and decapitation, a lower abdominal midline incision was made. The bladder was excised from each animal and through a longitudinal incision from the base to the dome each bladder was opened up to form a flat sheet. The base

and the top of the dome were carefully removed and 3–4 longitudinal strips, depending on bladder size, were cut from the bladder body. Tissues were suspended in 15 ml organ baths containing Krebs bicarbonate solution (118.3 mM NaCl, 11.7 mM D-Glucose, 24.9 mM NaHCO3, 4.7 mM KCl, 1.15 mM MgSO4, 1.15 mM KH2PO4 and 1.9 mM CaCl2) including 5 μM indomethacin (a cyclooxygenase inhibitor), maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. The strips were placed under 2 g of tension and left to equilibrate for one hour and the tension developed by the tissues was measured using isometric force transducers (Pioden Controls Ltd, UK). Following equilibration for 60 minutes, a cumulative concentration–response curve to the cholinergic receptor agonist carbachol (10^{-8} to 10^{-4} M) was obtained. Responses were compared between A, B, C and D groups bladder strips (15).

Tissue processing (Bladder Homogenates Preparation)

Wet weight of the bladder tissue samples was measured, then the samples were divided into pieces and transferred into tubes and homogenized to about 10% in 150 mM KCl at 4° C. The homogenates were centrifuged at 3000 rpm for 15 min, then separated into aliquots and frozen at -80°C until used for different biochemical studies.

Nitrite analysis method:

Following centrifugation, 0.25 mL of 0.3 M sodium hydroxide (1.2 g NaOH completed to 100 mL with distilled water) was added to 0.5 mL of supernatant. After five minutes of incubation at room temperature, 0.25 mL 5% zinc sulphate was added for deproteinization. The mixture was centrifuged for 20 min at 3000 g. One millilitre of ethanol (0º C) was added to 0.5 mL supernatant, incubated at 0º C for 30 min and then centrifuged at 14 000 g. for 5 min The final supernatant was used for nitrite determination (Sievers Model 280A NOA, Sievers Instruments Inc., USA). Standard solutions (25, 50, 75, 100 lM,and one and 10 mM) from a 100 mM stock solution, obtained by the dissolution of 69 mg sodium nitrite in 10 mL deionized water, were used for the standard curve. The concentrations were calculated from the supernatant of prepared tissue specimens by using a standard curve (16).

Nitrate analysis method

Total nitrate levels were measured with NO analyzer (Sievers Model 280A, Sievers Instruments Inc., USA). Tissue specimens were deproteinized with 1/2 volume of 96% ethanol. Saturated VCL3 (Vanadium Chloride) solution in 1 M hydrochloric acid was filtrated and 5 mL of this reagent was treated with nitrogen gas for 10 min in the purge pipette. Circulating water bath, which heats the reagent up to 95 ºC and cold water condenser was applied to purge pipette. Hydrochloric acid vapour was drained with gas bubbler equipment containing 15 mL 1 M sodium hydroxide. The velocity of gas flow in the chemiluminescence detector was controlled with needle-valve apparatus allowing a 6 Torr pressure. The materials, which convert nitrate to NO via VCL3 and HCL (Hydrogen Chloride) reagents, were injected into the purge pipette. NO product was drained from the reaction chamber and determined with ozone-induced chemiluminescence. A standard curve (16) was employed to calculate various levels of nitrate (10–100 uM).

Tissue MDA analysis

The MDA levels were assayed for products of lipid peroxidation (17). Following centrifugation, the supernatants were removed and 750 uL of the solution was added to 750 uL of 0.67% thiobarbituric acid (TBA). The samples were heated at 100º C for 15 min, cooled at room temperature and centrifuged at 4000 g. Absorbances were measured at 532 nm. The bladder tissue lipid peroxidation levels were expressed as MDA equivalence with 1.56 X 10^5/M coefficients (18).

Tissue GSH analysis

In the samples, the level of GSH was measured as described early (19). Tissue protein was obtained by the biuret's method (20). To 200 μL of the supernatant, 8 ml of pH 6.8 phosphate buffer, 78 ml of 1 N NaOH, and 100 μL Ellman's solution were added and read at 412 nm after standing for 5 min. The activity (a) was calculated from a standard containing 15.34 g GSH/100 ml. GSH levels were presented as μmol/ g protein.

Determination of Glutathione Peroxidase (GPx) Activity:

GSH-Px activity was measured according to the method of Paglia and Valentine (21). In the presence of hydrogen peroxide, GSH-Px catalyse oxidation of glutathione. Oxidized glutathione is converted into the reduced form in the presence of glutathione reductase and NADPH, while NADPH is oxidized to NADP. Reduction in the absorbance change per minute and by using the molar extinction coefficient of NADPH, the GSH-Px activities of bladder tissues were calculated. GPx activity was expressed as substrates (nmol NADPH) transformed min−1 mg protein−1.

Superoxide dismutase activity was measured by the

inhibition of pyrogallol autoxidation at 420 nm for 3 min according to the method of Marklund & Marklund (22). The activity was expressed as units per gram of tissues.

