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THE EFFECTS OF N-ACETYL-L-CYSTEINE ON OXIDATIVE PARAMETERS in TESTICULAR TISSUE OF STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract

The protection of N-Acetyl-L-cysteine (NAC) was investigated in the streptozocin-induced diabetes model in rat testicular tissues. In vivo biochemical mechanism was determined by measuring antioxidant parameters. The rats were randomly divided into 4 groups such as control, diabetes, diabetes+NAC and NAC. A single dose of intraperitoneal streptozotocin (IP STZ) (60 mg / kg body weight) was given to animals fasted overnight. Before injection and 72 hours after injection, blood glucose levels were measured from the tail vein with a glucometer. The blood glucose levels above 250 mg / dL were considered diabetic. Animals in groups 3 and 4 did not receive any treatment for four weeks after the diabetes model was established. Four weeks later, subjects in Groups 3 and 4 were given an IP injection of NAC (60 mg / kg, prepared in 0.9% NaCl solution daily) for 7 days. At the end of the applications, all animals were sacrificed and tissues were taken for antioxidant measurements. While the malondialdehyde (MDA) level increased in STZ-induced diabetic rat's testicular tissues, it was brought to the control level with NAC treatment. The glutathione (GSH) and nitric oxide (NOx) level, superoxide dismutaze (SOD) and caalase (CAT) enzyme activities were found significantly reduced in the diabetic group. However, NAC treatment resulted in elevated these parameters.

Key Words: diabetes, streptozotocin, N-acetyl cysteine, nitric oxide.

STREPTOZOTOSİN İLE İNDÜKLENEN DİYABETİK SIÇANLARIN TESTİS DOKULARINDA OKSİDATİF STRES PARAMETRELERİ ÜZERİNE N-ASETİL-L-SİSTEİNİN ETKİLERİ

Öz

Sıçan testis dokularında streptozosin kaynaklı diyabet modelinde N-Asetil-L-sisteinin (NAC) koruması araştırıldı. Antioksidan parametreler ölçülerek in vivo biyokimyasal mekanizma belirlendi. Sıçanlar rastgele kontrol, diyabet, diyabet+NAC ve NAC olmak üzere 4 gruba ayrıldı. Bir gece aç bırakılan hayvanlara tek doz intraperitoneal streptozotosin (IP STZ) (60 mg/kg vücut ağırlığı) verildi. Enjeksiyondan önce ve enjeksiyondan 72 saat sonra, bir glukometre ile kuyruk damarından kan şekeri seviyeleri ölçüldü. 250 mg/dL'nin üzerindeki kan şekeri seviyeleri diyabetik olarak kabul edildi. Grup 3 ve 4'teki hayvanlar, diyabet modeli oluşturulduktan sonra dört hafta boyunca herhangi bir tedavi görmediler. Dört hafta sonra, Grup 3 ve 4'teki deneklere 7 gün boyunca IP enjeksiyonu NAC (60 mg/kg, günlük %0.9 NaCl solüsyonunda hazırlanmıştır) verildi. Uygulamalar sonunda tüm hayvanlar sakrifiye edildi ve antioksidan ölçümleri için dokuları alındı. STZ ile diyabetik sıçanların testis dokularında malondialdehit (MDA) düzeyi yükselirken, NAC tedavisi ile kontrol düzeyine getirildi. Diyabetik grupta glutatyon (GSH) ve nitrik oksit (NOx) düzeyi, süperoksit dismutaz (SOD) ve caalaz (CAT) enzim aktiviteleri anlamlı olarak azalmış bulundu. Ancak, NAC tedavisi bu parametrelerin yükselmesine neden oldu.

Aahtar Kelimeler: diyabet, streptozotosin, N-asetil sistein, nitrik oksit.

1. INRODUCTION

Diabetes is a disease caused by disorders in carbohydrate, fat and lipid metabolism. The most important finding is hyperglycemia. High levels of glucose in the organism can be chemically recycled by combining with proteins it turns into glycosylation products, and this conversion increases in direct proportion to the blood glucose level. These changes trigger oxidative stress, causing an increase in reactive oxygen species. The oxidative effect can cause direct or indirect damage to DNA, cell dysfunction, inflammation and changes at the vascular level (1,2). In addition to many acute and chronic complications, diabetes also has negative effects on the male reproductive system. It causes many disorders such as decrease in sexual function, testicular deformation, spermatogenesis disorders (3-5). Oxidative stress refers to its overproduction by various harmful stimuli with high activity, such as reactive species (ROS) in the body. The main feature of cellular oxidative stress is the continuous increase regulation of ROS levels and it turns out that oxidative stress is a factor that promotes the development of diabetes (6,7).

