Potential chemoprotective effect of melatonin in cyclophosphamide- and cisplatin-induced testicular damage in rats

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Objective: To evaluate the effect of melatonin on cyclophosphamide (CP)- and cisplatin-induced testicular toxicity with use of sperm parameters and biochemical and histopathologic approaches.
Design: Experimental study.
Setting: Vakif Gureba Hospital, Istanbul, Turkey.
Animals: Six-week-old adult male Wistar albino rats (N = 48).
Intervention(s): Cyclophosphamide was administered to rats by gavage at a single dose of 100 mg/kg body weight, only once. Cisplatin was injected intraperitoneally at single doses of 7 mg/kg/d for five consecutive days. Melatonin was both administered separately and coadministered with CP and cisplatin intraperitoneally at a dose of 10 mg/kg body weight.
Main Outcome Measure(s): Body and testicular weight, epididymal sperm characteristics, plasma T, and histopathologic structure of the testicular tissue were determined. Malondialdehyde (MDA) and reduced glutathione (GSH) levels and glutathione peroxidase (GSH-Px) activity were assessed in testicular tissue.
Result(s): Body and testicular weight, epididymal sperm count, motility and morphology, plasma T levels, the activities of GSH-Px, and GSH levels were significantly decreased whereas the level of MDA was significantly increased in rats of the CP and cisplatin group. Melatonin treatment increased GSH levels and GSH-Px activity, decreased MDA level in testicular tissue, and increased plasma T level. Cyclophosphamide and cisplatin caused irregular seminiferous tubules, reduction of seminiferous epithelial layers, significant maturation arrest, and perivascular fibrosis. Melatonin significantly improved histopathologic changes.
Conclusion(s): Melatonin may prevent CP- and cisplatin-induced testicular damage. (Fertil Steril® 2009;92:1124–32. ©2009 by American Society for Reproductive Medicine.)

Key Words: Melatonin, cisplatin, cyclophosphamide, oxidative stress, antioxidant, testicular damage, testis, rat

Cyclophosphamide (CP), a cytotoxic alkylating agent, is used extensively as an antineoplastic agent for the treatment of various cancers, as well as an immunosuppressive agent for organ transplantation, multiple sclerosis, systemic lupus erythematosus, and other benign diseases (1). However, its full clinical utility is limited because of several adverse effects including reproductive toxicity in humans and experimental animals (2). Cyclophosphamide treatment in patients is associated with oligozoospermia and azoospermia, as well as biochemical and histologic alterations in the testes and epididymis of rats and humans (3–6). Moreover, disturbance in gonadotropin secretion, testicular damage, and decreased plasma T levels are found in patients undergoing treatment with CP (7, 8).

Cisplatin is another highly effective antineoplastic DNA alkylating agent in treatment of various solid tumors including cancers of the testis, bladder, ovary, cervix, endometrium, head, neck, and lung (9). Its wide spectrum of clinical uses is limited, because cisplatin is known to cause adverse effects on testes, kidneys, peripheral nerves, and the inner ear, but the impairment of kidney function by cisplatin is recognized as the main side effect and the most important dose-limiting factor (10, 11).

The biochemical basis of CP and cisplatin toxicity is believed to relate to free radicals generated in these tissues. The cellular mechanisms by which CP and cisplatin cause testicular injury are poorly understood; however, numerous studies have shown that CP and cisplatin treatment are associated with induction of oxidative stress by generation of free radicals and reactive oxygen species (ROS) (12–14).When produced in excessive amounts, the ROS stimulate DNA fragmentation and a loss of sperm function associated with peroxidative damage to the mitochondria and sperm

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membrane. Further, spermatozoa are more susceptible to peroxidative damage because of a high concentration of polyunsaturated fatty acids and low antioxidant capacity (15).

The combination of CP and cisplatin with biologic compounds having antioxidant properties may contribute to the protection of cells and tissues against hazardous effects of ROS and other free radicals induced by these cytotoxic drugs. Melatonin, a secretory product of the pineal gland, is a well-known antioxidant and free radical scavenger (16, 17). The aim of the present study was to investigate the protective effects of melatonin on CP- and cisplatin-induced lipid peroxidative damage, histopathologic changes, and antioxidant status of the rat testis.

MATERIALS AND METHODS

Chemicals

Cyclophosphamide was purchased from Baxter Oncology GmbH, Frankfurt, Germany. Cisplatin was obtained from Mayne Pharma Plc, Warwickshire, United Kingdom, and melatonin was obtained from Sigma Chemical Co., St. Louis, MO.

