

Effects of alpha lipoic acid and vitamin C supplementation on transforming growth factor alpha (TGF α) and transforming growth factor beta 2 (TGF β 2) secretion in testicular tissue of diabetic rats*

Sevda ELİŞ YILDIZ^{1**}, Buket BAKIR², Hamit USLU³, Ebru KARADAĞ SARI⁴, Musa KARAMAN⁵

¹School of Health Sciences, Kafkas University, Kars, Turkey

²Health Services Vocational School, Namık Kemal University, Tekirdağ, Turkey

³Atatürk Health Services Vocational School, Kafkas University, Kars, Turkey

⁴Department of Histology-Embryology, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey

⁵Department of Pathology, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey

Received: 09.07.2014 • Accepted: 28.10.2014 • Published Online: 12.01.2015 • Printed: 09.02.2015

Abstract: The effects of alpha lipoic acid and vitamin C supplementation on TGF α and TGF β 2 secretion in the testicular tissue of streptozotocin-induced diabetic rats were examined by immunohistochemical methods. Rats were divided into 5 groups; group 1 (control), 2 (diabetic control), 3 (diabetes + alpha lipoic acid), 4 (diabetes + vitamin C), and 5 (diabetes + alpha lipoic acid + vitamin C). Hematoxylin and eosin staining was performed in order to examine testicular tissue histopathologically. The streptavidin-biotin-peroxidase complex method was used for histological examination and determination of TGF α and TGF β 2 localization in the testicular tissue. Regarding fasting blood glucose levels, the lowest blood glucose level was detected in group 3. Fewer pathological histopathological findings were observed in groups treated with alpha lipoic acid, vitamin C, and alpha lipoic acid + vitamin C compared to the diabetes group. TGF α and TGF β 2 secretions increased in groups 3, 4, and 5 compared to group 2. In conclusion, exogenous administration of alpha lipoic acid, vitamin C, and alpha lipoic acid + vitamin C increased TGF α and TGF β 2 secretion in streptozotocin-induced diabetic rats.

Key words: Alpha lipoic acid, diabetes, vitamin C, TGF α , TGF β 2

1. Introduction

Diabetes mellitus (DM) is a degenerative disease causing alterations in carbohydrate homeostasis that, in turn, affect the function of the male reproductive system (1). DM is divided into 2 groups, type I and type II. Type I diabetes is caused by lack of insulin secretion. Type II diabetes is due to increased insulin resistance or lack of insulin receptors in target tissues (2).

Oxidative stress increases in diabetes due to overproduction of reactive oxygen species (ROS) and decreased efficiency of antioxidant defenses (3). To control the flux of ROS, aerobic cells have developed an antioxidant defense system with enzymatic and nonenzymatic components (4). Common antioxidants include vitamins A, C, and E; glutathione; superoxide dismutase, catalase, and glutathione peroxidase enzymes; and several bioflavonoids that fight against different types of free radicals in synergy (5).

Alpha lipoic acid (ALA) is one of the antioxidants that is effective in all steps of the antioxidant defense system such as inhibiting radical metabolite production, scavenging generated radicals, repairing resulting cell damage, halting chain reactions producing secondary radicals, and maximizing endogenous antioxidant capacity (6). ALA is synthesized in the liver, heart, and kidney tissues of humans (7).

Vitamin C, also known as ascorbic acid, is an important antioxidant in biological systems, with molecular formula $C_6H_8O_6$ and greyish white color. As humans are unable to synthesize vitamin C, they need this vitamin in their diet (8).

Transforming growth factor α (TGF α) is a member of the EGF family of cytokines that are synthesized as transmembrane precursors and characterized by the presence of one or several EGF structural units in their

* This research was presented at XIIth National Histology and Embryology Congress with International Contribution, P: 089, 27-30 May, 2014, Ankara, Turkey.

** Correspondence: sevdaelis36@hotmail.com

extracellular domain (9). TGF α exerts identical biological effects on proliferation, differentiation, and differentiated function in a wide variety of target cells (10).

Transforming growth factor β (TGF β) is formed by inactive peptide dimers with 25 kDa molecular weights and is widely expressed in the mammalian body (11,12). TGF β family has 3 members, TGF β 1, TGF β 2, and TGF β 3 (13). This family regulates numerous biological activities, including cell proliferation, differentiation, adhesion, apoptosis, extracellular matrix (ECM) production, and early embryo development and immunity (14).