Determination of CAT Activity

CAT-like activity was measured by the decrease in the concentration of hydrogen peroxide after incubation with various volumes of the homogenates using the assay and methods described by Zini et al (23). The presence of hydrogen peroxide was assessed using horseradish-peroxidase-dependent oxidation of phenol red to a blue derivative. After incubation at the room temperature, horseradish peroxidase and phenol red were added to react with the remaining hydrogen peroxide. The absorbance was read at 630 nm.

Active caspase-3 determination

Active caspase-3 was measured using a quantitative immunoassay kit (R & D Systems). A biotinilated inhibitor is bound to the large subunit of the active caspase-3. The inhibitor does not bind to the inactive caspase-3. The addition of streptoavidine during the ELISA allows the quantification of active caspase-3. The bladder caspase-3 activity was determined by a colourimetric method (24).

Cell death detection ELISA

Cell death detection ELISA was performed according to the manufacturer's instructions. Optical density was read using an ELISA reader at 405 nm (25).

Statistical Analysis

Data were expressed as (mean ± SE) for all parameters. The data were analyzed using GraphPad Prism data analysis program (GraphPad Software, Inc., San Diego, CA, USA). For comparison of statistical significance between different groups Student Newman-Keuls t-test for paired data were used. For multiple comparisons, one-way analysis of variance (ONE- WAY-ANOVA) test followed by the east Significant Difference (LST). Correlations were assessed using Spearman's non-parametric correlation coefficient □ as described by Knapp and Miller (26). A value of $P \leq 0.05$ was considered statistically significant.

Results

A summary of some of the characteristics of the rats used in this study is shown in Table 1. The serum glucose levels were significantly higher in the diabetic group than in the A, C, and D groups ($p<0.001$). The serum glucose levels of group D were significantly higher than those of group A and C ($p< 0.05$).

Table (1) showed that all control, C and D groups rats showed an increase in body weight, as the initial body weight of each of these groups had significantly increased than their final body weights ($p<0.001$).Whereas the initial body weight of the diabetic rats was significantly different than the final body weights ($p<0.05$). The D group had increase in body weights significantly than B groups ($p<0.001$) but still significantly lowered than A and C ($p<0.05$).

Bladder weights at the end of the experiment were increased significantly in the diabetic rats by approximately 2 times than A, C and, D groups ($p<0.001$). After vitamin E treatment, bladder weights of group D were not significantly different than those of A and C groups.

Contractile response to carbachol: Carbachol, which is a cholinergic receptor agonist, caused concentration-dependent contractions on all bladder strips tested when added to the isolated organ baths cumulatively (10^{-9} to 10^{-3} mol/L). The contractile responses in tissues from diabetic animals were significantly greater than in the age matched control tissues, C and D groups ($P< 0.001$). Treatment of group D with vitamin E improved the contractile responses of the bladder strip of D groups but still significantly higher than A and C groups ($p< 0.05$) (Fig. 1). There were no significant differences between the contractile responses in bladder strips from control and C groups.

Effects on tissue levels of nitrite and nitrate

Diabetic bladder strips had increased levels of nitrite and nitrate significantly higher than those of the other studied groups ($p<0.001$). Levels of nitrite and nitrate of D group after treatment with vitamin E were not significantly elevated different from those of A and C groups.(Fig 2)

Relation to MDA, GSH and antioxidants (SOD, CAT and GPx)

In the diabetic group (figure 3A), MDA levels of bladder tissues were found to be significantly higher than those of the other A ,C and, D groups ($p < 0.001$). Treatment of group D with vit E significantly reversed the elevations in MDA levels of the tissues, but still significantly higher compared with A and C group ($p < 0.01$).

Diabetes caused significant reduction in GSH levels of urinary bladder tissues when compared to the control and C groups ($p< 0.001$). GSH levels have been increased significantly after vit. E treatment compared to the diabetic group ($p<0.001$), but still significantly lower than those of A and C groups Fig 3B with $P<0.01$.

Table 1. Plasma glucose levels, body weights and bladder weights of all groups

	A	B	C	D
Plasma glucose (mg/dl)	93.3±5.5	248.6±20.7[a,b,c]	91.8±6[NS]	107±17.35[d'e]
Initial body weight (gm)	225.4±16.38[f]	230.3±13.16[g]	227.3±13.96[h]	229.7±12.69[i]
Final body weight (gm)	293.6± 18.58	256.1±17.57[a,b,c]	293±18.62[NS]	277±14.9[d'e]
Baldder weight (mg)	121.4± 4.81	244 ± 9.17[a,b,c]	122.3± 5.9[NS]	129.7±12.1[d'e]

Data are expressed as mean ± SD.
a: $p < 0.001$ compared to A group b: $p < 0.001$ compared to C group c: $p < 0.001$ compared to D group
d: $p < 0.05$ compared to A group e: $p < 0.05$ compared to C group NS: non significant compared to A group
f: $p < 0.001$ compared to final body weight of group A.
g: $p < 0.05$ compared to final body weight of group B.
h: $p < 0.001$ compared to final body weight of group C.
i: $p < 0.001$ compared to final body weight of group D.

