

Protective role of lycopene on cisplatin-induced changes in sperm characteristics, testicular damage and oxidative stress in rats

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Abstract

The aim of this study was to investigate the possible protective role of lycopene on cisplatin (CP)-induced spermiotoxicity using quantitative, biochemical and histopathological approaches. Adult male Sprague–Dawley rats were randomly divided into four groups. The control group received physiological saline; animals in cisplatin group received only cisplatin; pre-treatment group received a 10-day of lycopene before administration of cisplatin while animals in post-treatment group received a 5-day of lycopene following administration of cisplatin. Cisplatin (7 mg kg^{-1}) was intraperitoneally (i.p.) injected as a single dose and lycopene (4 mg kg^{-1}) was administered by gavage in corn oil. Traits of reproductive organs; sperm characteristics, testicular histological findings, plasma testosterone levels and the testicular tissue oxidative status were determined.

Administration of cisplatin to rats decreased sperm concentration ($p < 0.05$) and sperm motility ($p < 0.001$), increased total abnormal sperm rates ($p < 0.05$) as compared with the control group. While a marked normalization was achieved only in sperm concentration with lycopene in pre-treatment group, significant normalizations were achieved in the sperm concentration, sperm motility, total abnormal sperm rates in post-treatment group. No significant differences in levels of testosterone were observed among all groups. An increase in testes malondialdehyde concentrations ($p < 0.05$) and glutathione peroxidase activities ($p < 0.001$) were detected while significant decreases in glutathione levels ($p < 0.001$) in cisplatin alone group when compared to control group. While pre-treatment with lycopene restoring only malondialdehyde concentrations, its post-treatment caused normalization in both malondialdehyde and glutathione levels when compared with the cisplatin alone group. On the other hand, significant increases were determined in GSH-Px activities in all experimental groups when compared with the control group.

Although the mechanism is not clear, the results from this experimental study suggest that the lycopene have a possible protective effect against cisplatin-induced spermiotoxicity, effect of giving lycopene after cisplatin being superior to the giving it before cisplatin.

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1. Introduction

Chemotherapy has improved the quality of life of cancer patients and given hope for remission. Despite successes, even the most effective anti-cancer drugs may

cause unwanted lesions [1–3]. Cisplatin (*cis*-diamminedichloroplatinum-II, CP) is a widely prescribed anticancer drug. Activity has been demonstrated against a variety of neoplasm's, particularly in the head and neck, testis and ovary, bladder and small-cell lung cancers. High doses of CP can damage different tissues such as kidney, liver and testes. Impairment of renal function is recognized as the main side effect of CP and the most important dose-limiting

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factor [4–7]. In addition, genotoxicity has been shown in different animals by chromosome aberration, sister chromatid exchange and micronucleus assays in bone marrow and spermatogonia. Owing to the relative spermotoxicity of CP, almost all the human patients show temporary or permanent azoospermia [1,8–12].

Pathogenesis of renal, hepatic, testicular damage following CP exposure is generally ascribed to oxidative damage. CP causes lipid peroxidation and decreases the activity of enzymes that protect against oxidative damage in these tissues. The administration of antioxidants such as Vitamin E, selenium, Vitamin C, carotenoids and others may protect against xenobiotic-induced damage [8,9,13–15]. Lycopene, an aliphatic hydrocarbon, is one of the 600 known naturally occurring carotenoids. Recently, lycopene in tomatoes has attracted attention due to efficient antioxidant properties and free radical scavenging capacity [16–19].

The aims of the present study were to investigate the effects of CP on sperm characteristics, plasma testosterone levels, histopathological and biochemical changes related to oxidative stress in testes and to examine the protective effect of lycopene on these parameters.

2. Materials and methods

2.1. Chemicals

Cisplatin (10 mg/10 ml, Code 1876A) was purchased from Faulding Pharmaceuticals Plc (Warwickshire, UK), lycopene 10%FS (Redivivo TM, Code 7803) from DSM Nutritional Products (İstanbul, Turkey). The other chemicals were obtained from Sigma (St. Louis, MO, USA).