The present study investigated the effects of ALA and vitamin C supplementation on TGF α and TGF β 2 secretion, which have antioxidant and growth, development, differentiation, and also regulatory properties, respectively, in testicular tissues of rats with diabetes by immunohistochemical methods.

2. Materials and methods

2.1. Animals and care conditions

This study was approved by the Mehmet Akif Ersoy University Local Ethics Committee for Animal Experiments under the 12/03/2014, 11/68 protocol number.

At the beginning of the experiment 50 male Sprague-Dawley rats (310–365 g, 4–5 months old) were randomly divided into 5 groups of 10 animals each. The rats were kept in standard cages at 22 ± 2 °C and were exposed to a 12-h light cycle. Administrations were started after a 1-week adaptation period and were made each day between 1700 and 1800.

2.2. Experimental design

Group 1 (control) (n = 10): animals were fed ad libitum, group 2 (diabetes control) (n = 10): 50 mg/kg streptozotocin (STZ) dissolved in 50 mL of citric acid + 40 mL of disodium hydrogen phosphate buffer (pH 4.5) was administered intraperitoneally (ip). In order to ensure uniform group standards, saline was given by oral gavage to the animals throughout the study. Three days after STZ administration, fasting blood glucose levels of tail vein blood of rats fasted 8 h were measured by glucometer, and animals with fasting blood glucose of 250 mg/dL were labeled type I diabetic. Group 3 (diabetes + alpha lipoic acid) (n = 10): diabetic rats, as a result of administration of 50 mg/kg ip of STZ, were given 80 mg/kg of alpha lipoic acid orally for 21 days. Group 4 (diabetes + vitamin C) (n = 10): diabetic rats, as a result of administration of 50 mg/kg ip of STZ, were given 60 mg/kg of vitamin C orally for 21 days. Group 5 (diabetes + alpha lipoic acid + vitamin C) (n = 10): diabetic rats, as a result of administration of 50 mg/kg ip of STZ, were given 80 mg/kg of alpha lipoic acid and 60 mg/kg of vitamin C orally for 21 days.

2.3. Body weights

Live body weights of rats in all groups were measured before and after the experimental period. All rats were sacrificed by cervical dislocation under diethyl ether anesthesia, and then testicular tissue samples were taken.

Statistical Package for the Social Sciences 18.0 (SPSS) software was used to compare variants among groups. Possible differences were determined by using ANOVA and Tukey's test. The confidence interval in statistical analysis was $P < 0.05$.

2.4. Determination of blood glucose levels

Before starting the study (day 0), the animals were fasted for 8 h, venous blood samples were taken from tail veins, and a glucometer (Yasee, GLM-76, Taiwan) was used for determination of fasting blood glucose levels. Three days after STZ administration, fasting blood glucose levels of tail vein blood of rats fasted 8 h were measured, and animals with fasting blood glucose of 250 mg/dL were included in the study. Similarly, blood glucose levels were measured on days 10, 17, and 24 of the study following 8 h of fasting. Starting from the third day of STZ administration, ALA and vitamin C were administered orally to the experimental groups for 21 days.

2.5. Histopathology

Testicular tissue samples were fixed in 10% formaldehyde for 24 h and processed routinely for embedment in paraffin. Then, 5- μ m thick serial sections were obtained and stained with hematoxylin and eosin (H&E), following deparaffinization and rehydrating, to demonstrate the general structure of the testis.

2.6. Immunohistochemistry

For immunohistochemical staining, the streptavidin-biotin-peroxidase complex method was utilized on 5- μ m thick sections. After deparaffinization and rehydration of the sections, endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 20 min. Then the sections were washed in 0.01 M phosphate buffered saline (PBS) solution, pH 7.4. The tissue sections were incubated with polyclonal rabbit anti-TGF α (cat. no. GF10; Merck, USA) and polyclonal goat anti-TGF β (sc-90; Santa Cruz, CA, USA) diluted 1:100 in PBS. Sections were incubated with biotinylated goat anti-rabbit and rabbit anti-goat IgG for 30 min and peroxidase conjugated streptavidin (1:300) (P0397; Dako Corp., Carpinteria, CA, USA) for 30 min. Broad spectrum antibody [Invitrogen Histostain Plus Broad Spectrum (AEC), ref. 85.9943] was used as chromogen followed by hematoxylin counterstaining. Sections were mounted with Immuno Mount and examined under light microscopy (Olympus BX51; Shinjuku, Tokyo, Japan). Rabbit serum without primer antibody served as negative control. TGF α and TGF β -2 staining intensities were judged as (-) negative; (+) slight; (++) moderate, and (+++) intense immunolabeling.

