Lycopene prevents experimental priapism against oxidative and nitrosative damage

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Introduction

Priapism is a persistent and often painful penile erection in the absence of sexual stimulation1,2. Although it has rarely occurred in domestic animals, it is a problem for human3,4. The etiology of priapism related to use of some pharmacological agents such as antipsychotics and recreational drugs2,5. Also it can be associated with hematological disorders, metabolic disorders, trauma, tumors, neurological disorders and bites of the spider and scorpion2,6. Firstly, priapism can cause progressive fibrosis, edema and drying of the erectile tissue and then it can lead to erectile dysfunction2. Previous studies suggested that neuronal nitric oxide (NO) levels increased during the priapism4,8. Also, it was demonstrated that abnormally high NO levels can result in the formation of reactive oxygen species (ROS) leading to oxidative stress in tissue and reproductive system. This situation may cause severe infertility after priapism treatment. Therefore, it follows that antioxidant therapy may prevent side effects of priapism in the reproductive system.

Lycopene is a main carotenoid and is the most potent antioxidant among the various common carotenoids9. It is synthesized by microorganisms and plants red fruit and vegetables including tomatoes, watermelons, pink-grapefruits, apricots, papaya, guava and rosehip10,11. Humans and animals do not synthesize lycopene and, thus, depend on dietary sources mainly tomatoes12. Recently, many studies reported the beneficial effects of lycopene against cancer such as prostate and toxicities of antineoplastic agent (e.g. nephrotoxicity, hepatotoxicity) due to its highly efficient antioxidant and free radical scavenger properties13,15. The antioxidant activity of lycopene is assayed by inhibition

Abstract. – OBJECTIVE: Priapism is a persistent and often painful penile erection in the absence of sexual stimulation. It can cause progressive fibrosis, edema and drying of the erectile tissue and then it can lead to erectile dysfunction. Previous studies suggested that, neuronal nitric oxide levels increased during the priapism. High NO levels can result in the formation of reactive oxygen species (ROS) leading to oxidative stress in tissue and reproductive system. The aim of this study was to evaluate oxidative and nitrosative effects caused by priapism in cavernosal tissue and serum, and determine beneficial effects of lycopene on ischemic priapism.

MATERIALS AND METHODS: 32 rats were randomly divided into four groups and the first group being as the control. In the second group, experimental ischemic priapism was formed for an hour and then 1 hour reperfusion was provided. In the third group, lycopene was intraperitoneally given at the dose of 10 mg/kg. In the fourth group, lycopene were administered to rats with experimental priapism.

RESULTS: Priapism caused a significant increase in TBARS (thiobarbituric acid reactive substances) and NO levels and a significant decrease in the levels of GSH, CAT, GPx and SOD in serum and cavernosal tissue of rats. However, lycopene significantly increased GSH, CAT, GPx and SOD levels but decreased formation of TBARS production and NO in rats with priapism.

CONCLUSIONS: Our findings indicated that ischemic priapism lead to significant oxidative and nitrosative damage in cavernosal tissue and serum samples of rats. However lycopene treatment eliminates these negative effects induced by priapism. For this reason, we suggested that lycopene may be used in the treatment of priapism.

Key Words: Priapism, Lycopene, Oxidative stress, Nitrosative stress, Cavernosal tissue.

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of thiobarbituric acid reactive substances (TBARS) formation and induction of antioxidant defense system. Atessahin et al determined that lycopene prevents oxidative damage in testis tissue of rat. Similarly, many authors claimed that prevention of oxidative stress by lycopene results in chemotherapeutic effect against many cancer types such as prostate. Since oxidative stress is one of the important results of priapism, it is, therefore, reasonable to consider that lycopene may play a role in the cellular defenses against oxidative stress induced by priapism.

Thus, in the current study, we aimed to determine of oxidative and nitrosative damage caused by ischemic priapism in serum and cavernosal tissue and to find out whether or not lycopene has any beneficial effect against priapism in adult rats.

**Materials and Methods**

**Chemicals**

Lycopene 10% FS (Redivivo TM, Code 7803) was obtained from DSM Nutritional Products (Istanbul, Turkey). (Warwickshire, UK). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

**Animals**

Thirty two healthy adult male Spraque-Dawley rats (between 2-3 months old and 250-300 g. in weight) obtained from the Experimental Animal Institute, Malatya-Turkey for this experiment. Animals were kept in sterilized polypropylene rat cages, in 12-h light-dark cycle, at an ambient temperature of 21°C. Diet and water for them were given ad libitum. Experiments were performed based on Animal Ethics Guidelines of Institutional Animals Ethics Committee.