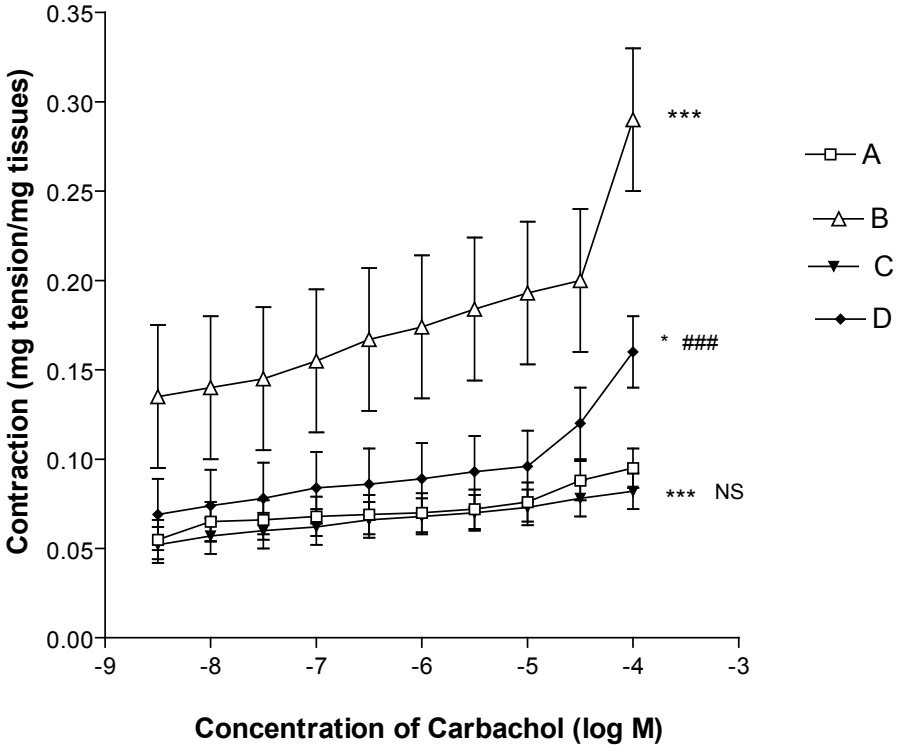


Figure 1. Effect of carbachol (10^{-8} to 10^{-4} M) on the contractile reponses of bladder strips of rats from control ,diabetic ,C and treated group with vit.E. Each group consists of 10 rats. ***$p < 0.001$,**$p<0.01$ compared with A group. ### $p < 0.001$, ## $p<0.01$ as compared with B group. ++ $p<0.01$ as compared to C group.
(*) Significance versus control group.(#) Significance of values versus group B, ($^{+}$) Significance of values versus group B ***/ (###) significant difference at P<0.001. NS :non significant as compared to group A. Data is presented as mean±SD

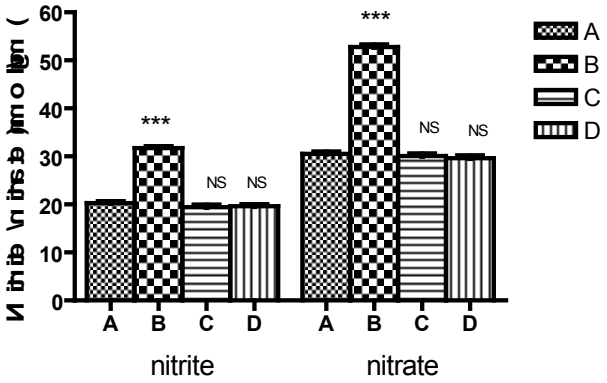


Figure 2. Tissue levels of nitrite (NO_2^-) and nitrate (NO_3^-) of all studied groups . Each column represents the mean ± S.D. Each group consists of 10 rats. (*) Significance versus control group .p<0.001 as compared to A group. NS : non significant as compared to group A.