Animals

In this study, 48 healthy, 6-week-old adult male Wistar albino rats (5–6 weeks old, 340–350 g) were used. The animals were kept under standard laboratory conditions (12 hours light, 12 hours dark, 26°C–28°C) for at least 1 week before the experiment, and those conditions were preserved till the end of the experiment. Animal cages were kept clean, and feed and water were given regularly every day. All experiments in this study were performed in accordance with the guidelines for animal research from the National Institutes of Health and were approved by the Local Committee on Animal Research.

Study Design and Treatment

The rats were randomly divided into eight groups consisting of six animals each. Cyclophosphamide was administered to rats by gavage in a single dose (100 mg/kg body weight), only once. Cisplatin was injected intraperitoneally (IP) in single doses (7 mg/kg/d) for five consecutive days. The doses of CP and cisplatin were selected according to previous studies that demonstrated significant damage in sperm parameters and testicular toxicity in rats (18, 19). Melatonin was dissolved in 5% ethanol and injected IP (10 mg/kg body weight). The doses of melatonin were 10 mg/kg/d body weight and were selected on the basis of the results of recent studies in which the antioxidant action of this agent was apparent (20, 21). Group 1 served as control and received a single-dose (IP) injection of isotonic saline solution (1 mL). Group 2 rats received melatonin in a single and only one dose. Group 3 rats were treated with CP alone. Rats in group 4 were treated with cisplatin alone. Group 5 rats received CP plus melatonin. Group 6 rats received cisplatin plus melatonin. The total dose of melatonin administered to rats was divided into two doses and injected 2 hours before and after the administration of CP or cisplatin. Groups 7 and 8 received CP plus 5% ethanol and cisplatin plus 5% ethanol, respectively (the melatonin vehicles).

Sample Collection

The testicular toxicity of CP can be detected within 2 weeks and that of cisplatin can be detected within 7 weeks after the treatment with a sufficient dose in rats (18, 22, 23). Therefore, 14 days after receiving CP and 40 days after receiving the last dose of cisplatin, rats were weighed and killed under anesthesia (ketamine 200 mg/kg body weight, IP). The abdomen was reached with an abdomen middle line cut, and then orchiectomy was performed. After weighing testes, one of the testes was stored at −80°C for enzymatic evaluation. The other testis was fixed with Bouin's solution for histopathologic examination. Blood samples were collected from the aorta and separated into plasma for biochemical examinations.

Biochemical Studies

Epididymal sperm analysis Epididymal sperm analysis was performed according to the World Health Organization (WHO) guidelines (24). Sperm count, sperm motility, and sperm morphology were assessed by optical microscopy, according to WHO criteria (24). One epididymis was minced fine with scissors in 4 mL physiologic saline solution at 37°C and then filtered through a piece of gauze. One drop of filtrate (sperm suspension) was placed on a slide, and a coverslip was placed over the droplet. At least 10 microscopic fields were observed at ×400 magnification, and the percentage of motile sperm was recorded. Sperm motility was expressed as a percentage of motile sperm of the total sperm counted.