**Experimental Protocol**

All operations were performed under sterile conditions. The animals were anesthetized with xylazine (1 mg/kg ip.) and ketamine injection (50 mg/kg, ip) during the ischemic priapism and reperfusion period. Priapism was accomplished with the modified method described by Ulucak et al and Sanli et al. Briefly, a 50-cc syringe was used for the vacuum erection device and before application of vacuum to the penis, a constriction band, which was cut from 16 Fr Foley catheter in 2 mm slices, was loaded around the tip of the vacuum erection device. Then, the tip of the syringe was placed to the base of the penis and withdrawn gently to induce erection in the rat penis. After induction of erection in sufficient grade, the constriction band was placed to the base of the penis by slipping off the syringe (Figure 1).

The animals were divided into four groups with each containing eight rats. The first group was kept as the control group and given only distilled water as carrier. In the second group, ischemic priapism was formed during 1 hour. Then, the band was removed from the base of the penis and the penile tissue was allowed to reperfuse for one hour. In the third group, lycopene was injected intraperitonally (10 mg/kg) to rats without priapism. In the fourth group, lycopene was administered at 30th min of priapism period and then reperfusion was provided for one hour in this group. End of the reperfusion period, the animals were sacrificed and cavernosal tissues were immediately removed and dissected over ice-cold glass. The homogenization of cavernosal tissues was carried out in a teflon glass homogenizer with 150 mM KCl (pH 7.4) to obtain 1:10 (w/v) dilution of the whole homogenate. Blood samples were collected from the left ventricle with an injector under anesthesia. Sera were obtained by centrifugation (3000 g, 20 minutes, at 4°C) of whole blood. Tissue and serum samples were stored at –50°C in a deepfreeze until analysis.

**Biochemical Assay**

The levels of homogenized tissue and serum TBARS, as an index of lipid peroxidation, were determined by thiobarbituric acid reaction using the method of Yagi. The product was evaluated spectrophotometrically at 532 nm and results are expressed as nmol/g tissue and nmol/ml. The reduced
glutathione (GSH) levels content of the testis homogenate was measured at 412 nm using the method of Sedlak and Lindsay\textsuperscript{21}. The GSH level was expressed as nmol/ml. SOD (superoxide dismutase) activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction due to $O_2^-$ generated by the xanthine/xanthine oxidase system\textsuperscript{22}. One unit of CuZn-SOD activity was defined as the amount of protein causing 50% inhibition of the NBT reduction rate. The product was evaluated spectrophotometrically at 560 nm. Results are expressed as IU/mg protein. Catalase (CAT) activity of tissues was determined according to the method of Aebe\textsuperscript{23}. The enzymatic decomposition of H$_2$O$_2$ was followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit time was used as a measure of CAT activity. Tissue protein content was determined according to the method developed by Lowry et al using bovine serum albumin as standard\textsuperscript{24}.

**Determination of NO**

Serum nitric oxide (NO) level was determined by the evaluation of its stable oxidation products; nitrite (NO$_2^-$), and nitrate (NO$_3^-$). Serum nitrite levels were measured by the Griess reaction\textsuperscript{25}. Reduction of nitrate to nitrite was accomplished by catalytic reaction using cadmium. The nitrite produced was determined by diazotization of sulfanilamide and coupling to naphthylethylene diamine. Absorbance of this complex was measured at 545 nm. A standard curve was established with a set of serial dilutions of sodium nitrite. Linear regression was made by using the peak area from the nitrite standard. The resulting equation was then used to calculate the unknown sample concentrations. Results were expressed as moles per liter serum\textsuperscript{26}.

**Statistical Analysis**

The degree of significance was set at $p < 0.001$. One-way analysis of variance (ANOVA) and post hoc Tukey-HSD test were used to determine the differences among the groups. All the analyses were carried out using the SPSS/PC (Version 11.5; SPSS, Chicago, IL, USA) software package program. Data are presented as mean±standard error of means (SEM).

**Results**

The cavernosal tissue SOD, GSH, CAT and TBARS levels are given in Table I. SOD, CAT and GSH levels were significantly decreased, whereas TBARS levels were significantly increased in rats with priapism compared with control and other groups. There were no significant changes between the control and lycopene group. On the other hand, in lycopene+priapism group SOD, CAT, GSH levels were increased, and TBARS levels were decreased compared with that of priapism group. When given together with priapism, lycopene brought SOD, CAT, GSH and TBARS levels closer to the control level.

