Vol.55, n. 4: pp. 527-536, July-August 2012
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

# Evaluation of Lipid Profile and Oxidative Stress in STZ-Induced Rats Treated with Antioxidant Vitamin

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## ABSTRACT

*The present study investigated the effect of supplementation of vitamin E on streptozotocin (STZ)-induced diabetic rats by measuring blood glucose, changes in body weight, food and water intake, lipid profile, serum urea and creatinine level, and antioxidant enzyme activity. Male Wistar rats were divided into four groups: control rats (GI); rats receiving vitamin E (GII); STZ-induced diabetic rats (GIII) and STZ-induced diabetic rats treated with vitamin E (GIV). Vitamin E reduced ($p<0.05$) blood glucose and urea, improved the lipid profile (decreased the serum levels of total cholesterol, LDL cholesterol, VLDL cholesterol and triacylglycerols, and increased HDL cholesterol) and increased total protein in STZ-induced diabetic rats (GIV). Vitamin prevented changes in the activity of SOD and GSH-Px and in the concentration of lipid hydroperoxide. These results suggested that vitamin E improved hyperglycaemia and dyslipidaemia while inhibiting the progression of oxidative stress in STZ-induced diabetic rats.*

**Key words:** vitamin E, lipid profile, oxidative stress, STZ.

The World Health Organization (WHO) has predicted that the number of patients with diabetes worldwide will double by the year 2025, from the current number of approximately 150 million to 300 million (Coskun et al. 2005). Diabetes mellitus (DM) is associated with the production of reactive oxygen species (ROS) and consequently oxidative stress, which promotes not only an alteration in the cellular redox state (Coskun et al. 2005) in the presence of chronic hyperglycaemia, but also reduces the ability of tissues to utilize carbohydrates, leading to disturbances in the metabolism of fat and protein (Je et al. 2001).

Moreover, this aetiology is accompanied by an imbalance between the oxidant and antioxidant status, i.e., increased production of ROS and/or decline in antioxidant defense systems (Baydas et al. 2002; Young et al. 1995; Fakher et al. 2007).

In this context, experimental data have suggested that chronic high blood glucose levels contributed to the formation of ROS, through several mechanisms such as glucose autoxidation, the oxidation of protein (Bonnefont-Rousselot et al. 2000; Maritim et al. 2002) and non-enzymatic glycation of protein (Szaleczky et al. 1998), thus exacerbating oxidative stress. Streptozotocin induces experimental insulin-dependent diabetes mellitus (type 1) in animals through its cytotoxic effects on beta-cells of the pancreas, *via* a mechanism associated with the generation of ROS (Punitha et al. 2005; Evelson et al. 2005). It leads

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to a deficiency of insulin, which acts as a diabetogenic agent (Szkudeslski 2001).

Diabetes mellitus has been associated with an increased risk of mortality and prevalence of cardiovascular disease. Atherosclerotic cardiovascular disease is the main source of morbidity and mortality in patients with diabetes (Bray 2000). In addition, oxidative stress may occur as a consequence of abnormalities in glucose and lipid metabolism, which favour hyperglycaemia and dyslipidaemia. These phenomena are associated with the development of atherosclerosis and cardiovascular complications in diabetic patients (Bray 2000; Chertow and Edwards 2004).

Since numerous studies have indicated that hyperglycaemia in diabetes contributes to oxidative stress, it has been suggested that the nutritional supplementation of antioxidants might reduce the oxidative stress, and hence protect tissues from ROS damage (Coskun et al. 2005; Ramkumar et al. 2008; Sharma et al. 2000). Such supplementation may have a protective role and has been correlated with a decrease in the incidence of various degenerative diseases, such as diabetes and its complications (Ramkumar et al. 2008; Sharma et al. 2000). In this respect, treatment with antioxidant vitamins, especially vitamin E, is of special interest, and given the potential protective activity of antioxidants, vitamin E consumption may prevent the development of diabetes mellitus - type 1 (Bonnefont-Rousselot et al. 2000; Knekt et al. 1999; Matheus et al. 2008).