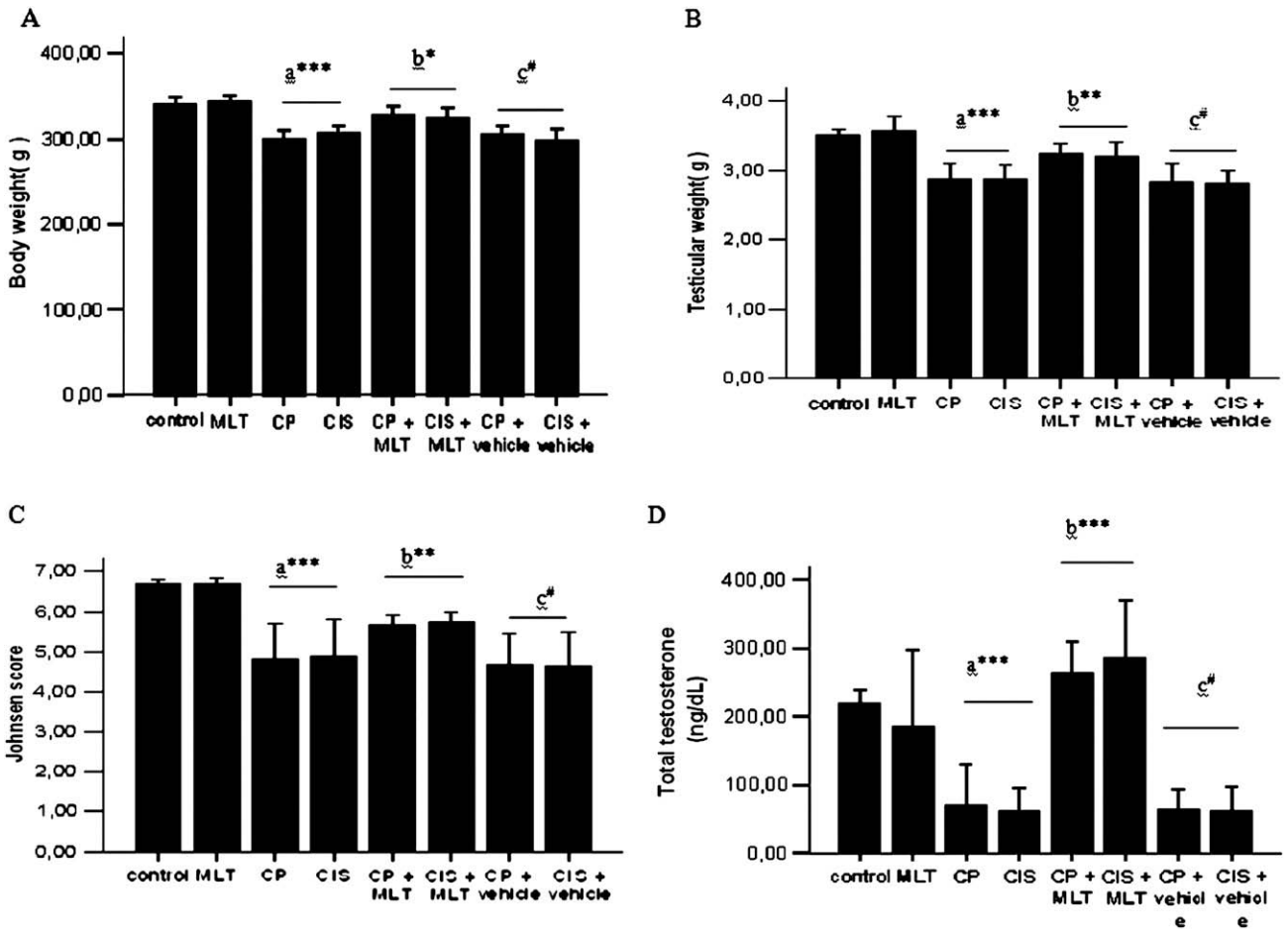
The epididymal sperm counts were obtained by the method described in the WHO manual (24). In brief, a 5-μL aliquot of epididymal sperm was diluted with 95 μL of diluent (0.35% formalin containing 5% $NaHCO_3$ and 0.25% trypan blue), and approximately 10 μL of this diluted specimen was transferred to each of the counting chambers of the hemocytometer, which was allowed to stand for 5 minutes in a humid chamber to prevent drying. The cells sedimented during this time and were counted with a light microscope at ×400. Sperm morphology was scored by determining the percentage of normal and abnormal forms after Diff-Quik staining (24).

Plasma T level The plasma T level was examined to evaluate chemotherapy-associated hypoandrogenism and was measured with the immunoenzymatic method according to the protocol described by Srivastava (25).

Lipid peroxidation level Testicular tissue was removed and homogenized in a Teflon-glass homogenizer with a buffer containing 1.5% potassium chloride to obtain 1:10 (wt/vol) whole homogenate. Malondialdehyde (MDA), formed as an end product of the peroxidation of lipids, served as an index

FIGURE 1

Groups: control, melatonin (MLT), CP, cisplatin (CIS), CP + MLT, CIS + MLT, CP + vehicle (CP + 5% ethanol vehicle for MLT), CIS + vehicle (CIS + 5% ethanol). (**A**) Body weight; (**B**) testicular weight; (**C**) Johnsen score; (**D**) total T levels in control, MLT, CP, CIS, CP + MLT, CIS + MLT, CP + vehicle, and CIS + vehicle groups. Control versus MLT ($P>.05$), control versus CP + MLT ($P>.05$), control versus CIS + MLT ($P>.05$), CP versus CIS ($P>.05$), CP + MLT versus CIS + MLT ($P>.05$). [a]Compared with control group; [b]compared with CP and CIS groups; [c]compared with CP and CIS groups. #$P>.05$; *$P<.05$; **$P<.01$; ***$P<.001$.