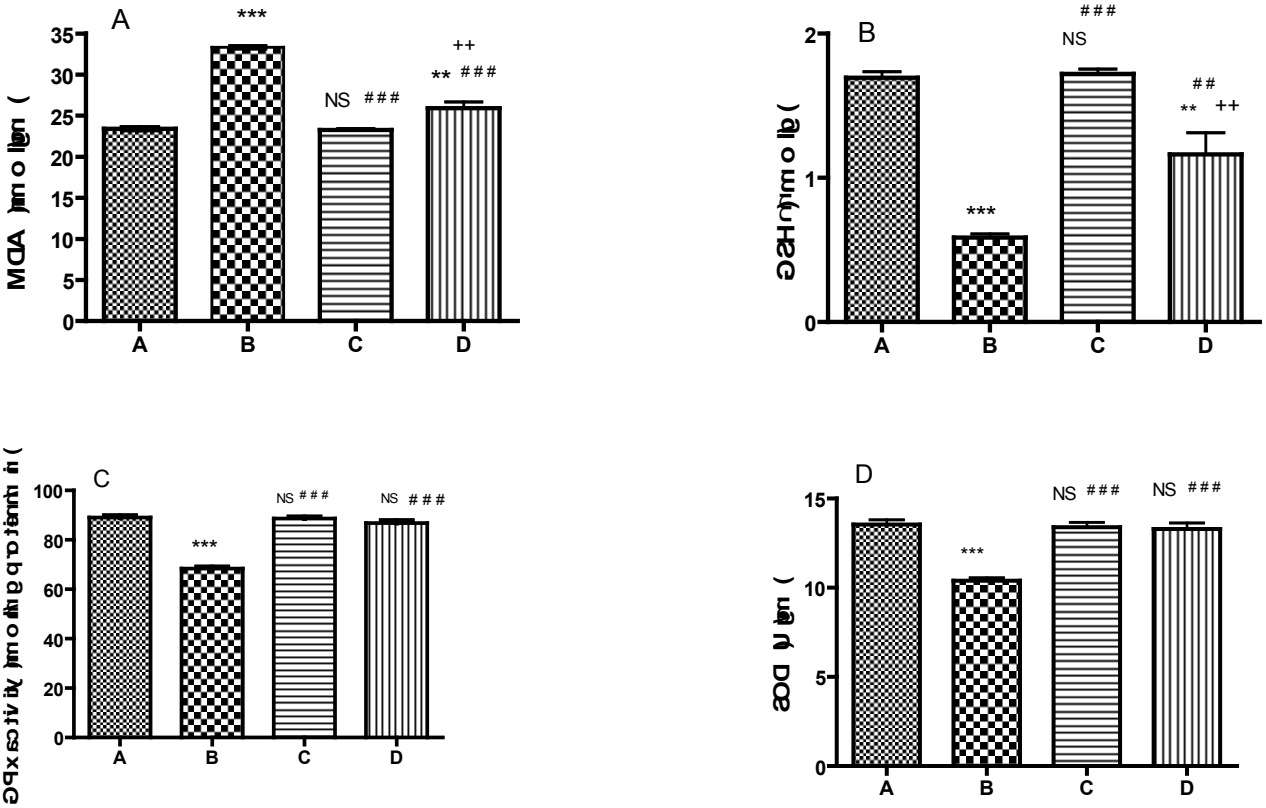


Figure 3. Tissue levels of MDA, GSH and antioxidant enzymes activities in all studied groups. Each group consists of 10 rats. ***p < 0.001,**p<0.01 compared with A group. ### p < 0.001, ## p<0.01 as compared with B group. ++ p<0.01 as compared to C group. (*) Significance versus control group.(#) Significance of values versus group B, ***/ (###) significant difference at P<0.001.NS :non significant as compared to group A.

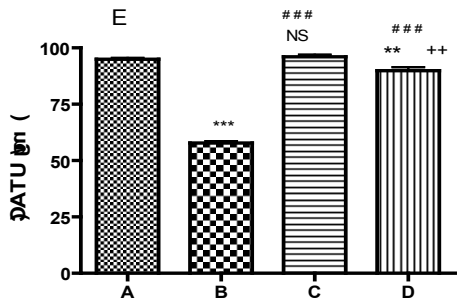


Figure 3E

Fig 3 C showed that significant reduction in GPx activities of diabetic bladder tissues compared to the control, C and D groups was detected (p<0.001). Vit E treatment of the diabetic rats reversed the changes in GPx activities back towards the normal levels and there were no significant differences between the activity of GPx in A, C and D groups.

The tissue levels of SOD of diabetic group were significantly lower than those of A,C and D groups (p < 0.001). Treatment of diabetic rats with vitamin E increased the SOD levels to the control levels as there was no significant difference between levels of D groups and those of A and C groups (Fig 3D).

The tissue levels of CAT of B groups were significantly lower than those A, C and D groups (p < 0.001).The tissue levels of CAT of D group were increased but still significantly lower than those of A and C groups with p < 0.01 (Fig 3E).

Effect on caspase-3 activation

There were more than 2 fold increases of caspase-3 activities in diabetic group compared to those of A and C groups (p < 0.001).Treatment with vitamin E suppressed activities significantly less than those of B group but still significantly higher than those of A and C groups with p< 0.01 (Fig. 4A).

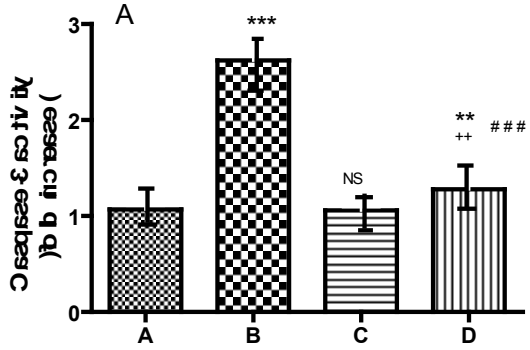
Effect on cell death

Cell death detection by ELISA was further employed to characterize apoptosis. The data (Fig. 4B) showed that diabetic rats had increase in DNA fragments in the cytoplasmic fraction of cells significantly higher than those of A and C groups (p<0.001). These levels had been decreased significantly in group D in comparison to those of group B (p < 0.001). The levels of group D were still significantly lower than those of group A and C (p< 0.001).