CAT, SOD, activities and TBARS, GSH levels in sera of rats are given in Table II. In priapism group, a significant decrease in SOD activities and GSH level was observed in the serum samples of rats compared with that of control and other groups. However, the TBARS and NO levels were significantly increased in the priapism group compared to other groups. In the lycopene+priapism group, TBARS, NO and GSH levels were near to control group value, but CAT and SOD activities remained unchanged compared with priapism group.

**Discussion**

Oxidative and/or nitrosative stress has an important role in the pathogenesis of ischemic priapism. It is largely known that the generation and...
activity of reactive oxygen (ROS) and nitrogen species (RNS) in the penis influence vascular homeostasis of this organ, with adverse effects exerted at cellular and molecular levels\(^1,8\). In this context, we thought that ROS scavengers and antioxidant agents could be beneficial in the clinical management of ischemic priapism. In this work we showed that ischemic priapism lead to oxidative and nitrosative damage in both cavernosal tissue and serum samples. On the other hand, lycopene treatment in ischemic priapism prevent oxidative and nitrosative stress and caused reoxygenation of the tissue.

TBARS, a degradation product from lipid hydroperoxidation, provides an index of peroxidation of lipids in biological tissues\(^27\). It has an important role in the etiology and pathogenesis of several diseases and disorders including ischemic priapism\(^7\). In our study the results showed that one hour priapism lead to a significant lipid peroxidation by increasing TBARS levels in both serum and corporal tissue in rats. Similarly, Uluocak et al\(^18\) determined that one hour priapism caused a significant increase in malondialdehyde level, a marker of oxidative stress, in rats. Besides, Kanika et al\(^2\) also observed that experimental priapism model lead to damage in corporal tissue proteins via increased lipid peroxidations. Previous studies\(^2,8\) confirm our results. Lycopene could decrease elevated TBARS levels induced by priapism and may prevent lipid peroxidations. In agreement with our findings, many previous studies clearly demonstrated that lycopene is a potent antioxidant and prevent lipid peroxidations\(^3,12,13,16\).

SOD, CAT, GPx (enzymatic) and GSH (nonenzymatic) are members of body antioxidant defense systems protecting against oxidative damage caused by free radicals production in normal physiological conditions and against molecules such as TBARS\(^28\). In the current study, it was shown that 1 hour priapism caused an attenuation in both enzymatic and nonenzymatic antioxidant defense systems via a decrease of SOD, CAT, GPx and GSH levels in rats. However, lycopene treatment significantly induced antioxidant capacity in serum and cavernosal tissue and could prevent oxidative damage caused by priapism. These results were confirmed by previous researches which showed that priapism lead to a decrease of antioxidant capacity in rats\(^19\). Besides several reports\(^29,30\) clearly indicated that the lycopene treatment increased antioxidant status in many diseases including cancer and toxication. Our results clearly showed that 1 hour priapism caused an imbalance in oxidant/antioxidant status and may lead to erectile dysfunctions due to cavernosal tissue damage in penis. However, lycopene can prevent oxidative damage in cavernosal tissue and may reverse the deleterious effects of priapism.

NO, known as endothelium-derived relaxing factor (EDRF), is an important cellular signaling molecule involved in many physiological and pathological processes\(^31\). The endothelium of blood vessels uses nitric oxide to signal the surrounding smooth muscle to relax, thus resulting in vasodilation and increased blood flow\(^32-34\). Also, NO has an important function in penile erection via coordination with corpus cavernosal smooth muscle relaxation\(^32\). In our study it was observed that priapism caused a significant increase in NO levels, but lycopene decreased elevated NO levels in serum. Elevation of NO may be due to ischemia conditions and elevated oxidative stress in penis. Similarly, Uluocak et al\(^18\) showed that priapism increase NO levels in serum and melatonin brought the levels of NO closer to normal levels. These findings agree with our findings.

### Conclusions

Oxidative and nitrosative stress play important role in many ischemic disorders such as pri-
apism. For that, if oxidative and nitrosative stress are prevented with any agent, the damage caused by priapism can be reversed. In this study, it was observed that an hour priapism caused ischemia and a significant oxidative and nitrosative damage via an increase of TBARS and NO levels and a decrease of SOD, CAT, GSH and Gpx levels in serum and cavernosal tissue in rats. Additionally, it was determined that lycopene eliminates oxidative and nitrosative damage in cavernosal tissue and serum due to their strong antioxidative properties. In conclusion because cavernosal damage may occur in erectile dysfunction in male, and therefore, quality of life may be impaired. Lycopene may be used for the clinical treatment of priapism.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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