

Evaluation of Protective Effects of Gallic Acid on Cisplatin-Induced Testicular and Epididymal Damage

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Research Article

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Abstract

The current study explored the beneficial effects of GA against testis and epididymis toxicity induced by CP treatment. Male rats were into 4 groups (n=7). Control (saline, intraperitoneal), Cisplatin (a single dose of 8 mg/kg/day cisplatin, intraperitoneal), Gallic acid (50 mg/kg Gallic acid orally for 10 days) and Cisplatin+Gallic acid groups. Total number of spermatogonia, Sertoli, Leydig cell and the total volume of testis, seminiferous tubule, interstitial area, and germinal epithelial thickness and numerical densities of caspase-3, Bax, Bcl-2, 8-OHdG immunopositive cells were calculated. Histopathological examination of the testis and epididymis was performed. MDA and CAT levels are measured in the testis. Also, the testosterone level was measured in the serum of the rats. As a result, a significant decrease was observed in all stereological data, Bcl-2 immunopositive cell number, CAT, and serum testosterone levels in the testis compared to the CP group control group, while a significant increase was observed in the number of caspase-3, Bax, and 8-OHdG immunopositive cells and the level of MDA. However, GA significantly improved these parameters. Our study reveals that GA may improve CP-induced male reproductive toxicity by reducing oxidative stress, suppressing apoptosis and DNA damage, and restoring structural and functional deterioration.

Introduction

Cisplatin (CP) is a powerful neoplastic drug widely used to improve various cancer such as bladder, lung, and testis cancer (Azarbarz et al. 2020). The anti-tumor effect of CP, a DNA-alkylating molecule, prevents the replication and transcription of DNA by breaking the DNA double strands (Ahmed et al. 2011). However, CP has side effects such as ototoxicity, nephrotoxicity, neurotoxicity, testicular toxicity, as well as significant anti-tumor effects (Elrashidy and Hasan 2020). It has been determined that oxidative stress, inflammation, and ischaemic injury are pathological conditions caused by CP toxicity in non-target tissues (Eren et al. 2020). The testis has become the target of chemotherapeutic agents due to the occurrence of spermatogenesis and spermiogenesis, where testicular cell division and morphological changes occur, and these agents can easily damage the testis (Ceylan et al. 2020). The permanent side effects of CP cause disruption of the Sertoli cell and seminiferous epithelium, resulting in impaired spermatogenesis (Whirledge et al. 2015). CP causes Leydig cell dysfunction leading to impaired testosterone secretion (El-shafaei et al. 2018). In addition, CP causes epididymal toxicity resulting in decreased epididymal sperm count (Kohsaka et al. 2020). Thus, infertility occurs in parallel with CP application (Mercantepe et al., 2018). However, although the underlying mechanism of CP on the testis and epididymis is not fully understood, one of its basic mechanisms is estimated to be related to its induction of reactive oxygen species (ROS) production and the decrease in the level of testicular antioxidants (Eren et al. 2020). Thus, The use of natural products is gradually increasing in the treatment of diseases and protection of tissues against intoxication of toxic substances. Various cytoprotective, antioxidant and functional foodstuffs have been applied to reduce or prevent CP-induced toxicity (Kohsaka et al. 2020).

Gallic acid (GA), a polyphenol compound found in many vegetables and fruits such as bananas, strawberries, sumac, green tea, oak bark, exhibits antioxidant, anti-inflammatory, anticancer effects, antiviral (Zhu et al. 2019). GA, widely used in medicine, cosmetics, and foods, attracts the attention of researchers because of

these effects (Dehghani et al. 2020). The antioxidant effect of GA ensures that the functions of biological systems are carried out healthily, and the balance between the oxidant and antioxidant mechanism is maintained (Shruthi et al. 2018). Also, its anti-inflammatory effect prevents or reduces the expression of inflammatory molecules and thus can be used in the treatment of inflammatory diseases (Kim et al. 2006). Previous studies have reported that GA exhibits curative effects on CP-induced nephrotoxicity and bone marrow genotoxicity (Akomolafe et al. 2014; Shruthi et al. 2018; Dehghani et al. 2020)

It is very important to protect male reproductive health against CP toxicity in chemotherapy. However, no adequate study has been found on the effects of GA on cisplatin-induced testis and epididymis toxicity. Therefore, in our study, it was planned to investigate the mechanism underlying the effects of GA in CP-induced male reproductive toxicity in detail by stereological immunohistochemical and biochemical evaluation.