The aim of the present study was to evaluate the effects of vitamin E on oxidative stress, lipid profile, as well as the renal dysfunction by measuring urea and creatine level, in normal and streptozotocin-induced diabetic rats.

## MATERIAL AND METHODS

### Experimental animals

Adult male Wistar rats (200±250 g) were housed at 25±3 $^{o}$C and humidity of 55±2%, under a constant 12 h light and dark cycle. Pellet food (Purina Labina, Campinas – SP, Brazil) and water were available *ad libitum.*The rats were divided randomly between four experimental groups (*n*=8) as follows: group I (GI): control rats; group II (GII): vitamin E; group III (GIII): STZ-induced diabetic rats and group IV (GIV): STZ-induced diabetic rats treated with vitamin E.

Streptozotocin (STZ – Sigma, St Louis MO, USA) dissolved in citrate - phosphate buffer at pH 4.5, was administered in a single dose of 60 mg,kg $^{-1}$ body weight; i.p. Hyperglycaemia was measured using a blood glucose (BG) monitor (Behringer Mannheim, Eli Lilly Ltd., São Paulo, Brazil), 48 h after STZ injection. STZ-treated rats with fasting blood glucose > 250 mg.dL$^{-1}$ were regarded as diabetic and selected for this experiment.

Three days after STZ administration, vitamin E (α-tocopherol acetate – Sigma T-1157) was administered intragastrically (gavage) once a week, at the dose of 440 IU.kg$^{-1}$ body weight (equivalent to 440 mg.kg$^{-1}$ body weight) for 30 days (Konen et al. 2000). Food (g) and water (mL) intake were measured daily. Body weight (g) was recorded weekly. After the experimental period (30 days), rats were deprived of food for 12 h, anaesthetized with 0.1 mL.100 g$^{-1}$ body weight of 10% cetamin chloridrate and killed by decapitation and blood was collected and centrifuged at 6000 rpm for 15 min to separate the serum.

### Measurement of glycaemia and dyslipidaemia

The biochemical parameters were measured using spectrophotometric methods with commercial enzymatic kits (CELM – Modern Laboratory Equipment Company, São Paulo, Brazil). Serum glucose was quantified enzymatically using the glucose oxidase and peroxidase method (Moura 1982)

The serum concentration of total cholesterol was determined by enzymatic methods using cholesterol ester/oxidase. The serum high-density lipoprotein (HDL) content was determined in the supernatant fraction after precipitation of the very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) with phosphotungstic acid and $MgCl_2$. LDL-cholesterol and VLDL-cholesterol values were calculated according to Friedewald's formula (Friedewald et al. 1972). The levels of triacylglycerol in the serum were estimated enzymatically after hydrolysis by lipoprotein lipase in glycerol, then to glycerol phosphate and then to dihydroxyacetone phosphate and $H_2O_2$, which in the presence of peroxidase was converted to aminophenazone.

The serum urea level was determined in the presence of urease, resulting in $CO_2$ and ammonia. The addition of phenol-hypochloride leads to an indophenol-blue complex with absorbance at 600

nm. Creatinine was assayed by reaction with picric acid in alkaline buffer to form a yellow-orange complex. The colour intensity, determined at 500 nm, was proportional to the creatinine concentration in the sample. Total protein was determined in the presence of biuret reagent.

**Estimation of oxidative stress markers**

Lipid hydroperoxide (HP) was estimated through the oxidation of ferrous ion, which in the presence of xylenol orange led to the formation of an $Fe^{-3}$ – xylenol orange complex, which was measured at 560 nm (Jiang et al. 1991). Superoxide dismutase (SOD, E.C.1.15,1.1) activity was assayed as described elsewhere (Crouch et al. 1981), through the inhibition of the reduction of nitro blue tetrazolium (NBT) in the presence of reduced nicotinamide adenine dinucleotide (NADH) and phenazine. The amount of enzyme that gave 50% inhibition of NBT reduction/mg protein was taken as one unit of enzyme activity.