2.2. Animals and treatments

This study used 24 healthy adult male Sprague–Dawley rats (8 weeks old weighing 190–250 g). The animals were obtained from the Fırat University Medical School, Experimental Research Centre, Elazığ, Turkey. They were kept under standard laboratory conditions (12-h light:12-h dark and $24 \pm 3^\circ\text{C}$) and fed standard commercial laboratory chow (pellet form, in the sack, Elazığ Food Company). Feed and water were provided ad libitum. Rats were divided into four groups of ($n=6$) for CP and or lycopene treatment. CP was injected intraperitoneally (i.p.) at 7 mg kg^{-1} , a dose that is well known to induce testicular toxicity in rats [1,6]. Lycopene was suspended in corn oil and administered by gavage at 4 mg kg^{-1} . This dose was guided by previous studies [16–18,20]. Isotonic saline (1.0 ml) and corn oil (0.5 ml) were the vehicles to administer CP and lycopene, respectively. Group 1 (control) received a single i.p. injection of saline following 10-day pre-treatment with corn oil. Group 2 received a single dose of CP following pre-treatment with corn oil for 10 days. Group 3 received a single dose CP following 10 days of lycopene pre-treatment. Group 4 received a single dose of CP before 5 days of lycopene post-treatment.

2.3. Sample collection

The rats in all groups were decapitated on the fifth day after CP injection. Blood samples were collected into tubes containing sodium oxalate (2%) and centrifuged at 3000 rpm for 10 min. The testes, epididymis, seminal vesicles and ventral prostate were removed, cleared of adhering connective tissue and assayed immediately. One of the testes was fixed in 10% formalin for histopathological examinations. Plasma and other testis samples were stored at -20°C until biochemical analyses.

2.4. Epididymal sperm concentration and motility

Spermatozoa in the epididymis were counted by a modified method of Yokoi et al. [21]. Briefly, the epididymis was minced with anatomical scissors in 5 ml of physiological saline, placed in a rocker for 10 min, and incubated at room temperature for 2 min. The supernatant fluid was diluted 1:100 with a solution containing 5 g sodium bicarbonate, 1 ml formalin (35%) and 25 mg eosin per 100 ml of distilled water. Total sperm number was determined with a hemocytometer. Approximately $10\text{ }\mu\text{l}$ of the diluted sperm suspension was transferred to each counting chamber and was allowed to stand for 5 min for counting under a light microscope at $200\times$ magnification. Sperm progressive motility was evaluated by an earlier method described by Sonmez et al. [22]. For this purpose, fluid was obtained from the caudal epididymis with a pipette and diluted to 2 ml with Tris buffer solution. The system was pre-warmed (35°C) and percentage of motility was evaluated visually at $400\times$ magnification. Motility estimations were performed from three different fields in each sample. The mean was used as the final motility score. The method by Evans and Maxwell [23] was used for determination of the percentage of morphologically abnormal spermatozoa after adapting the method for use in rats. According to this method, slides were prepared with India ink. A total of 300 sperm cells were counted on each slide under light microscope at $400\times$ magnification.

2.5. Biochemical assays

The testes was homogenized in Teflon-glass homogenizer with buffer containing 1.5% potassium chloride to obtain 1:10 (w/v) whole homogenate. Concentrations of malondialdehyde (MDA), as proceeding lipid peroxidation (LPO), were measured in the homogenate. Homogenates were centrifuged at $18,000 \times g$ (4°C) for 30 min to determine reduce glutathione (GSH) levels and glutathione peroxidase (GSH-Px) activities. MDA concentration was assayed according to a modified method of Ohkawa et al. [24] based on reaction with thiobarbituric acid and expressed as nmol g^{-1} tissue. Tissue GSH concentrations were measured by kinetic assay using a dithionitrobenzoic acid recycling method [25] and expressed as $\mu\text{mol g}^{-1}$ tissue. GSH-Px activity was determined as described [26] based on oxidation of reduced GSH

coupled to the appearance of NADPH by glutathione reductase measured 37 °C and 340 nm, expressed as $k\text{ g}^{-1}$, where k is the first-order rate constant. Protein concentrations were measured according to Lowry et al. [27]. Plasma testosterone levels (Immulite® Diagnostic Products Corporation, USA, catalogue no.: L2KTT) were determined by Chemiluminescence (CIA) method and expressed as ng ml^{-1} [28].