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of the intensity of oxidative stress. Malondialdehyde, referred to as a thiobarbituric acid–reactive substance, was measured with thiobarbituric acid at 532 nm in a spectrofluorometer, as described previously (26).

Glutathione peroxidase activity Glutathione peroxidase (GSH-Px) activity was measured according to Paglia and Valentine (27), by monitoring the oxidation of reduced nicotinamide–adenine dinucleotide phosphate (NADPH) at 340 nm. Enzyme units were defined as the number of micromoles of NADPH oxidized per minute and calculated with use of the extinction coefficient of NADPH at 340 nm (6.22×10^6/mol/cm). Results were reported as units per gram protein.

Glutathione level Reduced glutathione (GSH) was estimated by the method of Moron et al. (28), in which the color developed was read at 412 nm. Protein concentrations in all samples were measured by using the method of Lowry et al. (29).

Histopathologic Examination

Evaluation of spermatogenesis Testicular tissue (approximately 5–10 mg) was prepared for histologic examination. Semithin paraffin wax testicular tissue sections (4 μm thick) fixed in Bouin's solution were stained with hematoxylin and eosin (H & E) and examined under a light microscope (Olympus, Tokyo, Japan) at ×100, ×200, and ×400 magnification with use of standard techniques. To evaluate spermatogenesis, at least 40 seminiferous tubules were examined per slide, and each slide was scored with use of the Johnsen score (30), whereby seminiferous tubules are scored on a scale of 1 to 10,

TABLE 1

Effect of CP, cisplatin, and melatonin on epididymal sperm characteristics.

Parameters	Control	MLT	CP	CIS	CP + MLT	CIS + MLT	CP + V	CIS + V
Sperm count (10^6/mL)	155 ± 8.15	160 ± 7.8	79 ± 6.8[a]	77 ± 7.4[a]	111 ± 6.34[b]	110 ± 5.64[b]	78 ± 7.83[c]	76 ± 5.43[c]
Sperm motility (%)	70 ± 4.3	75.6 ± 5.4	38 ± 4.6[a]	35 ± 3.83[a]	54 ± 5.24[b]	55 ± 3.89[b]	36 ± 3.67[c]	37 ± 4.9[c]
Abnormal sperms (%)	8 ± 0.89	7.7 ± 0.67	40 ± 6.77[a]	39 ± 4.32[a]	19 ± 3.4[b]	21 ± 4.21[b]	39 ± 3.8[c]	40 ± 7.84[c]

Note: Values are expressed as mean ± SD for six rats in each group. MLT = melatonin, CIS = cisplatin, CP + V = cyclophosphamide + 5% ethanol vehicle for melatonin, CIS + V = cisplatin + 5% ethanol.

[a] $P<.001$, compared with control group.

[b] $P<.05$, compared with CP and CIS groups.

[c] $P>.05$, compared with CP and CIS groups.

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with tubules having complete inactivity scored as 1 and those with maximum activity (at least five or more spermatozoa in the lumen) scored as 10.

Evaluation of fibrosis To evaluate testicular fibrosis, specimens obtained from testis were embedded in paraffin, sectioned at 4-μm sections, and stained with Masson's trichrome. Specimens were scored after painting with, in brief, (−) no fibrosis, (+) fibrosis in <25% of total testicular tissue (mild), (++) fibrosis in 25% to 50% of total testicular tissue (moderate), (+++) fibrosis in >50% of total testicular tissue (serious) (31).

Statistical Analyses

Results of all groups were shown as mean values ± SD. Data were evaluated statistically with the Mann-Whitney *U* test. All results were compared one by one with other groups. $P<.05$ was accepted as a statistically significant value.

RESULTS

The biochemical and histopathologic results were similar for CP, cisplatin, and melatonin vehicle groups, and we decided to consider them without distinction and report only the CP and cisplatin groups.

Body Weight

Significant body weight loss was observed in the groups that received CP and cisplatin only compared with the control group ($P<.001$). Body weight was preserved by melatonin in the groups that received CP and cisplatin, and there was no statistically significant difference between the groups treated with chemotherapeutic agents plus melatonin and the control group ($P>.05$). There was no statistically significant difference between CP and cisplatin, when compared with each other in means of body weight lost ($P>.05$) (Fig. 1A).