Discussion

Several animal models have been employed to study the pathophysiology of type I, and type II diabetes mellitus. The most well established model is the streptozotocin (STZ)-induced diabetic rat model as this demonstrates many of the complications known to occur in human diabetes (27).

In this study the blood glucose levels of group D were significantly decreased than those of group Bafter vit E treatment. This finding is supported by Roldi et al (28), who reported that diabetic animals, that received vitamin E supplementation, exhibited a significant reduction



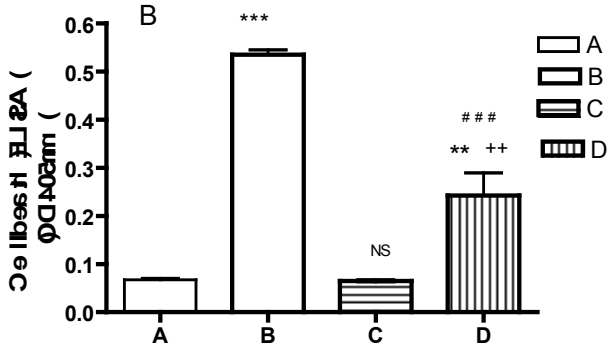


Figure. 4A: Caspase-3 activities in bladder tissues as a fold increase in the different groups. 4B: Cell death determined by ELISA. The ELISA detection was processed. The histone-associated DNA fragments were presented as the optical density at 405 nm. Each group consists of 10 rats. ***p < 0.001, **p<0.01 compared with A group.
p < 0.001, ## p<0.01 as compared with B group. NS: non significant as compared to group A.++ p<0.01 as compared to C group

in glycemia compared to controls indicating a positive effect of this antioxidant on blood glycemia. Shirpoor et al. (29) reported that diabetic rats that received vitamin E have a significant reduction in blood glucose and glycated hemoglobin compared with untreated diabetic animals.

In this study there was a significant increase of bladder weights in the diabetic rats than the other studied groups. These findings are in agreement with the conclusions of Nsabimana and Ojewole (30), and Atalik et al. (31). Another report by Itoh et al. (32) concluded that bladder hypertrophy may be due to physiological adaptation to the increased urine volume, and that bladder function is impaired by neurogenic factors rather than by myogenic factors. The increased bladder capacity may be due to an adaptation to polyuria and/or the diabetes itself. These changes have been attributed to increased smooth muscle cells and urothelium, which plays an important role in the filling and distension mechanisms of the bladder, with a reduction of the collagen content as an early event, 3 days after STZ injection (33) and these results are supported by Beshay et al. (34) and Pitre et al. (33) who reported the development of bladder dysfunction in this model of rats, and was manifested by increased bladder capacity and mass as a compensatory mechanism to increased urine output.The bladder smooth muscle cell is the contractile unit responsible for normal bladder function. The parasympathetic nervous system plays an important role in the functional regulation of bladder smooth muscle. Histochemical and functional studies have shown that there is a diabetes-induced alteration in the cholinergic innervation and/or changes in the response of bladder smooth muscle to cholinergic agonist in experimental animals (35). Changes in the contractile responses after 8 weeks of diabetes in this study were observed which exhibited increase of the contractile response to muscarinic agonist carbachol. The tissues from the diabetic animals were also supersensitive to carbachol. Latifpour et al. (36) noted that the densities of muscarinic receptors were significantly increased in bladder smooth muscle 8 weeks after the induction of diabetes.

In this study, diabetic rat bladder is heavier in weight and has altered contractile function compared with the normal bladder. The changes of diabetic bladder were observed by Tong, Chin & Cheng (37) who thought that these changes may be due to both excessive diuresis and the metabolic effects of diabetes on both nerve and muscle tissue. In support to these results Liu and Daneshgari (38) found in their study that diabetes caused a significant reduction of body weight compared with controls, although the bladders of diabetics weighed more than the controls. They reported that hypertrophy in diabetes is likely to be induced by diuretic effects of the disease. They concluded that such bladder remodeling could inevitably lead to distortion of the normal pattern of innervation, and other local neurogenic and myogenic changes, which may be the cause of diabetic bladder dysfunction. In support of these results, it was reported that carbachol-induced phasic activity was increased in diabetic rat bladder strips (39).

In another study, Vahabi et al. (14) reported that the pathophysiology of detrusor overactivity remains incomplete. Although it is still unclear whether the uninhibited spontaneous contractions that are observed urodynamically in patients with detrusor overactivity are associated with the phasic activity seen in vitro, it is clear that in animal models and patients with detrusor overactivity this phasic activity is increased, and the mechanisms underlying this are not yet understood.

As NO is rapidly degraded within 5–6 sec, its stable end products -nitrite and nitrate- have been used to evaluate NO levels in tissues (40). This study, found that bladder tissue nitrite and nitrate levels were significantly higher in the diabetic than in the other studied groups. The increased levels of NO had been explained by Hunt et al. (41) as they found that hyperglycemia has been shown to increase free radical production. However, Wong et al (42) reported that advanced glycation end products (AGEs), protein-bound oxidation products of sugars, can also produce oxygenated free radicals. As AGEs have been reported to induce iNOS expression in some cell cultures, it is possible that AGEs induce iNOS in diabetic bladder smooth muscle cells in vivo by similar mechanisms.