NAC, which is the reduced glutathione precursor; It is used in many researches due to its antioxidant capacity, increasing glutathione level and decreasing reactive oxygen species. Today, it is used in individuals as a mucolytic agent that can be taken orally or by intravenous infusion of nebulizer information. When its therapeutic potential is examined, it becomes a research topic in many diseases as an antidote for certain toxins, as a bioprotective against oxidative stress and ischemia [8]. Orally used NAC is readily available and can be used due to its easy supply. It is easily available from many pharmacies and health food stores (8,9). In many studies, it has been shown that NAC increases the antioxidant capacity associated with its increase in GSH levels and decreases the proportion of reactive oxygen species (10-13). It is used in oxidative stress-induced treatments, reducing the glycemic index and reducing complications of diabetes. During diabetes, NAC prevented the toxic effects of oxidative stress and has been shown in many studies as a complementary therapy (14,15).

This study aimed to; determine the protective effects of NAC in the STZ-induced diabetes model and express these effects with oxidative stress parameters in the testicular tissue.

2. MATERIALS AND METHODS

Chemicals

All chemicals used in the analyzes were obtained from Sigma and Merck.

Experimental procedure

Animals

The animals taken for the experiment were obtained from a legal seller, Saki Experimental Animals. The permission to use the animals was given by Giresun University (Giresun University Experimental Animals Local Ethics Committee: 23.09.2020/E-45965).

Treatment

Experimental animals were divided into four groups as control, DM, DM+NAC and NAC. Animals that were fasted overnight were administered sodium citrate to induce diabetes. Blood glucose levels were determined with a blood sample taken from the tail vein before and after the application. Blood glucose levels above 250 mg/dL were considered as patients. Animals in groups 3 and 4 remained diabetic for one month. After this period, they were treated with NAC for one week. One day before the end of the experiment, fasting blood glucose was measured in the fasting animals and they were sacrificed. (IP ketamine hydrochloride (80 mg/kg) and xylazine hydrochloride (10 mg/kg)). Biochemical analyzes were performed on testes removed after sacrification.

Biochemical investigation

Lipid peroxidation (LPO) determination

The malondialdehyde (MDA) level in the testis was determined according to the spectrophotometric method of Buege and Aust (1978) as an indicator of lipid peroxidation, (16).

Total glutathione (GSH) determination

Glutathione was measured in testicular tissues according to Elman's method (17).

The NOx Level

NOx levels in the samples were determined by the spectrophotometric method of Miranda et al. (2001) (18).

Superoxide dismutase (SOD) activity

SOD activity in the testis was determined using the method of Sun et al. (1988). (19).

Catalase (CAT) activity

The method developed by Aebi (1984) was used to determine CAT activity (20).

Statistical analyses

Statistical analysis was performed using SPSS (version 22.0). All values are expressed as mean ± SD; $P < 0.05$ was considered significant.

3. RESULTS

Antioxidant system enzymes were measured and determined in testicular tissues in order to evaluate the mechanisms of oxidative and antioxidative systems. Results are shown in Table 1, Figures 1 and 2. In the STZ-induced diabetes model, it was found that the LPO level of the diabetic group increased compared to the control group($p<0.05$). In contrast to, it is seen that the applied NAC brought this increase to a level close to the control group. In addition, in the Diabetes + NAC group, diabetes induced by STZ, was almost as effective as the NAC group (Figure 1). Likewise, we determined that other oxidative stress parameters, GSH level, CAT, SOD and NOx activities also decreased with STZ administration. NAC application has increased this decrease significantly ($p<0.05$). On the other hand, it increased the enzyme activities up to the control level thanks to NAC in the diabetic NAC group. In line with these results; NAC given to testicular tissues showed protective properties against diabetes.

Table 1: Effects of NAC and healthy groups on the amount of GSH and LPO, activities of SOD, NOx and CAT in streptozocin-induced diabetes rat testicular tissue.