Material And Method

Chemicals and antibodies

Caspase-3 (sc-56048), Bax(sc-20067), Bcl-2 (sc-7382), 8-OHdG (sc-393871) were obtained from Santa Cruz Biotechnology. Gallik acid (CAS: 149-91-7) was obtained from Sigma Aldrich and Cisplatin was obtained from Koçak Farma company (İstanbul, Turkey). The HRP kit and other chemicals required for immunohistochemical examination were obtained from Thermo Fisher Scientific.

Experimental animals and drug treatment

The protocol of our study was confirmed by Van Yuzuncu Yil University Animal Experiments Local Ethics Committee (decision number: 2021/02–25) and all methods were performed to guidelines of the committee. In this study, a total of 28 adult male Wistar rats weighing 200 ± 20 g were used, and the animals were purchased from the Van Yüzüncü Yil University Experimental Animals Research and Application Center. Rats were randomly divided into four groups ($n = 7$). Control (C) group was received 0.5 ml saline by intraperitoneal (IP). CP group was applied single dose of 8 mg/kg/day CP (IP) on the 5th day of study. GA group was received 50 mg/kg GA by gastric gavage for 10 days. CP + GA group was received 50 mg/kg GA by gavage for 10 days and CP was received a single dose of 8 mg/kg (IP) on the 5th day of the study. The doses of CP and GA were designed according to a previous study (Tomar et al. 2017; Abarikwu et al. 2020). Rats were housed under standard conditions of temperature ($25 \pm 2^\circ\text{C}$), relative humidity ($50 \pm 10\%$), and 12 h light/12 h dark cycle. The animals were fed with a standard pellet diet (ad libitum).

Stereological analysis

To evaluate stereological parameters, testis was fractionated into fractions (f) by the systematic random sampling rules (Cahill et al., 1996). For this, the testis was divided into four equal parts ($f_1 = 1/4$). One of the four pieces of the testis was randomly selected and then embedded in paraffin. 4 μm thick sections were cut for stereological examination. The first section was taken randomly, and the next sections were taken every 50 steps ($f_2 = 1/50$). An average of 10–13 sections was taken. After the sections taken were stained with

Hematoxylin-Eosin (H-E), examined by light microscopy (Olympus BX53, Japan) using a camera (Olympus DP27, Japan) and microscope imaging systems (Olympus CellSens Entry, Japan).

Calculation of the total number of cells

The physical dissector counting method was used to calculate the total number of spermatogonia, Sertoli, and Leydig cells according to previous studies (Altındağ and Özdek 2021). The following formula was used to calculate the total cell number:

$$N = N_v \times V_{\text{final}}$$

Where N_v is the numerical density of the cell of interest (cells/unit volume) and V_{final} is the total volume of the testis (Gundersen 1986; Mayhew and Gundersen 1996).

Estimation of the total volume

The point-counting method of the Cavalieri principle was used to calculate the total volume of the testis, germinal epithelial volume, and interstitial space volume. For this, the total numbers of points hitting the all testis, germinal epithelial, and interstitial space were counted using the point-counting probe. The following formula was used to calculate the volume:

$$V_{\text{ref}} = \sum P \times a/p \times h$$

Where “V” is the volume of the structure, “ $\sum P$ ” is the total number of points hitting the structure, “a/p” is the area covered by one point, and “h” is the height of disector. To calculate the total volume of the tissue, the reference volume is multiplied by the fraction ratios (f1 and f2) and the shrinkage rate. After the histological tissue processing stages, the volume of tissue is usually shrunk. The testis shrinkage rate was calculated according to previous studies (Altındağ and Özdek 2021).

Estimation of the height of the germinal epithelium

The height of germinal epithelium was estimated according to previous studies (Altındağ and Özdek 2021). For this, the following formulas were used:

$$S_{V(\text{epithelium}/\text{ref})} = 2 \times \sum I / (\sum P \times l/p)$$

$$\text{The height of germinal epithelium} = V_{V(\text{epithelium}/\text{ref})} / S_{V(\text{epithelium}/\text{ref})}$$

Where “ $\sum I$ ” is the total number of intersection points of the luminal surface of the epithelium and the test lines, “ $\sum P$ ” is the total number of points hitting testis and “l/p” is the length of a test line. The total surface area is calculated by multiplying the surface density by the total volume (Noorafshan 2014; Altındağ and Özdek 2021)

Histological preparation

At the end of the experiment, rats were anesthetized by xylazine (10 mg/kg) and ketamine (50 mg/kg) IP. For stereological, immunohistochemical, and histological examination, the testis and epididymis tissues were removed by an incision made from the scrotal region and fixed in 10% buffered formalin. Testis and

epididymis were embedded in paraffin after tissue processing stages. Sections of 4 µm thickness taken from paraffin blocks were stained with hematoxylin-eosin (H&E).

The histological examination was made according to the Johnsen score (Johnsen, 1970) For this, 15–20 areas were evaluated by random sampling in 8–10 sections taken from each animal in the groups.