In the present study, the levels of MDA, an end product of lipid peroxidation, are significantly increased in bladder tissues. This observation is in agreement with a previous study by Şener et al (43), in which elevated levels of lipid products were increased from 40 to 80 % above basal values as a result of oxidative stress.

The present results demonstrate that vitamin E treatment decreased the levels of MDA, nitrite, nitrate, and increased levels of GSH and the antioxidants activities of D group. These data possibly provide evidence that increased NO production mediates smooth muscle cell oxidative damage and the defective antioxidant defense system may contribute to the development of diabetic bladder dysfunction and thus is involved in the pathogenesis of bladder dysfunction in diabetic rats. Reduction in the enzyme activity observed in this study may reflect a reduction in the cellular defense to oxidants and an increased susceptibility of the bladder tissue to oxidative damage.

Treatment with vitamin E improved the phasic activity in D group which possibly indicates that elimination of lipid and protein oxidation -as two important sources of free radical generation by vitamin E- may result in the recovery of the cell membrane to its normal physiologic state, and the insulin binds to the cell readily. In support to this finding Saito et al. (44) reported that although the precise mechanism of impaired contractile function has not been clarified, it is known that the oxidants alter contractile responses of tissues to various agents. For example, oxidants are known to alter vascular reactivity of coronary endothelial cells through the impairment of signal transduction system (45). In addition Cai & Kang (46) found that in both clinical and experimental models of diabetes, ROS -induced oxidative stress is considered to be a key participant in causing tissue injury. In another study, Beshay and Carrier (47) reported that in diabetics, hyperglycemia might impair the enzyme activity through different possible mechanisms such as protein glycation, oxidation, and glycoxidation.

Our results demonstrated that in group D vitamin E treatment decreased MDA, nitrite, nitrate elevations significantly and reversed them back toward the control levels, and decreased the levels of GSH, SOD, CAT, and glutathione-Px activities of diabetic bladder tissues. Thus vitamin E could be protective against organ damage by preserving the cellular integrity.

Caspases that participate in the apoptosis cascade have been divided into initiators and executioners. The exact order of the executioners and the position of specific caspases in the apoptotic pathway are still under investigation. Caspases-3, -6, and -7 are generally accepted to be executioners. Caspase-3 is considered a key effector caspase in many cells and mediates the cleavage of itself, other downstream caspases and other caspases (48).

Increased activities of caspase -3 and cell death in diabetic bladder were decreased by vitamin E treatment back toward the normal levels. Taken together, these criteria to characterize apoptosis suggest that cell death caused by diabetes occurs by apoptosis. In support to our study, Beshay and Carrier (47) found a significant increase in the number of apoptotic cells of smooth muscle cell layers of diabetic bladder compared with controls. Moreover, Loeken (49) and Shirpoor et al. (50) reported that it was determined in some studies -examining different tissues- that vitamin E decreased apoptosis in DM.

Sylvester (51) reported that antiapoptotic effects of vitamin E are primarily associated with its antioxidant activity. However, vitamin E has also been shown to induce protective effects and prevent apoptosis in some experimental model systems. Mehmet et al. (13) reported that vitamin E, known as an antioxidant, decreased apoptosis in urothelial cells of the urinary bladder in STZ-induced diabetic rats.

Conclusions

In summary our study revealed that vitamin E (known as an antioxidant), decreases apoptosis in tissues of the urinary bladder and has protective effects on detrusor contractility in STZ-induced diabetic rats. These data suggest that vitamin E treatment may be beneficial in delaying the progression of diabetic bladder dysfunction in this experimental animal model. Thus, vitamin E treatment in diabetic rats may be useful as a therapy to prevent oxidative damage. Oxidative stress plays a role in the pathogenesis of diabetes-induced bladder dysfunction in a rat model

References

1. Ueda T, Yoshimura N, Yoshida O. Diabetic cystopathy: relationship to autonomic neuropathy detected by sympathetic response. J Urol.1997;157:580-4.
2. Abrams, P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U, Van Kerrebroek P, Victor A, Wein A. The standardization of terminology of lower urinary tract function: report from the standardization sub-committee of the International Continence Society. Neurourol. Urodyn. 2002;21:167–8.
3. Stevens L, Chapple CR and Chess-Williams R. A comparison of muscarinic receptor-mediated function in the normal and neurogenic overactive bladder. J. Urol. 2004a;171(Suppl.):143(P535).
4. Stevens L, Chapple CR. And Chess-Williams R. Muscarinic receptor function in the idiopathic overactive ladder. J. Urol. 2004b;171(Suppl.):140 (P527).
5. Yenilmez A, Ozcifci M, Aydin Y, Turgut M, Uzuner K, Erkul A. Protective effect of high dose thiamine (B1) on rat detrusor contractility in streptozotocin induced diabetes mellitus. Acta Diabetol. 2006;43:103-8.
6. Khan MA, Thompson CS, Calvert RC, et al. Decreased urinary bladder apoptosis in a rabbit model of diabetes mellitus. Urol Res.2002;30:79-83.
7. Kubota Y, Nakhara T, Mitani A, Maruko T, Sakamoto k, Ishii k. Augmentation of rat urinary bladder relaxation mediated by beta 1-adrenoceptors in experimental diabetes. Eur. J. Pharmacol. 2003;467:191-5.