Glutathione peroxidase (GSH-Px, E.C. 1.11,1.9) activity was determined indirectly by measuring the consumption of NADPH during the reduction of oxidized glutathione (GSSG) in a reaction catalyzed by glutathione reductase, One unit of enzyme was defined as the amount required to oxidize 1 μmole GSH/min, which corresponded to 0.5 μmole NADPH oxidized/min (Nakamura et al. 1974),

**Statistical analysis**

Data were expressed as means ± standard deviation (S.D.). Tukey' analysis was used to test for differences among the means when analysis of variance (ANOVA) indicated a significant *P* value<0,05 (Zar 1996).

**Ethics committee**

All the experimental animals were treated according to a protocol approved by the Ethics Committee for Conduct of Animal Studies at the Institute of Biological Sciences, University of São Paulo State (UNESP), and conforming to the principles and guidelines of the Canadian Council on Animal Care as outlined in the "*Guide to the Care and Use of Experimental Animals"*.

## RESULTS

Diabetic animals exhibited a significant ($p<0.05$) decrease in body weight (GIII) when compared with normal rats (GI). Dietary vitamin E supplementation (GIV) led to a significant ($p<0.05$) increase in body weight. However, vitamin E did not influence the body weight in normal rats (GII) when compared to the control rats. Diabetic rats showed significantly ($p<0.05$) higher intake of food and water when compared with the control group. Food intake was significantly decreased in group IV, along with water intake, when compared with group III. Serum glucose was significantly elevated ($p<0.05$) in diabetic rats compared with the normal control rats. Vitamin E, orally administered, also led to a marked decrease ($p<0.05$) in the serum glucose of the diabetic rats (GIV) at the end of the study. However, supplementation with vitamin E in the normal rats (GII) did not have any significant ($p<0.05$) effect on the fasting serum glucose level in this study.

**Table 1 -** Effect of supplementation with vitamin E on body weight, food intake, water intake and serum glucose concentration in normal and STZ-induced diabetic rats, after 30 days of treatment.

| Groups | body weight (g) | food intake (g rat $^{-1}$ day $^{-1}$) | water intake (mL rat $^{-1}$day $^{-1}$) | glucose (mmol/L) |
|---|---|---|---|---|
| GI | 404.40 ± 20.56 b | 23.56 ± 2.05 a | 33.22 ± 0.91 a | 5.54 ± 0.41 a |
| GII | 440.64 ± 44.80 b | 27.54 ± 1.72 b | 37.81 ± 3.81 a | 5.16 ± 0.45 a |
| GIII | 309.95 ± 17.39 a | 43.42 ± 1.89 d | 180.55 ± 25.80 c | 17.29 ± 2.18b |
| GIV | 447.49 ± 41.6 b | 35.22 ± 0.51 c | 88.38 ± 6.38 b | 5.34 ± 0.47 a |

Values are given as mean±SD for eight rats in each group. Statistical evaluation was carried out using ANOVA followed by Tukey. Values not sharing a common superscript letter differ significantly at $p<0.05$. GI: Control; GII: Vitamin E; GIII: Diabetic; GIV: Diabetic + Vitamin E.

The changes in the lipid profile of normal and diabetic rats are illustrated in Table 2. Untreated diabetic rats were characterized by a significant elevation in the levels of total cholesterol, LDL cholesterol, VLDL cholesterol and triacylglycerols, compared with the normal

animals. Diabetic rats treated with vitamin E (GIV) showed near normal levels of total cholesterol, VLDL cholesterol and triacylglycerols. Dietary vitamin E caused a significant decrease in the serum level of LDL cholesterol, when compared with diabetic rats (GIII). Significantly ($p<0.05$) decreased levels of serum HDL cholesterol were observed in diabetic rats when compared with the non-diabetic rats (GI). Oral administration of vitamin E to diabetic rats significantly increased serum HDL cholesterol, when compared with diabetic rats.