Treatments	N	Amount of MDA (nmol/g tissue)	Amount of GSH (nmol/mg tissue)	CAT Activity (mmol/min/mg tissue)	SOD Activity (mmol/min/mg tissue)	Amount of NOx (nmol/g tissue)
Control (Healthy)*	6	17.34±0.92*	7.18±0.22*	8.82 ± 0.16*	48.05±6.29	51.44±6.38*
Diabetes	6	32.12±2.04	2.15±0.43	4.36±0.43	43.37 ±5.21	19.45±2.32
NAC (N-acetyl systein)	6	15.33±0.36*	6.23±0.07*	7.48±0.14*	49.84 ±5.49	48.65±5.16*
Diabetes+ NAC	6	21.17±0.68 *	4.89±0.19*	6.76±0.38*	44.95±7.16	41.29±4.57*

Means in the same column by the same letter are not significantly different to the One-way ANOVA ($p<0.05$).Mean damage index ± SE of six animals in each group.

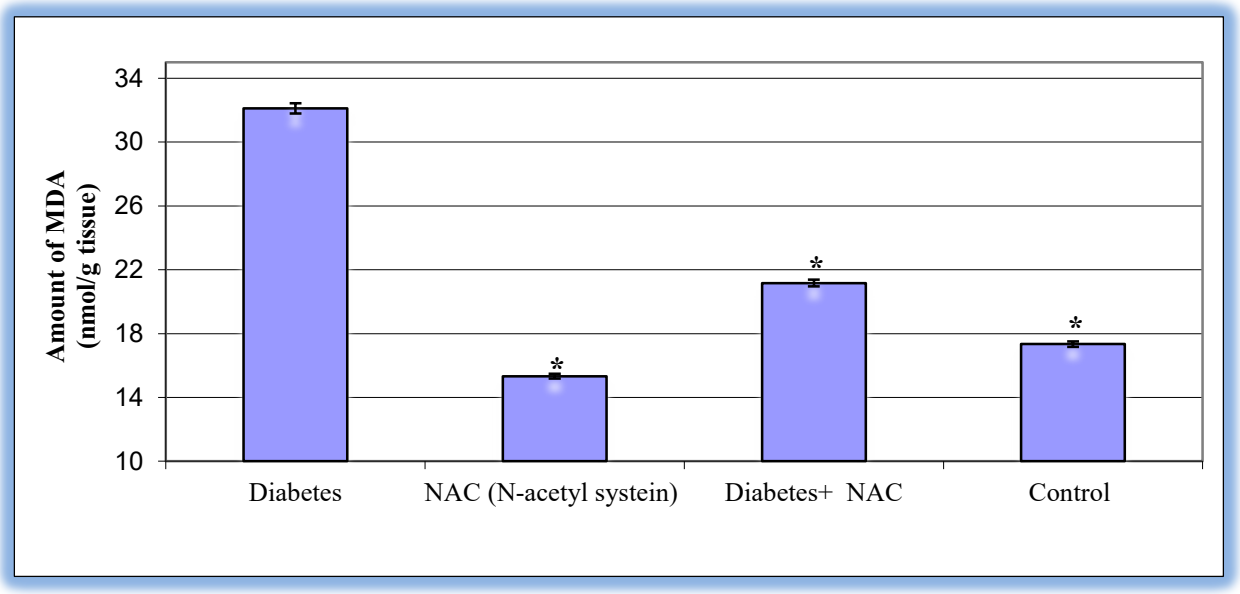

Fig. 1. Effects NAC on the amount of malondialdehyde (MDA) in rat's streptozocin-induced diabetes model in rat testicular tissues. Means in the same column by the same letter are not significantly different to the One-way ANOVA.