Immunohistochemical preparation

The Sections of 4 µm thickness taken from paraffin blocks were deparaffinized and dehydrated. After sections were incubated in 3% Hydrogen peroxide (H₂O₂), in citrate buffer (ph 6.1), in Ultra V Block, in antibodies of caspase-3 (1:50), Bax (1:50), Bcl-2 (1:50), and 8-OHdG (1:50), in Biotinylated Goat Anti-Polyvalent, and Streptavidin–peroxidase conjugate respectively. Diaminobenzidine (DAB) was used as a chromogen, and then stained with Mayer's hematoxylin. Cell numerical density was estimated by counting caspase-3, Bax, Bcl-2, and 8-OHdG positive cells using the physical dissector counting method, which is a stereological method by a light microscope (Olympus BX53, Japan) and cellSens Software imaging systems (Olympus, Japan). The formula below was used for this:

$$N_v = \frac{\sum Q^-}{\sum P \times a/\text{frame} \times h}$$

Where $\sum Q^-$ is the total number of immunopositive cells counted in all the dissector, $\sum P$ is the total number of counting frames, a/frame is the area of counting frame, h is the height of disector

Measurements of serum testosterone

At the end of the study, blood samples taken from the heart with an injector were centrifuged at + 4°C at 3000 rpm for 10 minutes. Serum testosterone was evaluated in an autoanalyzer (Abbott Architect ci16200,) using commercial kits (Abbott Architect 2nd Generation Testosterone Reagent Kit).

Measurement of MDA and CAT in testis

It was measured the activity of CAT to evaluate the antioxidant and was measured the content of MDA to determine the lipid peroxidation. To measure the oxidative stress-associated enzymes, testis tissues were homogenized by a homogenizer. After homogenates were centrifuged, analysis of CAT and MDA were evaluated by a spectrophotometric method according to the previous studies (Lartillot et al. 1988; Dubovskiy et al. 2008)

Statistical analysis

Statistical analyses were accomplished by using SPSS 21.0 software. Differences between groups were evaluated by the one-way analysis of variance (ANOVA) followed by the Tukey post hoc. $p \leq 0.05$ was accepted as statistical significant. All data were expressed as mean \pm standard deviations.

Results

Total number of testicular cells

As in the Table 1, the total number of spermatogonia, Sertoli, and Leydig cells were significantly decreased in rats of CP group compared to the control group, while these parameters were significantly increased in the CP + GA group compared to the CP group ($p < 0.05$) (Table 1).

The total volume of testicular parameters

Compared to the control group, total testicular volume and total seminiferous tubule volume were significantly decreased in the CP group ($p < 0.05$), while no significant difference was observed in the total interstitial area volume. However, compared to the CP group, a significant increase was observed in the total testicular volume and total seminiferous tubule volume in the CP + GA group ($p < 0.05$) (Table 1).

Height of germinal epithelium

Height of germinal epithelium significantly decreased in the CP group compared to the control group, while a significant increase was observed in the CP + GA group compared to the CP group ($p < 0.05$) (Table 1).

Table 1
Effect of GA on stereological parameters in CP-induced rats.

| Groups | The total number of spermatogonia | The total number of Sertoli cells | The total number of Leydig cells | The total volume of testis (mm ³) | The total volume of seminiferous tubule (mm ³) | The total volume of interstitial (mm ³) | Height of germinal epithelial (μm) |
|---------|-----------------------------------|-----------------------------------|----------------------------------|-----------------------------------------------|------------------------------------------------------------|-----------------------------------------------------|------------------------------------|
| Control | 10128396 ± 980602 ^b | 6986982 ± 218182 ^b | 4668402 ± 341247 ^b | 1484 ± 85 ^b | 1169 ± 67 ^b | 294 ± 27 | 79.6 ± 7.4 ^b |
| CP | 8161542 ± 570060 ^a | 58660787 ± 472222 ^a | 3883100 ± 227340 ^a | 1241 ± 70 ^a | 956 ± 45 ^a | 276 ± 47 | 66.4 ± 5.3 ^a |
| CP + GA | 9331420 ± 456670 ^b | 6678004 ± 639402 ^b | 4457787 ± 369579 ^b | 1397 ± 23 ^b | 1106 ± 67 ^b | 284 ± 21 | 76.3 ± 4.3 ^b |
| GA | 10271330 ± 635569 ^b | 6768161 ± 645778 ^b | 4749709 ± 384872 ^b | 1477 ± 45 ^b | 1173 ± 86 ^b | 303 ± 32 | 83.1 ± 8.6 ^b |

Values are expressed as means ± SD. Significant differences as compared with the Control group at ^aP < 0.05, Significant differences as compared with the CP group at ^bP < 0.05.