2.6. Histopathological examinations

Formalin-fixed testes were embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin (H&E) for evaluation by light microscopy. The diameter and germinal cell layer thickness of the seminiferous tubule (ST) from five different areas of each testicle were measured using an ocular micrometer in a light microscope, and the average size and thickness of seminiferous tubule was calculated.

2.7. Statistical analyses

All values were given as mean $\pm$ standard error of measurement (S.E.M.). To determine the differences among all groups in the whole parameters with the exception of sperm motility and abnormal sperm rate were performed one-way analyses of variance (ANOVA) and post hoc Duncan test being used the SPSS/PC program (version 12.0). The values in the sperm motility and abnormal sperm rate were compared with the Chi-square test (χ^2) by using the MINITAB® software package program (version 14.0).

3. Results

3.1. Organ weights and dimensions

The values of testis weights and dimensions, epididymis and accessory glands weights are shown in Table 1. A significant difference ($p < 0.05$) was found in both right and

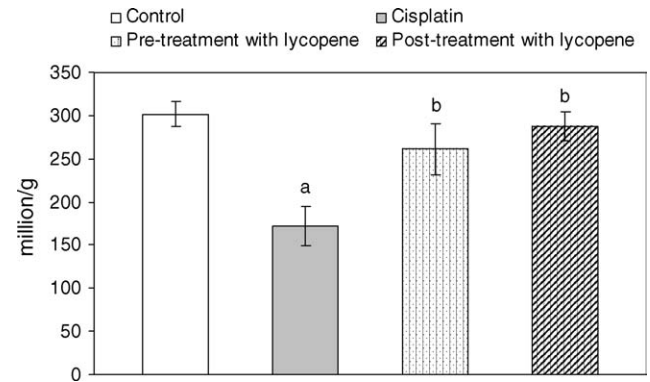


Fig. 1. Epididymal sperm concentration. $p < 0.001$ as compared with (a) control group and (b) cisplatin group.

left testes weights between control and other groups. There was a significant difference ($p < 0.05$) between the control and CP alone group with respect to the lengths of the right and left testes. When the weight of the epididymis was analyzed, all the groups receiving CP had significantly reduced epididymal weights (both right and left sides) as compared to control groups ($p < 0.05$). The weight of seminal vesicles and prostate tissue were also significantly lower in all experimental groups versus controls ($p < 0.05$).

3.2. Sperm characteristics and plasma testosterone levels

Epididymal sperm concentration (Fig. 1), sperm motility (Fig. 2), abnormal sperm rates (Fig. 3) and plasma testosterone levels (Table 2) are shown for CP and/or lycopene treatments. Lycopene prevented the CP-induced reduction in sperm concentration. The group receiving CP alone had significantly lower ($p < 0.05$) sperm concentration than the corresponding control group; however, this effect was absent in both lycopene groups and the post-treatment effect of

Table 1
Testes, epididymis, accessory glands weights and testes dimensions in all groups