**Table 2** - Effect of supplementation with vitamin E on lipidic profile in normal and STZ- induced diabetic rats, after 30 days of treatment.

| Groups | total cholesterol (mmol/L) | LDL-cholesterol (mmol/L) | VLDL-cholesterol (mmol/L) | triacyl-glycerols (mmol/L) | HDL-cholesterol (mmol/L) |
|---|---|---|---|---|---|
| GI | 2.66±0.25a | 0.95±0.28a | 0.40±0.04a | 0.88±0.08a | 1.30±0.29bc |
| GII | 2.82±0.28a | 0.80±0.26a | 0.39±0.04a | 0.85±0.08a | 1.62±0.24b |
| GIII | 7.02±0.42b | 5.67±0.42c | 0.71±0.07b | 1.55±0.16b | 0.64±0.12a |
| GIV | 2.99±0.18a | 1.48±0.16b | 0.39±0.04a | 0.86±0.09a | 1.12±0.16c |

Values are given as mean±SD for eight rats in each group. Statistical evaluation was carried out using ANOVA followed by Tukey. Values not sharing a common superscript letter differ significantly at $p<0.05$, GI: Control; GII: Vitamin E; GIII: Diabetic; GIV: Diabetic + Vitamin E.

In diabetic rats, lipid hydroperoxide increased, while the GSH-Px and SOD activity decreased significantly in comparison to normal control rats. The treatment of diabetic rats with vitamin E (GIV) led to a significant decrease in serum levels of lipid hydroperoxide. However, the antioxidant enzyme activity was significantly higher in the vitamin E-treated diabetic group (GIV) when compared to the untreated diabetic rats. Vitamin E administration to normal rats did not alter the activity of GSH-Px or SOD (Table 3).

The serum urea and creatinine concentration was significantly higher ($p<0.05$) in STZ-diabetic rats (GIII). Diabetic animals administered vitamin E (GIV) showed a significant decrease in the level of urea and creatinine (Table 3). The total protein level showed a marked reduction in the serum of diabetic rats (GIII); a significant increase in total protein content was observed after the treatment with vitamin E (GIV).

**Table 3 -** Effect of supplementation with vitamin E on lipid hydroperoxide (HP) concentration and glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities in normal and STZ-induced diabetic rats, after 30 days of treatment.

| Groups | HP (nmol/mL) | GSH-Px (U/mL) | SOD (U/mg protein) |
|---|---|---|---|
| GI | 0.29 ± 0.01 a | 142.22 ± 26.37 c | 21.10 ± 2.66 b |
| GII | 0.29 ± 0.01 a | 139.87 ± 20.78 c | 21.14 ± 3.34 b |
| GIII | 0.61 ± 0.03 c | 75.64 ± 18.15 a | 10.37 ± 1.96 a |
| GIV | 0.42 ± 0.03 b | 97.99 ± 20.47 b | 21.25 ± 2.50 b |

Values are given as mean±SD for eight rats in each group. Statistical evaluation was carried out using ANOVA followed by Tukey. Values not sharing a common superscript letter differ significantly at $p<0.05$. GI: Control; GII: Vitamin E; GIII: Diabetic; GIV: Diabetic + Vitamin E.

**Table 4** - Effect of supplementation with vitamin E on serum concentration of creatinina, urea and total protein in normal and STZ-induced diabetic rats, after 30 days of treatment.

| Groups | Creatinine (μmol/L) | Urea (mmol/L) | Total Protein (g/L) |
|---|---|---|---|
| GI | 62.48 ± 8.8 a | 8.84 ± 1.77 a | 73.0 ±8.0 b |
| GII | 161.92 ± 14.96 b | 7.59 ±0.92 a | 71.0 ± 8.0 b |
| GIII | 192.72 ±19.36 c | 16.64 ± 1.54 b | 43.0 ± 7.0 a |
| GIV | 176.00 ± 16.72c | 9.78 ± 1.63 a | 72.0 ± 5.0 b |

Values are given as mean±SD for eight rats in each group. Statistical evaluation was carried out using ANOVA followed by Tukey. Values not sharing a common superscript letter differ significantly at $p<0.05$. GI: Control; GII: Vitamin E; GIII: Diabetic; GIV: Diabetic + Vitamin E.