Testicular Weight

Significant testicular weight loss was observed in the group that received CP and cisplatin only compared with the control group ($P<.001$). Coadministration of melatonin in CP- and cisplatin-treated rats preserved testicular weight. The testicular weight was preserved only slightly better in the CP plus melatonin group, compared with the cisplatin plus melatonin group. However, there was no statistically significant difference between the groups that received cisplatin plus melatonin and CP plus melatonin ($P>.05$) (Fig. 1B).

Epididymal Sperm

Compared with the control group, the sperm count and percentage of motile sperm were significantly decreased, whereas the percentage of abnormal sperm was significantly increased in rats treated with CP and cisplatin alone ($P<.001$). Treatment with melatonin significantly prevented

TABLE 2

Effect of CP, cisplatin, and melatonin on the activities of antioxidant enzyme and on nonenzymic antioxidant in the testis.

Antioxidants	Control	MLT	CP	CIS	CP + MLT	CIS + MLT	CP + V	CIS + V
GSH-Px (IU/mg protein)	8.5 ± 0.8	9 ± 0.3	5 ± 0.5[a]	4.5 ± 0.5[a]	9 ± 0.2[b]	9.1 ± 0.18[b]	4.9 ± 0.15[c]	5.1 ± 0.15[c]
GSH (μmol/mL)	1.45 ± 0.08	1.4 ± 0.06	0.9 ± 0.1[a]	0.95 ± 0.1[a]	1.54 ± 0.4[b]	1.76 ± 0.2[b]	1.1 ± 0.04[c]	0.8 ± 0.3[c]
MDA (nmol/mL)	35.7 ± 3.6	35.2 ± 4.8	57.8 ± 13.4[d]	47 ± 8[d]	38 ± 6[e]	30 ± 9.4[e]	61 ± 8.7[c]	50 ± 8.3[c]

Note: Values are expressed as mean ± SD for six rats in each group. MLT = melatonin, CIS = cisplatin, CP + V = cyclophosphamide + 5% ethanol vehicle for melatonin, CIS + V = cisplatin + 5% ethanol.

[a] $P < .01$, compared with control group.

[b] $P < .01$, compared with CP and CIS groups.

[c] $P > .05$, compared with CP and CIS groups.

[d] $P < .05$, compared with control group.

[e] $P < .05$, compared with CP and CIS groups.

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the decline of sperm quantity and quality in CP- and cisplatin-treated rats ($P<.05$) (Table 1).

Testosterone Level

Plasma T levels were significantly lowered in CP- and cisplatin-treated rats compared with the control group ($P<.001$); coadministration of melatonin in CP- and cisplatin-treated rats significantly increased plasma T level ($P<.001$) (Fig. 1D).

Glutathione Peroxidase Activity and GSH Level

The activity of GSH-Px and GSH level in testicular tissue of CP- and cisplatin-treated rats were significantly lower than those in the control group ($P<.01$). Treatment with melatonin significantly elevated the activity of GSH-Px and GSH level ($P<.01$) (Table 2).

Malondialdehyde Levels

Malondialdehyde levels in the testicular tissue were found to be significantly higher in CP- and cisplatin-treated rats than in the control group ($P<.05$). Treatment with melatonin prevented elevation of MDA levels significantly in CP- and cisplatin-treated rats ($P<.05$) (Table 2).