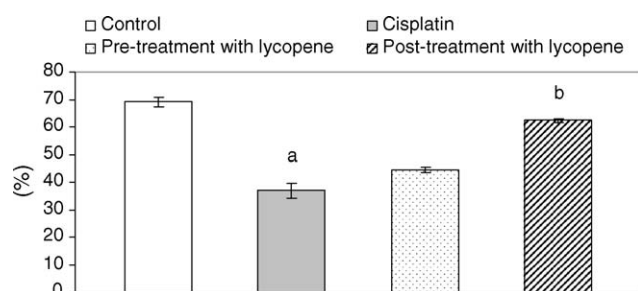
Parameters	Control	Cisplatin	Pre-treatment with lycopene	Post-treatment with lycopene
Testes weight (mg)				
Right	1240.0 $\pm$ 30.8 ^a	1060.0 $\pm$ 39.8 ^b	1110.0 $\pm$ 40.0 ^b	1130.0 $\pm$ 34.6 ^b
Left	1223.3 $\pm$ 31.9 ^a	1033.3 $\pm$ 54.2 ^b	1090.7 $\pm$ 52.5 ^b	1123.3 $\pm$ 45.8 ^b
Testes length (cm)				
Right	1.83 $\pm$ 0.02 ^a	1.66 $\pm$ 0.02 ^b	1.79 $\pm$ 0.04 ^{ab}	1.80 $\pm$ 0.02 ^{ab}
Left	1.83 $\pm$ 0.02 ^a	1.65 $\pm$ 0.08 ^b	1.81 $\pm$ 0.05 ^{ab}	1.78 $\pm$ 0.05 ^{ab}
Testes thickness (cm)				
Right	0.93 $\pm$ 0.06	0.89 $\pm$ 0.01	0.84 $\pm$ 0.02	0.91 $\pm$ 0.03
Left	0.91 $\pm$ 0.04	0.90 $\pm$ 0.00	0.91 $\pm$ 0.02	0.90 $\pm$ 0.00
Epididymis weight (mg)				
Right	363.3 $\pm$ 10.2 ^a	270.0 $\pm$ 18.6 ^b	286.7 $\pm$ 27.7 ^b	313.3 $\pm$ 25.0 ^{ab}
Left	366.7 $\pm$ 12.0 ^a	291.7 $\pm$ 12.5 ^b	293.3 $\pm$ 30.6 ^b	321.7 $\pm$ 20.1 ^{ab}
Accessory glands weight (mg)				
Seminal vesicles	595.0 $\pm$ 64.6 ^a	378.3 $\pm$ 32.3 ^b	461.7 $\pm$ 29.6 ^b	396.7 $\pm$ 34.9 ^b
Prostate	305.0 $\pm$ 13.4 ^a	218.3 $\pm$ 13.5 ^b	213.3 $\pm$ 09.5 ^b	248.3 $\pm$ 07.9 ^b

Different superscript letters (a–c) within same line showed significant ($p < 0.05$) differences among the groups.

Table 2

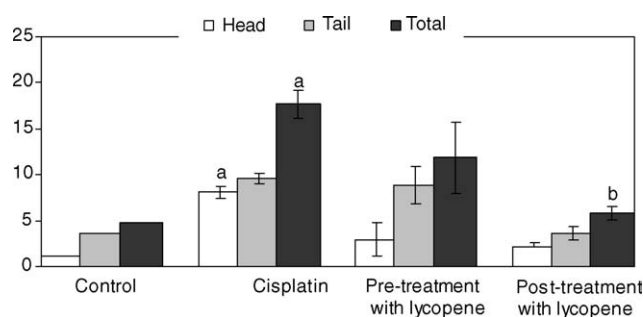
Testes tissue MDA, GSH and plasma testosterone levels and GSH-Px activities in all groups

Parameters	Control	Cisplatin	Pre-treatment with lycopene	Post-treatment with lycopene
Testes MDA (nmol ml ⁻¹)	1.29 ± 0.13	2.11 ± 0.45	1.55 ± 0.19	1.48 ± 0.43
Testes GSH levels (μmol mg protein ⁻¹)	0.16 ± 0.01 ^a	0.09 ± 0.01 ^b	0.09 ± 0.01 ^b	0.13 ± 0.01 ^a
Testes GSH-Px activities (μg protein)	2.96 ± 0.90 ^a	8.90 ± 2.17 ^b	12.17 ± 2.64 ^b	14.03 ± 1.30 ^b
Testosterone (ng ml ⁻¹)	2.99 ± 1.33	2.70 ± 0.91	0.49 ± 0.27	1.26 ± 0.78

Different superscript letters (a and b) within same line showed significant ($p < 0.001$) differences among the groups.Fig. 2. Sperm motility. $p < 0.001$ as compared with (a) control group and (b) cisplatin group.

lycopene was stronger. Effects of CP treatment on sperm motility had a similar effect pattern. CP alone had significantly lower ($p < 0.001$) sperm motility compared to the control group, but lycopene-treatment prevented the CP-induced reduction in sperm motility again the post-treatment being more effective (Fig. 2).

CP treatment did not cause significant tail abnormalities of sperm in any group. Only the CP treated group had significantly higher head abnormalities compared to the control group ($p < 0.05$). When total sperm abnormalities were analyzed, only CP treated animals had significantly higher level of abnormalities. Lycopene treatment prevented CP-induced total sperm abnormalities. While the pre-treatment group had a modest level of lower total sperm abnormalities, lycopene post-treatment had a comparable level of total sperm abnormalities to the control group, significantly lower than the only CP treated group (Fig. 3). No significant differences in levels of testosterone were observed in any group.