## DISCUSSION

The present study investigated the effects of treatment with vitamin E on the lipid profile, oxidative stress and renal outcome of STZ-induced diabetes in rats (Insulin dependent diabetes mellitus – IDDM type 1).Characteristic symptoms in STZ-induced diabetic rats included excessive water intake (polydipsia), increased food intake (hyperphagia), hyperglycaemia, and severe loss of body weight (Table 1). Increased water and food consumption are a direct result of the accumulation of glucose in the blood and increase in the urinary excretion of glucose (Punithavathi et al. 2008).

The present data indicated that vitamin E reduced the glycaemic index in diabetes, which was consistent with previous studies (Baydas et al. 2002; Bonnefont-Rousselot 2004). Vitamin E was able to reduce the food and water intake in diabetic rats. This could be due to the correction of glycaemia (Bonnefont-Rousselot et al. 2000), which was associated with an improved metabolic state in those animals. The reduction in body weight in untreated diabetic rats might be due to the proteolytic breakdown of structural protein (Kammlakkannan and Prince 2006; Franz et al. 2002) into amino acids, which were then oxidized, since the cells could not be bale to absorb blood glucose for use as a metabolic energy source (Sekar et al. 2005). In addition, these amino acids may be utilized as gluconeogenic precursors in the liver (Postic et al. 2004). Moreover, glycogenolysis (mobilization of glycogen) and lypolysis (triacylglycerol hydrolysis in the adipose tissue) might also contribute to the reduction in weight gain by diabetic rats (Sekar et al. 2005).

Adipose tissue weight and hepatic glycogen content decreased in the experimental STZ-induced diabetes in rats (Ruperez et al. 2008). The body weight was restored in the presence of vitamin E in STZ-induced diabetic rats, demonstrating its antidiabetogenic effect.The capacity of vitamin E to protect against the body weight loss could be attributed to its ability to reduce hyperglycaemia. This may be achieved *via* the suppression of hepatic gluconeogenesis and glucose output from the liver, which is associated with the inhibition of lipolysis in adipose tissue (Postic et al. 2004). These findings were consistent with the fact that vitamin E caused a reduction in the level of circulating glucagon in diabetic rats (Shamsi et al. 2006). It is essential to preserve glucose homeostasis, as it is a key part of the normal regulation of hepatic metabolic activities (Postic et al. 2004) and maintenance of blood glucose concentration within the normal range.

The increases in plasma triacylglycerol, total cholesterol, very low-density lipoprotein (VLDL) cholesterol and low-density lipoprotein (LDL) cholesterol, and the decrease in high density lipoprotein (HDL), shown in Table 2, indicated significant dyslipidaemia in untreated diabetic rats. Similar results were obtained in several studies in animal or experimental diabetes (Chertow and Edwards 2004; Cullen et al. 1999; Solano and Goldberg (2006). Sout (2005) considered diabetic dyslipidaemia and hyperglycaemia to be predictors of cardiovascular complications.

Several biochemical mechanisms have been proposed as responsible for the hypertriglyceridaemia in diabetes. These include an increase in the activity of hormone-sensitive lipase, which catalyses the mobilization of fatty acids from triacyglycerols stored in adipocytes (Cullen et al. 1999; Reynisdottir et al. 1997). Thus, the greater quantities of fatty acids returning to the liver are reassembled into triacylglycerols and secreted in VLDL. It has also been reported that the activity of lipoprotein lipase (an enzyme bound

to endothelial cells, which catalyses the hydrolysis of triacylglycerols in VLDL and chylomicrons (Reynisdottir et al. 1997; Kondo et al. 2007) is reduced in diabetes. This promotes diabetic hypertryglyceridaemia.