3. Results

3.1. Blood glucose levels

On the starting day (day 0), blood glucose levels of the groups were between 64 and 100 mg/dL. A significant increase was observed in blood glucose levels of group 1 towards the end of the study (day 0 and 24th day, $P < 0.01$; 10th day and 24th day, $P < 0.05$) (Table 1).

Significant increases were detected in blood glucose levels of all diabetic groups on day 3 ($P < 0.001$). Blood glucose levels increased day by day in group 2. A significant increase was detected on day 24 in group 4 ($P < 0.001$) (Table 1). Significant increases were found in blood glucose levels of group 3 between days 3 and 17, 3 and 24, 10 and 17, and 10 and 24 at $P < 0.001$ level. In group 5, the increase in glucose levels on day 24 compared to days 3 and 10 was significant ($P < 0.05$) (Table 2).

A significant decrease was determined in blood glucose levels of group 4 compared to group 2 on day 17 ($P < 0.01$). Nonsignificant decreases were detected in groups 3 and 5 on days 10 and 17, respectively ($P > 0.05$) (Table 2).

3.2. Body weight findings

Body weights of all groups were measured beginning on the 3rd, 10th, 14th, and 24th days of the study, and groups were evaluated within groups. A statistically nonsignificant

increase in body weights of the animals in group 1 was observed (3rd day, 10th day, 17th day, and 24th day; $P > 0.05$). A statistically significant decrease was observed in body weights of group 2 animals (3rd day, 10th day, 17th day, and 24th day; $P < 0.001$). Measurement of the body weights of group 3 revealed statistically significant decreases on the 3rd day ($P < 0.05$), 10th day ($P < 0.01$), 17th day, and 24th day ($P < 0.001$). Similarly, statistically significant changes were observed in the measurements of group 4 with levels of $P < 0.001$ on days 3, 10, 17, and 24. Statistically significant decreases at advanced levels were determined in the weights of group 5 (3rd day, $P < 0.05$; 10th day, 17th day, and 24th day, $P < 0.001$) (Table 3).

3.3. Histopathological results

No difference was observed in the histological structure of rats in group 1 (Figure 1a). In group 2, degenerative and necrotic changes were observed in some seminiferous tubules, Sertoli cells, and spermatogenetic cells. A reduction in number of spermatogenetic cells and collapse of seminiferous tubules due to necrosis were also observed (Figure 1b). Results in groups 3 and 4 were similar to those in group 1 (Figures 1c and 1d). In group 5 there was a marked reduction in degenerative and necrotic changes in seminiferous tubules and Sertoli cells and in spermatogenesis (Figure 1e).

Table 1. Comparison of blood glucose levels in all groups (mg/dL).

Glucose	Day 0 X ± SD	3 rd day X ± SD	10 th day X ± SD	17 th day X ± SD	24 th day X ± SD
Group 1	84.8 ± 4.6 ^b	86.5 ± 6.4 ^b	88.0 ± 5.4 ^{b*}	89.9 ± 6.7 ^{ab}	97.4 ± 10.2 ^a
Group 2	83.9 ± 5.3 ^c	307.8 ± 32.9 ^{b*}	326.8 ± 26.1 ^{b†}	377.5 ± 41.4 ^{a*}	402.4 ± 19.7 ^{a†}
Group 3	83.2 ± 9.1 ^c	323.5 ± 30.3 ^{b*}	316.6 ± 20.9 ^{b†}	369.8 ± 17.3 ^{a*}	393.5 ± 8.4 ^{a†}
Group 4	83.4 ± 6.4 ^c	311.8 ± 19.2 ^b	318.4 ± 50.1 ^b	311.1 ± 35.8 ^b	393.7 ± 5.9 ^a
Group 5	86.9 ± 10.5 ^c	329.6 ± 22.3 ^b	327.2 ± 16.9 ^b	344.1 ± 32.5 ^{ab}	375.2 ± 57.0 ^a

Different superscripts (^{a,b,c,*†}) in the same column indicate significant differences between groups. Group 1: a-b: $P < 0.01$; a-b*: $P < 0.05$. Group 2: b*-c, b†-c, a*-c, a†-c, a*-b*, b*-a†, a†-b†: $P < 0.001$; b†-a*: $P < 0.01$. Group 3: b*-c, b†-c, a*-c, a†-c, a*-b*, a†-b*, a*-b†, a†-b†: $P < 0.001$. Group 4: b-c, a-c, a-b: $P < 0.001$. Group 5: a-c, b-c, ab-c: $P < 0.001$; a-b: $P < 0.05$.