Spermatogenesis

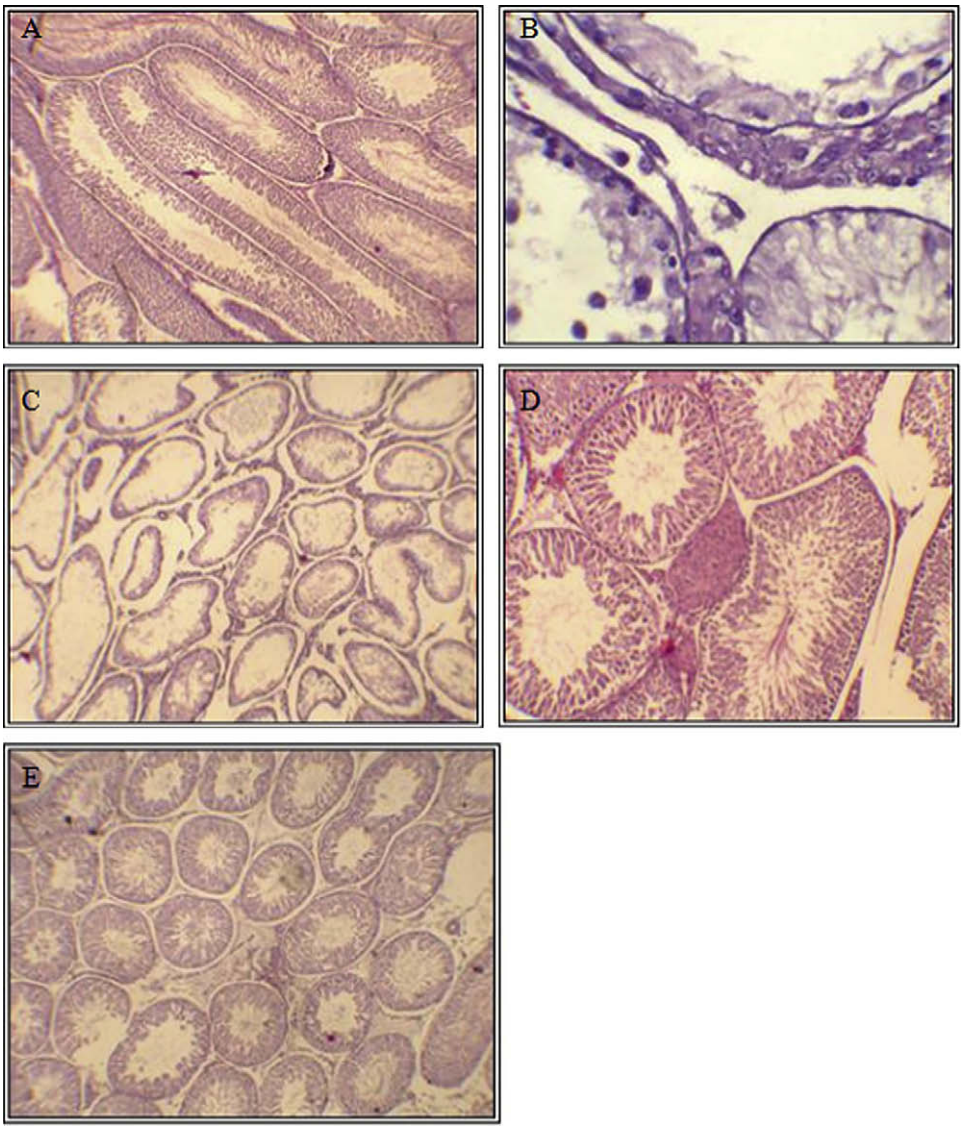
Rats in control and melatonin-only groups showed normal testicular architecture with the regular course of spermatogenesis and Sertoli cells (Fig. 2A). All rats treated with CP or cisplatin were characterized by a depletion of germ cells, irregular seminiferous tubules in which there were Sertoli cells only, and a few spermatogonia (Fig. 2B). Reduced seminiferous epithelial layers were found in numerous tubules, and irregular and diminished tubules containing a few germ cells also were seen (Fig. 2C). Significant maturation arrest and low Johnsen score were observed in the groups that received CP and cisplatin alone compared with the control group (Fig. 1C). Spermatogenesis was preserved significantly in the rats treated with CP plus melatonin and cisplatin plus melatonin. The morphologic characteristics of testes were comparable to those in control groups (Fig. 2D and E). There was no statistically significant difference between CP and cisplatin groups, when compared with each other in means of spermatogenesis.

Testicular Fibrosis

In the rats receiving CP and cisplatin mildly perivascular fibrosis and hyalinization of intertubular tissue were observed (Fig. 3A). Perivascular fibrosis and hyalinization of intertubular connective tissue were not determined in the control group and the groups that received melatonin together with CP and cisplatin (Fig. 3B).

FIGURE 2

Light microscopy of testes tissue from rat treated with CP and cisplatin alone or with melatonin. (**A**) Regular seminiferous tubules, normal intertubular gaps, and advanced-stage cells of spermatogenesis in control group (H & E, original magnification ×100). (**B**) Seminiferous tubules containing only Sertoli cells and spermatogonia and irregular seminiferous tubules in cisplatin-alone group (H & E, original magnification ×400). (**C**) Irregular and diminished seminiferous tubules containing a few germ cells in CP-alone group (H & E, original magnification ×100). (**D**) Highly regular seminiferous tubules showing spermatogenesis at level of spermatocytes in cisplatin plus melatonin group (H & E, original magnification ×200). (**E**) Regular and minimally diminished seminiferous tubules in CP plus melatonin group (H & E, original magnification ×100).