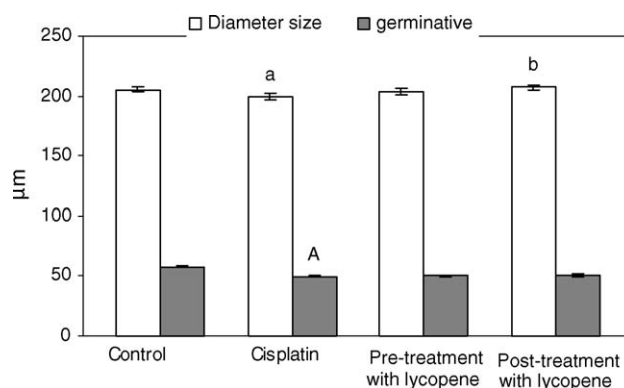
Fig. 3. Abnormal sperm rates. $p < 0.001$ as compared with (a) control group and (b) cisplatin group.

3.3. Lipid peroxidation and GSH-Px activity in testes tissue

The levels of testes MDA and GSH and the activity of GSH-Px are presented in Table 2. Although the CP alone group had significantly higher MDA levels compared to controls, both lycopene-treated groups showed a trend to lower MDA than CP alone, although not significant. There were significant decreases in the GSH levels in group CP compared to the control group. Although there was no significant difference between the CP-alone group and the lycopene-pre-treatment group with respect to the GSH levels, post-treatment with lycopene had significantly higher GSH levels. GSH-Px activities were significantly higher in all experimental groups compared to the control group.

3.4. Effect of cisplatin and lycopene treatment on testis histology

No degenerative or necrotic changes were detected in germinative and Leydig cells. The diameter of ST in the CP alone group appeared smaller than controls; however, the differences were not statistically significant in pre- and post-treatment with lycopene group compared to control group. Conversely, the thickness of the germinative cell layer at ST all of the administration groups was significantly ($p < 0.001$) smaller than control group. The diameter and germinative cell layer thickness of the ST are presented in Fig. 4.

Fig. 4. Diameter size and germinative cell layer thickness of seminiferous tubules in testes tissue. $p < 0.05$ as compared with (a) control group (b) cisplatin group (A) control group.

4. Discussion

Cisplatin-based chemotherapy results in damage of different tissues such as kidney, liver and testes. Recently, it has attracted more attention owing to impairment in testicular function following the chemotherapy [1,4,10,29,30]. Ishikawa et al. [29] reported that chemotherapy-induced gonadal toxicity and recovery of spermatogenesis are related to the type of drugs used, their total dose, and the duration of therapy. Some investigators [9–12] have reported that CP administration caused temporary or permanent azoospermia or oligospermia. The results of the present study indicated that CP administration at the dose of 7 mg kg⁻¹ resulted in both a significantly decrease in sperm concentration, sperm motility and increase in all of sperm abnormality rates in the rats.

Germinal epithelial damage leading to oligo- or azoospermia has long been a recognized consequence of treatment with chemotherapeutic agents, and there is also evidence of Leydig cells impairment following treatment. The mechanism of Leydig cells impairment following chemotherapy is not well known. There is no histological evidence of Leydig cell abnormalities on testicular biopsy after cytotoxic therapy. Chemotherapy may have a direct toxic effect on the Leydig cells, but there is also some evidence that germinal epithelial damage may indirectly affect the Leydig cell function. Some investigators observed that histological examinations in testes further indicate significant damage to Sertoli, Leydig and germ cell populations induced by CP [10–12,20,31]. Our findings, especially impairment in sperm characteristics and in histopathological findings are compatible with report of some workers and confirm the spermiotoxic effects of CP in rat testes.