Table 2. Comparison of blood glucose levels among all groups (mg/dL).

Glucose	Group 1	Group 2	Group 3	Group 4	Group 5
Day 0 (X ± SD)	84.8 ± 4.6	83.9 ± 5.3	83.2 ± 9.1	83.4 ± 6.4	86.9 ± 10.5
3 rd day (X ± SD)	86.5 ± 6.4 ^b	307.8 ± 32.9 ^a	323.5 ± 30.3 ^a	311.8 ± 19.2 ^a	329.6 ± 22.3 ^a
10 th day (X ± SD)	88.0 ± 5.4 ^b	326.8 ± 26.1 ^a	316.6 ± 20.9 ^a	318.4 ± 50.1 ^a	327.2 ± 16.9 ^a
17 th day (X ± SD)	89.9 ± 6.7 ^c	377.5 ± 41.4 ^a	369.8 ± 17.3 ^a	331.1 ± 35.8 ^b	344.1 ± 32.5 ^{ab}
24 th day (X ± SD)	97.4 ± 10.2 ^b	402.4 ± 19.7 ^a	393.5 ± 8.4 ^a	393.7 ± 5.9 ^a	375.2 ± 57.0 ^a

Different superscripts (^{a,b,c}) in the same column indicate significant differences between groups. Day 0: $P > 0.05$; 3rd day: a-b: $P < 0.001$; 10th day: a-b: $P < 0.001$; 17th day: a-c, b-c, ab-c, a*-c: $P < 0.001$, a*-b: $P < 0.01$, a-b: $P < 0.05$; 24th day: a-b: $P < 0.001$.

Table 3. The mean weight of rats in all groups among days (g).

Glucose	Day 0 X ± SD	3 rd day X ± SD	10 th day X ± SD	17 th day X ± SD	24 th day X ± SD
Group 1	365.4 ± 15.3	356.7 ± 37.7	366.3 ± 25.4	381.6 ± 30.7	390.6 ± 27.1
Group 2	317.5 ± 8.0 ^a	281.4 ± 17.8 ^b	270.2 ± 18.1 ^{bc}	261.9 ± 19 ^{bc}	252.8 ± 20.2 ^c
Group 3	306.3 ± 12.8 ^a	276.7 ± 23.7 ^b	268.3 ± 22.2 ^{bc}	248.9 ± 21.8 ^c	241.2 ± 24.4 ^c
Group 4	341.9 ± 5.1 ^a	310.8 ± 7.7 ^b	292.0 ± 14.6 ^c	278.2 ± 13 ^{cd}	270.9 ± 13.9 ^d
Group 5	341.5 ± 9.0 ^a	308.7 ± 19.0 ^b	288.1 ± 24.9 ^{bc}	270.2 ± 25.8 ^{cd}	244.6 ± 30.4 ^d

Different superscripts (^{a,b,c}) in the same column indicate significant differences between groups. Group 1: P > 0.05. Group 2: a-b, a-bc, a-c: P < 0.001; b-c: P < 0.01. Group 3: a-c, a-c*: P < 0.001; a-bc, b-c*: P < 0.01; a-b, b-c: P < 0.05. Group 4: a-b, a-c, a-cd, a-d, b-cd, b-d, c-d: P < 0.001; b-c: P < 0.01. Group 5: a-bc, a-cd, a-d, b-d, bc-d: P < 0.001; b-c: P < 0.01; a-b: P < 0.05.