In the present study, rats with STZ-induced diabetes that were treated with vitamin E showed a reduction in both VLDL, triacylglycerols and LDL, and an increase in HDL. This could be attributed to improved glycaemic control by a mechanism involving enhanced insulin action promoted by the vitamin E (Baydas et al. 2002; Jari et al. 1999). The supplementation with vitamin E has previously been positively correlated with decreased total cholesterol and triacylglycerol concentrations (Jain et al. 1978; Hamilton et al. 2000; Merzouk et al. 2004). Baydas et al. 2002) reported a negative association between vitamin E and serum cholesterol and triacylycerol levels.

The efficacy of vitamin E with regards reducing serum triacyglycerols and VLDL may be attributed to its protection of membrane-bound lipoprotein lipase against lipid peroxide (Mendez and Balderas 2001). The antihyperglycaemic effect of vitamin E and hence the improved diabetic state may lead to a reduction in the concentration of VLDL and consequently the LDL level, (Guo et al. 2002).

Bonnefont-Rousselot (Bonnefont-Rousselot et al., 2000) demonstrated the beneficial effect of better glycaemic control on the increase in oxidized LDL, promoted by ROS in diabetes. The chronic production of oxidized LDL induces foam cell formation, which results in atherosclerosis (Price et al. 2001).

This study demonstrated that the administration of vitamin E was able to prevent a reduction in HDL during the experimental period in STZ-induced rats. Thus, vitamin E may be protective against atherosclerosis and cardiovascular disease. Since there was a negative correlation between atherosclerotic cardiovascular disease and the plasma HDL level (Stephen 2004), the mechanism could involve reverse cholesterol transport.

Oral administration of vitamin E increased serum HDL (GII). One explanation for the association between the increased concentration of HDL and vitamin E could be that the antioxidant vitamin increased lecithin:cholesterol acyltransferase (LCAT) activity. Thus, raise the rate of cholesteryl estertransfer for within of HDL lipoprotein. Furthermore, LCAT activity may increase the transport of cholesterol ester by from peripheral tissues to the liver and stimulate the production and secretion of HDL for circulating (Mackness et al. 1993)

SOD is involved in the direct elimination of ROS, through dismutation of superoxide radicals (Punitha et al. 2005).GSH-Px catalyzes the conversion of $H_20_2$ to $H_20$ through the oxidation of reduced glutathione, *via* the glutathione redox cycle (Szkudeslski, 2001; Duarte et al. 2001). Under normal circumstances, sufficient amounts of reactive oxygen species are removed by the antioxidant defense systems. The excess formation of ROS, due to a depleted endogenous antioxidant system (Baydas et al. 2002; Young et al. 1995), for example, leads to a decrease in GSH-Px and SOD and an increase in lipid peroxidation. It exacerbates oxidative stress, mediated mainly by hyperglycaemia (Koo et al. 2001).

In the present study, lipid hydroperoxide increased, while GSH-Px and SOD activity decreased in diabetic rats (Table 3). These results were consistent with other reports of an increase in lipid hydroperoxides in the plasma of diabetic subjects (Ahmed 2005; Kakkar et al. 1995) and in animals with experimental diabetes (Desco et al. 2002; Ramesh and Pugalendi 2005). The decrease in antioxidant enzyme activity under diabetic conditions could be due to glycation of these enzyme, which occurred at persistently elevated blood glucose levels (Taniguchi, 1992). Glycation of SOD reduces its activity, leading to the insufficient dismutation of superoxide anions ($O^{-2}$) (Taniguchi 1992; Majithiya and Balaram 2005; Ravi et al. 2004), Studies by Young et al. (1995) demonstrated a correlation between improved glycaemic control and the inhibition of protein glycation, and hence an increase in SOD activity.