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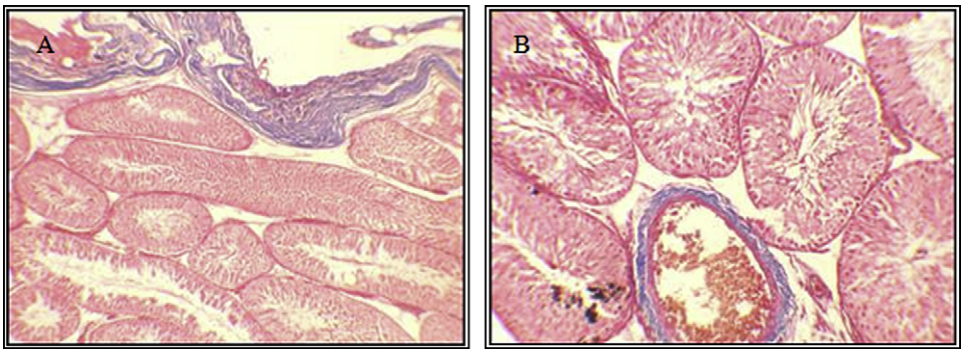
DISCUSSION

Cytotoxic chemotherapy has improved the survival rates in many conditions, particularly testicular malignancies. Treatment is, however, associated with significant morbidity, and testicular dysfunction is among the most common long-term side effects of this therapy (32). Many drugs used for chemotherapy, especially alkylating agents, have gonadotoxic effects, and their reproductive toxicity is associated with variables such as antineoplastic agent group, number of chemotherapeutic agents used, their total doses, treatment duration, and individual sensitivity (32, 33). The aim of the present multiagent chemotherapeutic protocols is to achieve a balance between the highest care results and the smallest side effects.

Effective anticancer therapy with cytotoxic drugs such as CP and cisplatin is limited by their reproductive toxicity

FIGURE 3

Light microscopy of rat testes from control (**A**) and cisplatin (**B**) groups. (**A**) Regular and fibrosis-free seminiferous tubules (Masson's trichrome, original magnification ×100). (**B**) Fibrosis in perivascular and interstitial area in cisplatin group (Masson's trichrome, original magnification ×200).



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that has been documented in the various experimental studies (2, 6, 34). Especially spermatogenic cells are targeted by these agents because of their high mitotic activity. It has been reported that male rats that received CP had a decrease in reproductive organ weights, impaired fertility, and altered growth and development of next generations (12, 35, 36). In the present study, administration of CP or cisplatin reduced testes weight when compared with control, confirming previous reports that CP and cisplatin decrease reproductive organ weights (22, 24). Because reductions in testis weight were due to marked parenchymal atrophy and histopathologic examination showed severe degeneration, necrosis, and reductions in seminiferous tubule and germinal cell thickness in the testes of rats treated with CP and cisplatin alone, the effects of these agents on the testis may be due to their specific toxic effects on the target organ and not the result of their general toxicity. We also observed body weight loss in rats treated with CP and cisplatin alone. The reduction in body weight may be due to malnutrition that occurs as a consequence of systemic toxic effects of CP and cisplatin. Melatonin prevented the reduction in both body and testis weight in the CP plus melatonin and cisplatin plus melatonin group rats when compared with CP and cisplatin alone. Plasma T levels were significantly lowered in CP- and cisplatin-treated rats in the present study. The decreased plasma T level in CP- and cisplatin-treated rats corresponds to the observation that CP inhibits testicular steroidogenesis (13), and the decreases in T level could be attributed to the impaired Leydig cells. Coadministration of melatonin in CP- and cisplatin-treated rats significantly increased the T level. The spermatogenic inhibition in CP- and cisplatin-treated rats indicated in the present study may be the result of lowered plasma T level. Besides hormonal alteration, the spermatogenic inhibition may also be due to the formation of free radical products in the testicular tissue as they exert a detrimental effect on spermatogenesis (13).