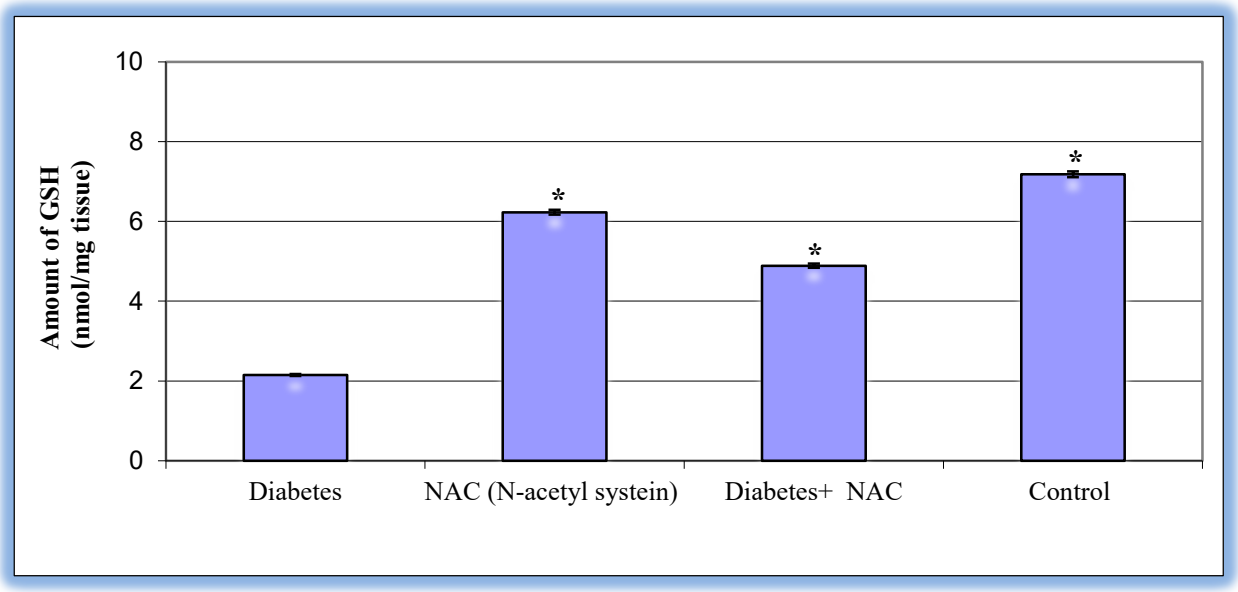

Fig. 2. Effects NAC on the amount of glutathione (GSH) in rat's streptozocin-induced diabetes model in rat testicular tissues. Means in the same column by the same letter are not significantly different to the One-way ANOVA.

4. DISCUSSION

Diabetes Mellitus (DM) is an endocrine disease that develops as a result of insulin deficiency or insulin resistance and manifests with hyperglycemia and glycosuria, and can cause acutations (21). Considering the studies conducted in recent years, the most important factor in the development of complications caused by diabetes is oxidative stress caused by the imbalance between free radical formation and antioxidant defense mechanism (22). It is known that oxidative stress plays an important role in the pathophysiology of male reproductive dysfunction and anomalies (21). Pharmacological treatment of diabetes is based on hypoglycemic drugs and insulin. However, recently, scientific studies have been carried out on traditional and alternative treatment methods for diabetes treatment. Antioxidants are thought to have significant effects on correcting oxidative stress, protein glycation and glucose metabolism, which are likely to be impaired in diabetes (23).

In experimental studies, the diabetes model in animals is created by virus, spontaneous or chemical agents. STZ is one of the chemical agents used for this purpose and is often used to model Type I diabetes (24). Especially in rat testes, it has been reported that a decrease in testicular weight, deterioration in the seminiferous tubule structure, atrophy in the seminiferous tubules and a decrease in spermatogenic serial cells have been reported [(25-28).

Free radicals cause oxidative damage to lipids, proteins and nucleic acids in the cell structure and damage DNA by disrupting the structures within the cell. Increased free radical levels in the body can lead to various diseases such as diabetes, coronary diseases, cancer, liver damage, cataracts (29,30). In addition, mitochondrial DNA mutations associated with oxidative stress have been reported in diabetic tissues (28). Damage to the mitochondria caused by oxidative stress can cause a decrease in the energy level required for sperm development (31,32). On the other hand, the antioxidants are molecules that prevent the destructive effects of free radicals and prevent reactions that can cause many diseases. In this study, NAC, one of the commonly used antioxidant agents and

known as glutathione storage, was used. NAC is often used as an antioxidant because it has sulfhydryl groups that scavenge free radicals. As it plays a role in detoxification, it protects the cell and cell components against oxidative stress. One of the most important non-enzymatic endogenous antioxidants is glutathione (33). Glutathione is a cysteine-containing tripeptide, the most abundant non-protein endogenous thiol in cells. In oxidation-reduction reactions, the mutual conversions between disulfide and sulfhydryl groups are used to destroy H_2O_2 from the cell before causing cellular damage. These reciprocal rotations are achieved by reducing and oxidizing the sulfur-containing compound GSH. Tissue GSH level is not only regulated by the enzymes involved in the synthesis, it is also very important that amino acids containing thiol are sufficient (33,34). NAC enhances the natural antioxidant defense by increasing the GSH concentration in cellular reduction. As shown in our study, the GSH level in rats with diabetes induced by STZ is quite low compared to the control group (Table 1 and Figure 2). However, with the applied NAC, GSH level was increased close to the control in both the diabetic + NAC group and the NAC group. Therefore, this decrease shows the oxidative stress and H_2O_2 in the tissue. In the present study, it may be suggested that because of the conversions between sulfhydryl and disulfide groups, NAC reduced oxidative stress. The results obtained are in line with the findings in the literature.