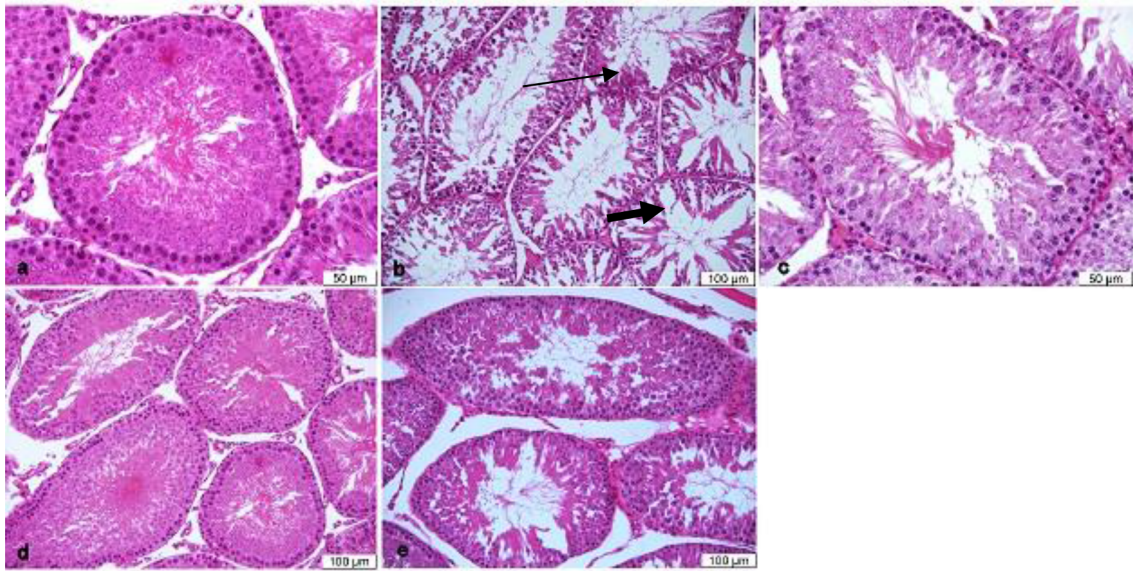


Figure 1. Rat testis. a. Group 1 (bar = 50 µm); b. group 2, degeneration (long arrow) and necrosis (thick arrow) (bar = 100 µm); c. group 3 (bar = 50 µm); d. group 4 (bar = 100 µm); e. group 5 (bar = 100 µm).

3.4. Immunohistochemical results

TGF α release was found only in Leydig cells in all groups. Regarding intergroup comparison, there was an intense and cytoplasmic reaction in group 1, slight and cytoplasmic reaction in group 2, mild and cytoplasmic reaction in group 3, and intense and cytoplasmic reaction in groups 4 and 5 (Figure 2).

TGF β immunoreactivity was observed in Leydig cells, spermatids, and spermatozoa in all groups. When groups were compared according to their reaction rates, there was intense and cytoplasmic reaction in Leydig cells and intense cytoplasmic and nuclear reaction in spermatids and spermatozoa of group 1; slight and cytoplasmic reaction in Leydig cells and slight cytoplasmic and nuclear reaction in spermatids and spermatozoa of group 2; 1 intense and cytoplasmic reaction in Leydig cells and

intense cytoplasmic and nuclear reaction in spermatids and spermatozoa of groups 3, 4, and 5 (Figure 3).

4. Discussion

Streptozotocin (STZ), structurally a nitrosourea derivative, is an antibiotic derived from *Streptomyces achromogenes* (15). The STZ-induced diabetic rodents exhibited frequent abnormal architectural changes in the seminiferous tubule lined by spermatogenic cell series, Sertoli cells, and Leydig cells, which leads to a decrease in plasma testosterone levels (16,17) and testicular atrophy (18).

Male infertility is a common threat nowadays, and it has increased rapidly because of hyperglycemia (11,19). The testes of diabetic animals showed frequent abnormal histology, and seminiferous epithelium cytoarchitecture as well as the occludin distribution pattern appeared altered.