Oxidative stress is the result of a redox imbalance between the generation of ROS and the compensatory response from the endogenous antioxidant network. There is no consensus concerning the changes in the activities of antioxidant enzymes in diabetic rats. Although some studies measuring the activities of SOD in diabetes mellitus showed reductions in the levels of these enzymes (Coskun et al. 2005; Cho et al. 2002; Sekeroglu et al. 2000), others reported increased activities in streptozotocin-induced diabetic rats (Yilmaz et al. 2004; Sanders et al.

2001). The increase in SOD activity could be due to its induction by increased production of superoxide, and $H_2O_2$ was reported to act as an

inducer of tissue SOD (Matkovics et al. 1977; Dias et al. 2005).

Lipid hydroperoxide, which has been used as an indirect biomarker to evaluate the oxidative stress, is increased in the serum of type 1 diabetic experimental models. This finding was consistent with the hypothesis that elevated levels of hydroperoxide in the serum increased the generation of ROS in diabetes mellitus (Merzouk et al. 2004).

In the present study vitamin E attenuated the increase in lipid hydroperoxide, accompanied by a concomitant increase in the activities of GSH-Px and SOD, which modulated the concentration of ROS. These data were consistent with studies reporting that vitamin E exerted a beneficial effect on the antioxidant enzyme activity in type 1 diabetes (Varvarovská et al. 2004; Musalmah et al. 2002). Indeed, antioxidant therapy could prevent a disturbance in the mechanism of protection against the deleterious cellular and biomolecular effects that led to alterations in the cell function. Since diabetes is associated with increased oxidative stress as a consequence of persistent hyperglycaemia, supplementation with vitamin E, and thus regulation of glycaemia, could have a protective effect against lipid peroxidation in diabetes.

The urea and creatinine levels, which have been considered significant markers of diabetic nephropathy, increased in the STZ-induced diabetic group (Table 3). The abnormally high levels of serum creatinine and urea were consistent with the impaired kidney function (Ronco et al. 2010). An increased urea concentration in diabetic rats is associated with greater protein catabolism. Ceriello et al. (2000) demonstrated a positive correlation between hyperglycaemia and the development of nephropathy. It has been demonstrated that the metabolic abnormalities observed in uncontrolled diabetes result in gluconeogenesis (Punithavathi et al. 2008) and consequently urea production, which are substantially enhanced in diabetes (Unger and Orci 1981). These results were consistent with the reduction in the total serum protein levels observed in STZ-induced diabetic rats (Table 3).

The treatment of diabetic rats with vitamin E (GIV) prevented increases in the biomarkers creatinine and urea, suggesting better renal function in this group. This could be due to its antioxidant property and also its ability to regulate the glycaemia, since diabetic hyperglycaemia led to renal dysfunction (Ceriello et al. 2000).

Although vitamin E reduced the occurrence of glomerular membrane damage and prevented the increases in serum creatinine level, animals of the GII showed higher creatinie level. Mc Ginley et al. (2009) reported that vitamin E reduced creatine kinase activity, enzyme that catalyzed the convertation the of creatinine in phosphoceratine. This resulted in increase of the serum creatine level. The ability of vitamin E to improve the glycaemic state might result in a reduction in glyconeogenase. This would reduce protein breakdown, correcting the level of protein observed in the serum of STZ-induced diabetic rats (GIV).

In conclusion, the administration of vitamin E reduced food and water intake, and led to lower creatinine and urea levels, ameliorated hyperglycaemia and body weight, and promoted the correction of lipid metabolism disorders and protein levels in the STZ-induced diabetic rats. The increases in the SOD and GSH-Px activity and reduced hydroperoxide levels could be due to the improvement of glycaemia promoted by vitamin E. Thus, the imbalance between the generation of ROS and enzyme activity be controlled in diabetic rats.

## ACKNOWLEDGEMENTS

This research was supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

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Received: March 28, 2011;
Revised: August 02, 2011;
Accepted: May 07, 2012.