8. Srinivasan S, Stevens M, and Wiley JW. Diabetic peripheral neuropathy: Evidence for apoptosis and associated mitochondrial dysfunction. Diabetes. 2000;49:1932-8.
9. Bonnefont-Rousselot D. Glucose and reactive oxygen species. Current Opinion in Clinical Nutrition" Metabolic Care. 2002;5:561-8.
10. Babaei-Jadidi R, Karachalias N, Ahmed N, Battah S, Thornalley PJ. Prevention of incipient diabetic nephropathy by high-dose thiamine and benfotiamine. Diabetes 2003;52:2110-20.
11. Hammes HP, Du X, Edelstein D, Taguchi T, Matsumura T, Ju Q, Lin J, Bierhaus A, Nawroth P, Hannak D, Neumaier M,Bergfeld R, Giardino I, Brownlee M. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. Nat Med. 2003;9:294-9.
12. Bermudez Y, Ahmadi S, Lowell NE, et al. Vitamin E suppresses telomerase activity in ovarian cancer cells. Cancer Detect Prev. 2007;31:119-28.
13. Ustuner MC, Kabay S, Ozden H, Guven G, Yucel M, Olgun EG, et al. The Protective Effects of Vitamin E on Urinary Bladder Apoptosis and Oxidative Stress in Streptozotocin-induced Diabetic Rat. Urology. 2010;7(4):902-6.
14. Vahabi B, Lawson K, McKay NG, Sellers DJ. Phasic activity of urinary bladder smooth muscle in the stozotocin-induced diabetic rat: Effect of potassium channel modulators. European Journal of Pharmacology. 2011;660:431-7.
15. Ng YK, de Groat WC and Wu HY. Muscarinic modulation of neonatal rat bladder spontaneous contractions. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2006;291:1049-59.
16. Tuncayengin A, Biri H, Onaran M, Şen İ, Tuncayengin O¨, Polat F, Erbas D and Bozkirli İ. Cavernosal tissue nitrite, nitrate, malondialdehyde and glutathione levels in diabetic and non-diabetic erectile dysfunction.International journal of Andrology. 2003;26:250-4.
17. Beuge JA and Aust SD. Microsomal lipid peroxidation. Methods in Enzymology. 1978;53:302 (abstract).
18. Casini, AF, Ferrali M, Pompella A, Maellaro E and Comporti M. Lipid peroxidation and cellular damage in extrahepatic tissues of bromobenze-intoxicated mice. American Journal of Pathology.1986;123,520-31.
19. Elman, GL. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics.1959; 82(1):70-7.
20. Burtis CA, Ashwood ER. Determination of Total Protein (biüret metod) in Tietz Textbook of Clinical Chemistry. 2nd ed. WB Saunders Company; 1994. p. 297-696.
21. Paglia DE and Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med.1967;70:158-69.
22. Marklund S and Marklund G. Involvement of the superoxide anion radical in autoxidation of pyrogallol as a convenient assay for superoxide dismutase. European Journal of Biochemistry. 1974 ;47:469-74.
23. Zini A, de Lamirande E, and Gagnon C: Reactive oxygen species in semen of infertile patients: level of superoxide dismutase- and catalase-like activities in seminal plasma and spermatozoa. Int J Androl.1993;16**:**183-8.
24. Kwon SH, Ahn SH, Kim YK, Bae GU, Yoon JW, Hong S, Lee HY, Lee, YW, Lee, HW and Han JW. Apicidin, a histone deacetylase inhibitor, induces apoptosis and Fas/Fas ligand expression in human acute promyelocytic leukemia cells. J. Biol. Chem. 2002;277:2073-80.
25. Li P, Dietz R and von Harsdorf R. Reactive oxygen species induce apoptosis of vascular smooth muscle cell. FEBS.1997;404:249-52.
26. Knapp GR and Miller MC.:Tests of statistical significance: Regression and Correlation. In: Clinical Epidemiology and Biostatistics 1st Edition. Williams and Wilkins, Baltimoe,Maryland; 1992. p. 255-74.
27. Stevens MJ, Dananberg J, Feldman EL, Lattimer SA, Kamijo M, Thomas TP, et al. The linked roles of nitric oxide, aldose reductase, and (Na+, K+)- ATPase in slowing of nerve conduction in the streptozotocin diabetic rat. J Clin Invest. 1994;94:853-9.
28. Roldi PL, Pereira VR, Eleandro A Tronchini EA, Rizo GV, Scoaris CR, Zanoni JN and Natali MR. Vitamin E (α-tocopherol) supplementation in diabetic rats: effects on the proximal colon**.** BMC Gastroenterology. 2009;9(88):1-8.
29. Shirpoor A, Ansari MHK, Salami S, Pakdel FG and Rasmi Y: Effect of vitamin E on oxidative stress status in small intestine of diabetic rat. World J. Gastroenterol. 2007;13:4340-4.