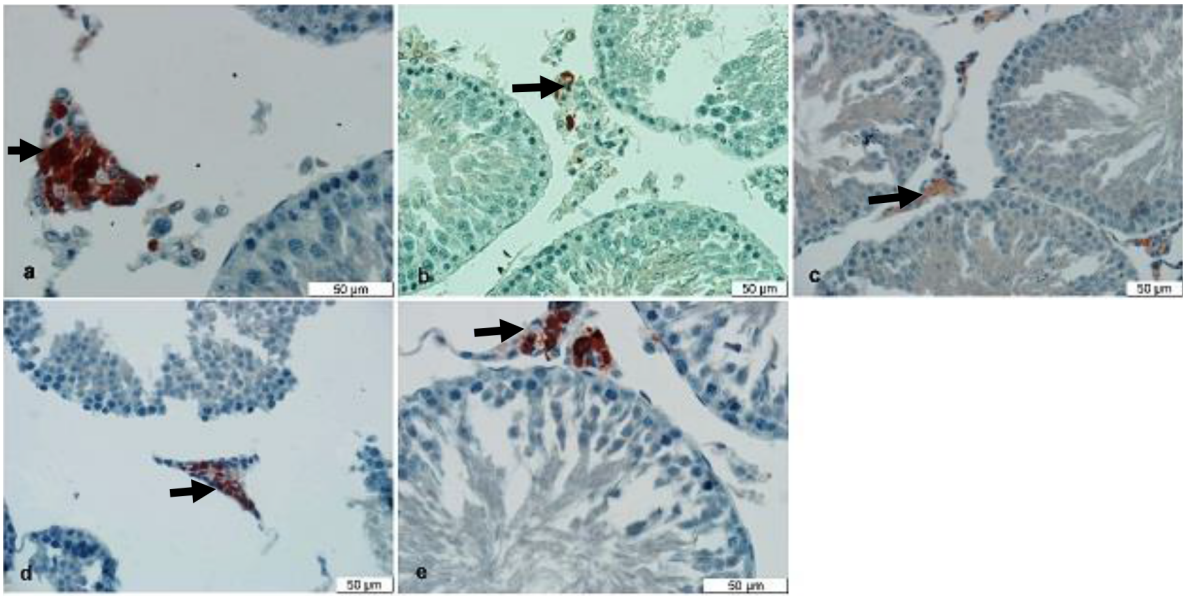


Figure 2. TGF α immunoreactivity in rat testis. a. Group 1; b. group 2; c. group 3; d. group 4; e. group 5, Leydig cell (arrow) (bar = 50 μ m).

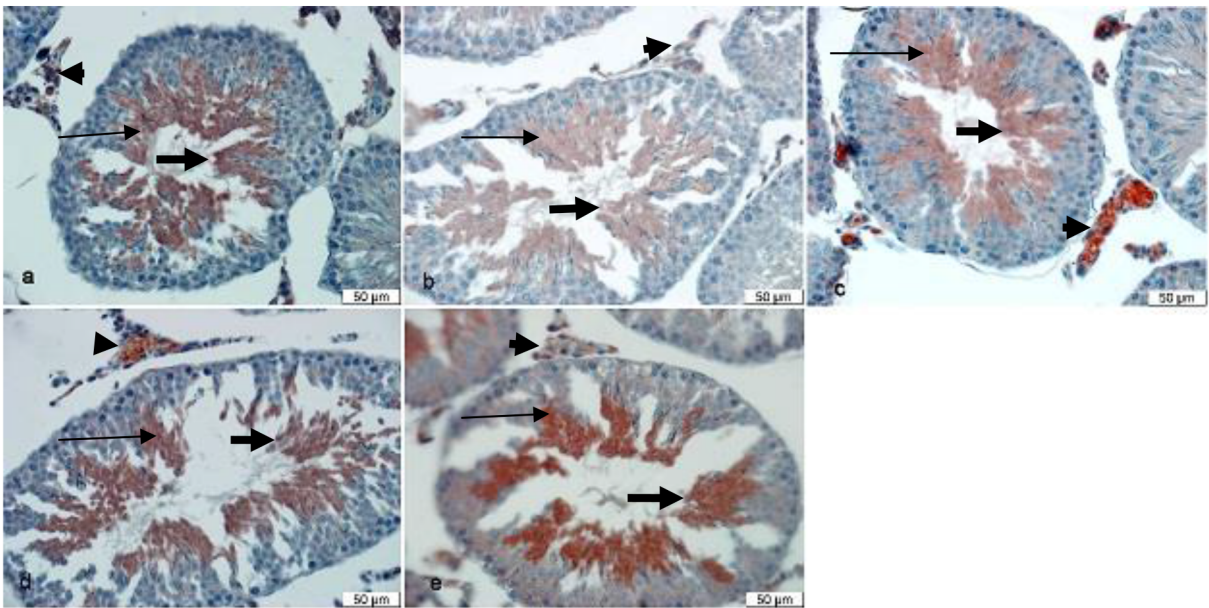


Figure 3. TGF α immunoreactivity in rat testis. a. Group 1; b. group 2; c. group 3; d. group 4; e. group 5, Leydig cell (arrow head), spermatid (long arrow), spermatozoon (thick arrow) (bar = 50 μ m).

Early occurrence of diabetes increased the percentage of animals with a high number of damaged tubules (16). Hassan et al. (20) reported there was germ cell depletion of the seminiferous epithelium and malorientation of spermatids. Some tubules showed greatly thickened walls and the lumen diameter had increased. The interstitial tissues appeared to have an increased area in the diabetic group. Results of our study agreed with results of these

previous studies. Our study indicated abnormal histology and necrosis in seminiferous tubules, Sertoli cells, and spermatogenic cells in diabetic rats, and administration of ALA and vitamin C decreased these abnormal changes.