Oxidative stress and free radical–induced damages have been implicated in the etiology of several toxic effects caused by CP and cisplatin (37). Oxidative stress is induced by oxidant substances commonly known as ROS (38). The main highly reactive ROS that have potential implications in reproductive biology are the superoxide anion, the hydroxyl radical, and hydrogen peroxide (39). Normally, the balance between ROS produced by pro-oxidant and that scavenged by antioxidant is maintained, and cellular damage arises when this equilibrium is disturbed (40). On the other hand, it has been reported that various antioxidants or free radical scavengers including vitamin C, lycopene, and melatonin decrease significantly the testicular damage induced by CP or cisplatin (12, 19, 20). In the present study, administration of CP and cisplatin decreased significantly epididymal sperm concentration and motility, confirming several studies (20, 34). The increased abnormal sperm morphology and maturation arrest also were observed in CP- and cisplatin-administered rats. The testicular tissues are very sensitive to ROS effects. The oxidative damage to polyunsaturated fatty acids of cell membranes of mammalian spermatozoa has long been considered to result in the impairment of membrane permeability (15). This results in damage of germ cells, spermatozoa, and mature sperm (38, 39). In our study, decreased sperm count and reduction in sperm motility observed in rats treated with CP and cisplatin may be due to the spermatogenic cell death and the toxic effects of these agents on the flagellum. It also has been reported that adenosine triphosphate (ATP) is an energy source for sperm motility, and its availability may be a limiting factor responsible for loss of sperm motility in CP- and cisplatin-treated rats (15, 41). The beneficial effect of melatonin at the mitochondrial level has been described, which includes stimulatory effects of the indolent on ATP production (15). Significant increases in sperm count and motility and decreases in the number of dead and abnormal spermatozoa were observed in CP plus melatonin and cisplatin

plus melatonin group rats when compared with CP and cisplatin alone. These preventive and ameliorative effects of melatonin probably relate to its free radical scavenging ability and stimulatory effect of ATP production.

Increased morphologic defects and production of abnormal and dead sperms also may be due to the direct toxicity of CP and cisplatin, because cellular DNA is a primary target of CP and cisplatin in their antineoplastic and toxic activity (42). Melatonin prevented the CP- and cisplatin-induced increases in abnormal sperm. The rationale for the mechanism of the antimutagenic effects of melatonin is its ability to scavenge free radicals that cause oxidative DNA damage.

Additionally, spermatozoa are particularly susceptible to lipid peroxidation because of the high concentration of polyunsaturated fatty acids in their plasma membrane required to give the plasma membrane fluidity, which is needed for sperm motility and participation in the events associated with fertilization (43). Lipid peroxidation has been suggested to be closely related to CP- and cisplatin-induced testicular damage, and MDA is a good indicator of the degree of lipid peroxidation. In the present study, we observed a significant increase in MDA content of testicular tissue in rats treated with CP and cisplatin alone, and melatonin administration reduced MDA level.

Reduced glutathione is one of the most important molecules in the cellular defense against chemically reactive toxic compounds or oxidative stress. Decreased cellular GSH levels and capacity for GSH synthesis sensitize cells to certain drugs. Reduced glutathione synthesis is induced in cells exposed to oxidative stress as an adaptive process. Therefore, interest has been focused on compounds that act as antioxidants and are capable of stimulating GSH synthesis. In the present study, treatment with melatonin in part reserved the increase in the GSH levels. The present study also shows a decrease in activity of antioxidant enzymes, namely GSH-Px, as well as the level of GSH in rats treated with CP and cisplatin alone. The decrease in activity of the antioxidant enzymes may predispose the sperms to increased free radical damage, because GSH-Px has been considered the primary scavenger of H_2O_2 (15). In addition to scavenging free radicals, melatonin also reduced their generation (44). Additionally, the antioxidant actions of melatonin probably derive from its stimulatory effect on enzymic antioxidants such as GSH-Px (45).

In the present study, coadministration of melatonin with chemotherapeutic agents prevented the side effects and damage to testis induced by these drugs. However, melatonin also may decrease the efficacy of chemotherapy based on these agents. Many studies have shown that intracellular glutathione and nonprotein thiols, increased by melatonin, can quench platinum, which may cause resistance to cisplatin chemotherapy (46, 47). This is supported by our observation that rats receiving CP and cisplatin together with melatonin had less body weight loss, which is supposed to be one side effect of these chemotherapeutic agents. Another experimental study may be designed to evaluate whether melatonin decreases the efficacy of antitumor chemotherapy in a tumor model.

In conclusion, the findings of the present study demonstrate that oxidative stress caused by free radicals plays an important role in the development of CP- or cisplatin-induced testicular damage, and melatonin has a potent protective effect against the testicular toxicity of these agents. Although lacking tumor specificity, melatonin may be a potential therapeutic agent for impairment of testicular function that is induced by cytotoxic chemotherapies such as CP and cisplatin.

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