30. Nsabimana AM and Ojewole AO. Diabetes mellitus and responses of the urinary bladder to acetylcholine: an in vitro study. J. Smooth Muscle Res. 2010;44:133-50.
31. Atalik KE, Okudan N, Gokbel H, Kalkan S and Gokhan C.Diabetes and cooling-induced bladder contraction: an in vitro study.J Smooth Muscle Res. 2010;46(4):175-83.
32. Itoh H, Morikawa A and Makino I. Urinary bladder dysfunction in spontaneously diabetic Chinese hamsters.Diabet Res Clin Pract. 1994;22:163-70.
33. Pitre DA, Ma T, Wallace LJ, Bauer JA. Time-dependent urinary bladder remodeling in the streptozotocin-induced diabetic rat model. Acta Diabetol. 2002;39:23-7.
34. Beshay E, Rehman K, and Carrier S. Diabetes induced bladder dysfunction in a rat model. BJU.2002; Int 90 (suppl 2):286-92.
35. Ichiyanagi N, Tsuji T, Masuda H, Kihara K, Goto M, Azuma H. Changed responsiveness of the detrusor in rabbits with alloxan induced hyperglycemia: Possible role of 5- hydroxytryptamine for diabetic bladder dysfunction. J Urol. 2002;168:303–7.
36. Latifpour J, Gousse A, Kondo S, Morita T, Weiss RM. Effects of experimental diabetes on biochemical and functional characteristics of bladder muscarinic receptors. J Pharmacol Exp Ther.1989;248:81-8.
37. Tong YC, Hung YC, Lin SN, Cheng JT. Alternation in urinary bladder synaptosomal neurotransmitter concentrations in two-week streptozotocin- induced diabetic rats. Neurosci Lett. 1996;206:165-8.
38. Liu G and Daneshgari F. Alterations in neurogenically mediated contractile responses of urinary bladder in rats with diabetes. Am J Physiol Renal Physiol. 2005;288:F1220-6.
39. Vahabi B, McKay NG, Lawson K, Sellers DJ. The role of c-kit positive interstitial cells in mediating phasic contractions of bladder strips from streptozotocin(STZ)-induced diabetic rats. BJU Int. 2011 May;107(9):1480-7. doi: 10.1111/j.1464-410X.2010.09507.x.
40. Stevens LA, Sellers DJ, McKay NG, Chapple CR and Chess-Williams R. Muscarinic receptor function, density and G-protein coupling in the overactive diabetic rat bladder Autonomic & Autacoid. Pharmacology. 2006;26:303-9.
41. Hunt JV, Smith CCT, and Wolff SP: Autoxidative glycation and possible involvement of peroxide and free radicals in LDL modification by glucose. Diabetes. 1990;39:1420-4.
42. Wong A, Dukic-Stefanovic S, Gasic-Milenkovic J, Schinzel R, Wiesinger H, Riederer P, Münch G. Anti-inflammatory antioxidants attenuate the expression of inducible nitric oxide synthase mediated by advanced glycation endproducts in murine microglia". Eur J Neurosci. 2001;14:1961-7.
43. Şener G, Şehirli O¨, Paskaloğlu K, Ayanoglu-Dülger G, Alican I. Melatonin treatment protects against ischemia/reperfusion-induced functional and biochemical changes in rat urinary bladder. Journal of Pineal Research. 2003;34,226-30.
44. Saito M,Wada K, Kamisaki Y,Miyagawa I. Effect of ischemia-reperfusion on contractile function of rat urinary bladder: possible role of nitric oxide. Life Sciences. 1998;62:149-56.
45. Masuda H, Tsujii T, Okuno T, Kihara K, Goto M, Azuma H. Involvement of accumulated endogenous NOS inhibitors and decreased NOS activity in the impaired neurogenic relaxation of the rabbit proximal urethra with ischemia. British Journal of Urology. 2001;133:97-106.
46. Cai L and Kang Y J. Cell death and diabetic cardiomyopathy. Cardiovascular Toxicology. 2003;3:219-28.
47. Beshay E and Carrier S. Oxidative stress plays a role in diabetes-induced bladder dysfunction in a rat model. Urology. 2004;64:1062-7.
48. Kwon SH, Ahn SH, Kim YK, Bae, GU, Yoon JW, Hong S, Lee HY, Lee YW, Lee HW and Han JW. Apicidin, a histone deacetylase inhibitor, induces apoptosis and Fas/Fas ligand expression in human acute promyelocytic leukemia cells. J. Biol. Chem. 2002;277:2073–80.
49. Loeken MR. Advances in understanding the molecular causes of diabetes-induced birth defects. J Soc Gynecol Invest. 2006;13:2-10.
50. Shirpoor A, Salami S, Khadem-Ansari MH, Ilkhanizadeh B, Pakdel FG, Khademvatani K. Cardioprotective effect of vitamin E: rescues of diabetes-induced cardiac malfunction, oxidative stress, and apoptosis in rat. J Diabetes Complications. 2009;23:310-6.
51. Sylvester PW. Vitamin E and apoptosis. Vitam Horm. 2007;76:329-56.