A decrease in body weight was observed in the STZ-treated group during the experiment period (21). In our study, a significant decrease in body weight in the diabetes-induced group was also detected.

ALA can improve insulin sensitivity, accelerate recovery of pancreatic β -cells, and restore the metabolic alterations associated with diabetes (22). Packer et al. (22) reported that ALA increases glucose uptake through translocation of the glucose transporter to plasma membranes, a mechanism that is shared with insulin-stimulated glucose uptake. Moini et al. (23) reported that an insulin receptor is a potential cellular target for ALA action, with the mechanism of action of ALA as an insulin sensitizer. This mechanism occurs through auto-phosphorylation of insulin receptors and oxidation of thiol groups present in insulin receptor β -subunits by the oxidized form of ALA, which may be required for insulin-stimulated glucose transport. Moreover, administration of ALA to streptozotocin-induced diabetic rats was reported to lower fasting glucose levels (5). Our study indicated that ALA has an effective protective role in diabetic rats, as ALA decreased blood glucose levels of streptozotocin-induced diabetic animals.

Vitamin C is a major antioxidant that is essential for scavenging of toxic ROs in both blood and tissues (24). Vitamin C was reported to reduce blood glucose levels in type I diabetes (25). At the same time, vitamin C infusion increases the body's glucose utilization by enhancing absorption of glucose in both healthy and diabetic individuals (26). Compared to the diabetes control group in our study, vitamin C supplementation in diabetic groups decreased blood glucose levels and increased secretion of TGF α and TGF β , which help eliminate the negative effects of diabetes on liver tissue and have antioxidant properties.

No study has been found regarding the effects of antioxidant administration on TGF α and TGF β secretion in testicular tissue in diabetes. Immunolocalization studies in testicular tissue have indicated that Leydig cells express significant amounts of TGF α (27,28). Teerds et al. (28) localized TGF α by immunohistochemistry in adult Leydig cells in the rat. Millena et al. (29) reported that LH induces the expression of TGF α and EGFR in immature rat Leydig cells, which, in turn, may exert autocrine effects on steroidogenesis in Leydig cells. Furthermore, TGF β s modulate paracrine/autocrine actions (30). Similar to the above-mentioned studies, our study found that TGF α was being secreted from Leydig cells and TGF β 2 from Leydig, spermatids, and spermatozoa cells. Secretion was reduced in the diabetes-induced group; however, vitamin C and ALA administration increased TGF α and TGF β 2 secretion.

Diabetes may develop as a result of a metabolic problem but also may occur as a result of free radical damage to the organism. With an antioxidant defense system, the deleterious effects of free radicals are eliminated or minimized. We think that ALA and vitamin C supplementation in order to reduce or eliminate the oxidant effect in streptozotocin-induced diabetic rats increases TGF α and TGF β 2 secretions, which have growth, development, and regulatory roles in the organism and, therefore, can help to eliminate the negative effects of diabetes, such as infertility, on testicular tissue.

References

1. Sexton WJ, Jarow JP. Effect of diabetes mellitus upon male reproductive function. *Urology* 1997; 49: 508–513.
2. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2004; 27: 5–10.
3. Amaral S, Oliveira PJ, Ramalho-Santos J. Diabetes and the impairment of reproductive function: possible role of mitochondria and reactive oxygen species. *Curr Diabet Rev* 2008; 4: 46–54.
4. Naziroğlu M. Enhanced testicular antioxidant capacity in streptozotocin induced diabetic rats. Protective role of vitamins C and E and selenium. *Biol Trace Elem Res* 2003; 94: 61–71.
5. Maritim AC, Sanders RA, Watkins JB, 3rd. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol* 2003; 17: 24–38.
6. Karaca EG. Lipoik asit: Evrensel antioksidan. Afyon Kocatepe University J Sci 2008; 8: 231–246 (in Turkish).
7. Kramer K. Nutraceuticals in Health and Disease Prevention. Marcel Dekker Incorp 2001; 8: 113.
8. Duarte TL, Lunec J. When is an antioxidant not an antioxidant? A review of novel actions and reactions of vitamin C. *Free Radic Res* 2005; 39: 671–686.
9. De Larco JE, Todaro GJ. Sarcoma growth factor (SGF): specific binding to epidermal growth factor (EGF) membrane receptors. *J Cell Physiol* 1980; 102: 267–277.
10. Schroeder JA, Lee DC. Transgenic mice reveal roles for TGF- α and EGF receptor in mammary gland development and neoplasia. *J Mammary Gland Biol Neoplasia* 1997; 2: 119–129.
11. Wang F, Shi R, Zhao L. Expression and significance of TGF- β 1 and VEGF in formation of new blood vessels after rabbit corneal suture. *Recent Adv Ophthalmol* 2008; 28: 96–99.
12. Lee SH, Leera HS, Jeong SM, Lee K. Bevacizumab accelerates corneal wound healing by inhibiting TGF- β 2 expression in alkali-burned mouse cornea. *BMB Reports* 2009; 42: 800–805.
13. Ohtomo K, Ebihara N, Matsuda A, Tokura T, Funaki T, Murakami A. Role of TGF- β 1 in tissue eosinophilia associated with vernal keratoconjunctivitis. *Experimental Eye Res* 2010; 91: 748–754.
14. Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB. Myofibroblasts, paracrine cells important in health and disease. *Am J Physiol* 1999; 277: 1–9.
15. Szkudelski T. The mechanism of alloxan and streptozotocin in action in beta cells of rat pancreas. *Physiol Res* 2001; 50: 536–546.