Adverse effects of short and long-time CP treatment on testicular function have previously been documented in the rat, mouse and in human [10,11]. CP has also been shown to reduce intratesticular testosterone, and to decrease sperm motility and count. It has been reported that chemotherapy causes persistent dysfunction of Leydig cells, despite normal testosterone levels. When there is a complete Leydig cell failure, the LH concentrations rise and testosterone levels fall down [30,32]. Testosterone production, however, may be reduced in rats with seminiferous tubular damage, even though the total testosterone levels remain within the normal range [12,30]. On the other hand, CP is highly mutagenic, inducing chromosome aberrations in humans and rats [9,10]. We observed both a decrease in sperm motility and sperm concentration, and increase in abnormal sperm rates. Testosterone levels in the CP group were not significantly different than the control group, but appeared lower in pre- and post-treatment lycopene treated groups. Also, the histopathological findings in this group supported that CP does not induce complete Leydig cell failure. But, decreases in diameter size and germinative cell layer thickness of ST in group CP suggests that this drug caused spermiotoxicity. Thus, CP might partially suppress sper-

matogenesis, causing abnormalities in spermatozoa and ST atrophy.

Reactive oxygen species (ROS) and oxidative damage to biomolecules as a mechanism of drug cytotoxicity [13–15] may contribute to male infertility by reducing sperm function [1,6,32]. Bhat et al. [20] reported that CP (8 mg/kg) increased lipid peroxidation in rat testes. The loss of sperm function is attributed to peroxidation of unsaturated fatty acids in the sperm plasma membrane. Hydrogen peroxide, for example, directly affects sperm functions at fertilization in a dose- and time-dependent fashion. Testes lipid peroxidation determined TBARs production was reported to increase in several days after a single dose of CP [3,20,32]. In the present investigation, a significant increase in testes MDA concentrations and GSH-Px activities, decrease in GSH levels, were observed 5 days after CP administration.

Carotenoids are well known scavengers of singlet oxygen (¹O₂) and other excited species. During ¹O₂ quenching, energy is transferred from ¹O₂ to the lycopene molecule, converting it to the energy-rich triplet state. Trapping of other ROS, like OH•, NO₂• or peroxyxynitrite, in contrast, leads to oxidative breakdown of the lycopene molecule. Thus, lycopene may protect in vivo against oxidation of lipids, proteins and DNA [32,33]. Lycopene has been shown to have the highest antioxidant activity among the carotenoids in cell protection against hydrogen peroxide and nitrogen dioxide radicals. In addition, lycopene has been reported to attenuate oxidative stress and exert anticancer effects both in vitro and in vivo [16–19,34].

In the present study, we found 4 mg kg⁻¹ lycopene significantly improved sperm quality in animals treated with CP. To our knowledge, there is no use of lycopene in a similar protocol and therefore we cannot compare the dose regime used in this study with other(s). With regards to the difference between the duration of pre- and post-lycopene treatments, to avoid a possible time-dependent decline from CP-induced spermiotoxicity the post-treatment was limited to 5 days. Pre- and post-treatment with lycopene significantly inhibited the increase MDA and GSH depletion in the testis induced by CP exposure. Post-treatment with lycopene more significantly remediated testicular function than pre-treatment with lycopene. The possible explanation for the protective effects of lycopene against CP-induced increase in LPO its ability to react with the oxygen metabolites. Gupta and Kumar [35] evaluated that the effect of oral lycopene therapy in men with idiopathic infertility and found improvement in male infertility and especially in sperm characteristics. Silva et al. [6] suggested that pre-treatment with another carotenoid, bixin, reduced the total number of chromosome aberrations and inhibited the increase in lipid peroxidation induced by CP. Our study showed a significant decrease in sperm concentration, sperm motility and increase in abnormal sperm rates in CP treated rats and rescue of these parameters with lycopene, especially in the post-treatment group. A rational mechanism for potential anti-carcinogenic and anti-mutagenic effects of β-carotene and other carotenoids is free radical scav-

enger activity and suppression of oxidative DNA damage. We suggest that the protective effects of pre- and post-treatment lycopene against CP-induced abnormal sperm rates is attributed to the antioxidant properties of lycopene. These observations might also indicate that lycopene has protective and therapeutic effects on CP induced oxidative stress.

In conclusion, this study found that CP treatment markedly impaired testicular function in rats through a mechanisms that could be significantly rescued with pre-or especially post-treatment with lycopene. Our results indicate that lycopene may have a potential for clinical applications such as impairment of testicular function by cytotoxic chemotherapeutics.

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