Free radical levels are difficult to measure in vivo due to their high reactivity, short half-life and low concentration. Therefore, secondary reaction products of oxidative damage such as MDA and lipid peroxides, which are indicators of lipid peroxidation, are used in clinical practice to determine the damage. MDA is the end product of lipid peroxidation, a type of aldehyde containing three or more double bonds, formed by the peroxidation of fatty acids. Therefore, measurement of the amount of MDA reflects the degree of lipid peroxidation in tissues. MDA causes the formation of superoxide anion and hydrogen peroxide by causing molecular oxygen depletion. These products are known to cause damage to cells and tissues. Since MDA in biological environments is both free and bound to sulfur (SH) and amine (NH_2) groups of macromolecules such as protein and nucleic acids, total (free and bound) MDA is generally evaluated (35). The MDA formed causes the cross-linking of the compounds in the membrane by affecting the ion exchange through the cell membranes. It causes the enzyme activity to change by decreasing the ion permeability (36). In diabetes studies, it has been reported that MDA levels of diabetic rats increased in parallel with the increased oxidative stress and lipid peroxidation compared to the control groups (37,38). Likewise, in the current study, MDA level increased in testicular tissues and cellular damage occurred in diabetes induced by STZ. The cellular repair was achieved through the NAC treatment which reducing this increase to the control level (Table 1 and Figure 1). The findings are consistent with the studies in the literatüre (35-38).

Other antioxidant enzyme systems that neutralize superoxide and hydrogen peroxides in tissues are superoxide dismutase and catalase enzymes. While the most important enzyme that deactivates superoxides is SOD, the enzyme that converts H_2O_2 to H_2O and removes the damaging effect of H_2O_2 with weak radical character is catalase. Eliminates the damaging effect of the weak radical H_2O_2 (39). However, H_2O_2 is considered one of the reactive oxygen species and plays an important role as a radical source. It can react with superoxide in the presence of transition metals such as H_2O_2, Fe^{+2} and Cu^{+2} to form a highly reactive hydroxyl radical (40). Therefore, tissues have antioxidative mechanisms to regulate the amount of H_2O_2. It is natural that there are increases in the activity of the CAT enzyme in order to reduce the amount of H_2O_2, which becomes excessive in tissue damage and traumatic situations. In the diabetes model, the decrease in catalase enzyme activity and increase with NAC application is due to the adjustment of the amount of H_2O_2. Likewise, the increase in SOD activity is because it tries to neutralize hydrogen peroxides. Therefore, the results are consistent with the literature (Table 1) (39-40).

Nitric oxide is a molecule that can play both a prooxidant and an antioxidant role (41,42) suggested that nitric oxide may be involved in the etiology of Type I diabetes. Accordingly, macrophages activated for an autoimmune reason cause damage to the islet cells of the pancreas by

releasing large amounts of NO. This feature has been investigated on experimental diabetes models in animals and it has been reported that the damage to the pancreas is reduced by applying NOS inhibitors (43). In normal physiological conditions, NO released from the endothelium plays a role in many processes such as vascular tone, coagulation and inflammation. Endothelial damage is among the most important reasons for the decrease in NO levels in diabetes. Hyperglycemia activates the production of Protein Kinase C (PKC), which causes the production of advanced glycosylation products. PKC activation causes endothelial dysfunction by causing an increase in oxygen radicals. NADPH is one of the cofactors of the nitric oxide synthase enzyme. Under physiological conditions, NADPH is supplied from the pentose phosphate pathway. Since this pathway is inhibited in hyperglycemia, it will again cause a decrease in NO synthesis [44]. Considering the results of our study, the decrease in NO level in the diabetes group can be explained by the increase in destruction or decrease in its production. The reduction in nitric oxide level was significantly increased in both groups with the applied NAC.

Conclusions

NAC therapy reduces testicular oxidative stress induced by diabetes and supports the antioxidant system. This result may suggest a promising alternative treatment for the male reproductive system disorders that develop with diabetes.

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The author's contribution

A.K: The care of the animals, drug administration, pharmacological support,

O.A.B: The biochemical analysis, statistics of data and interpretation of results

Conflict to interest

All authors declare that there are no conflicts of interest.

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