16. Ricci G, Catizone A, Esposito R, Pisanti FA, Vietri MT, Galdieri M. Diabetic rat testes: morphological and functional alterations. *Andrologia* 2009; 41: 361–368.
17. Cai L, Chen S, Evans T, Deng DX, Mukherjee K, Chakrabarti S. Apoptotic germ-cell death and testicular damage in experimental diabetes: prevention by endothelin antagonism. *Urol Res* 2000; 28: 342–347.
18. Shrilatha B, Muralidhara. Occurrence of oxidative impairments, response of antioxidant defences and associated biochemical perturbations in male reproductive milieu in the streptozotocin-diabetic rat. *Int J Androl* 2007; 30: 508–518.
19. Andy P, Luiz R, Marco M, Luciana A. Relation between diabetes mellitus and male fertility. *Einstein* 2009; 7: 407–410.
20. Hassan GMA, Moneium TA. Structural changes in the testes of streptozotocin-induced diabetic rats. *Suez Canal Univ Med J* 2001; 4: 17–25.
21. Hassan AA, Hassouna MM, Taketo T, Gagnon C, Elhilali MM. The effect of diabetes on sexual behavior and reproductive tract function in male rats. *J Urol* 1993; 149: 148–154.
22. Packer L, Kreamer K, Rimbach G. Molecular aspect of lipoic acid in the prevention of diabetes complications. *Nutrition* 2001; 17: 888–895.
23. Moini H, Packer L, Saris NE. Antioxidant and prooxidant activities of α -lipoic acid and dihydrolipoic acid. *Toxicology and Applied Pharmacology* 2002; 182: 84–90.
24. Opara EC. Oxidative stress, micronutrients, diabetes mellitus and its complications. *J R Soc Health* 2002; 122: 28–34.
25. Cunningham JJ. The glucose/insulin system and vitamin C: implications in insulin dependent diabetes mellitus. *J American Coll Nutr* 1998; 2: 105–108.
26. Paolisso G, D'Amore A, Balbi V, Volpe C, Galzerano D, Giugliano D, Sgambato S, Varricchio M, D'Onofrio F. Plasma vitamin C affects glucose homeostasis in healthy subjects and in non-insulin-dependent diabetics. *Am J Physiol Endocrinol Metab* 1994; 266: 261–268.
27. Caussanel V, Tabone E, Mauduit C, Dacheux F, Benahmed M. Cellular distribution of EGF, TGF and their receptor during postnatal development and spermatogenesis of the boar testis. *Mol Cell Endocrinol* 1996; 123: 61–69.
28. Teerds KJ, Rommerts FF, Dorrington JH. Immunohistochemical detection of transforming growth factor—alpha in Leydig cells during the development of the rat testis. *Mol Cell Endocrinol* 1990; 12: 69.
29. Millena AC, Reddy SC, Bowling GH, Khan SA. Autocrine regulation of steroidogenic function of Leydig cells by transforming growth factor. *Mol Cell Endocrinol* 2004; 224: 29–39.
30. Skinner MK. Secretion of growth factors and other regulatory factors. In: Russell LD, Griswold MD, editors. *The Sertoli Cell*. Bolesta, Clearwater, FL, USA: Cache River Press; 1993; 1: 237–248.