Histopathological evaluations

Testis and ductus epididymis tissues were observed to have a normal histological structure in the control group. In the ductus epididymis compared to the control group, fewer sperm cells in the tubular lumen and edema in the area between the ducts were observed in the CP group (Fig. 1). In the testicular CP group, it was

observed that atrophy in the seminiferous tubule, decreased sperm number in the tubule lumen, and a decrease in the number of tubular epithelial layers (Fig. 1). GA application improved these changes in both testis and epididymis. In addition, according to the Johnsen testicular biopsy score results, a lower testicular score was observed in the CP group compared with the control group. On the other hand, the testicular biopsy score of the CP + GA group was higher than the CP group (Fig. 3).

Immunohistochemical evaluations of Caspase-3, Bax, Bcl-2, and 8-OHdG

As given in Table 2, just a few caspase-3, Bax, and 8-OHdG immunopositive cells were observed in the control and GA groups. A significant increase in the numerical density of caspase-3, Bax, and 8-OHdG immunopositive cells and a significant decrease in the numerical density of Bcl-2 immunopositive cells were observed in the CP group compared to the control group. But, the numerical density of caspase-3, Bax, and 8-OHdG immunopositive cells significantly decreased in the CP + GA group, while the numerical density of Bcl-2 immunopositive cells significantly increased ($p < 0.05$) compared to the CP group (Fig. 2, Table 2).

Table 2
Effect of GA on the numerical density of immunopositive cells (cell number/mm³) in CP-induced rats

| Groups | Caspase-3 | Bax | Bcl-2 | 8-OHdG |
|---------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Control | 2,01 ± 0,82 ^b | 1,86 ± 0,69 ^b | 160,01 ± 13,58 ^b | 3,43 ± 0,96 ^b |
| CP | 112,29 ± 9,30 ^a | 73,86 ± 7,47 ^a | 38,71 ± 8,69 ^a | 121,43 ± 8,94 ^a |
| CP + GA | 32,71 ± 7,32 ^{a,b} | 25,43 ± 6,78 ^{a,b} | 80,43 ± 9,78 ^{a,b} | 41,71 ± 6,68 ^{a,b} |
| GA | 3,29 ± 0,76 ^b | 1,71 ± 0,76 ^b | 184,14 ± 11,01 ^b | 4,29 ± 0,76 ^b |

Values are expressed as means ± SD. Significant differences as compared with the Control group at ^aP < 0.05, Significant differences as compared with the CP group at ^bP < 0.05.

Serum testosterone levels

Compared to the control group, testosterone level significantly decreased in animals of the CP group ($p < 0.05$). However, the decreased testosterone level significantly increased in CP + GA group ($p < 0.05$) (Fig. 3).

Effects of GA on MDA and CAT levels in testis

When compared with the control group, it was observed that the MDA level significantly increased and the CAT level significantly decreased in the CP group ($p < 0.05$). On the other hand, the MDA level significantly decreased and the CAT level significantly increased in the GA + CP group compared to the CP group ($p < 0.05$) (Fig. 3).

Discussion

The current study revealed that CP treatment caused a decrease in the total number of Sertoli, Leydig, and spermatogonia cells and total testicular volume, as well as structural deterioration in the testis and epididymis. It also caused a significant increase in apoptosis, oxidative stress, and DNA damage indexes. Due to these effects of CP, there have been impairments in testosterone secretion and spermatogenesis. However, GA significantly improved the side effects induced by CP, by reducing testicular structural deformities, apoptosis, DNA damage, oxidative stress, and improving testosterone secretion.

Chemotherapeutic agents are considered to be among the high-risk factors of infertility (Nna et al. 2020). Typically, infertility occurs after chemotherapy and radiotherapy. CP, an antineoplastic agent, is widely used to treat tumors. It causes cytotoxic effects in proliferating cells by damaging the binding of DNA base pairs. Excessive cell proliferation in spermatogenesis makes the testis more sensitive to these agents (Mercantepe et al. 2018). Therefore, protecting the testicles from CP-induced toxicity has become an important issue in chemotherapy with CP (Afsar et al. 2017). Previous studies have reported that CP-induced testicular toxicity can be prohibited with treatment of antioxidants such as melatonin, vitamin C, and resveratrol (Reddy et al. 2016; Almeer and Abdel Moneim 2018; El-shafaei et al. 2018). Additionally reported that GA has protective effects against CP-induced nephrotoxicity, ototoxicity and genotoxicity of the bone marrow (Akomolafe et al. 2014; Shruthi et al. 2014; Kilic et al. 2019). However, in our literature review, no study was found on the effects of GA on testicular and epididymal damage induced by CP.

It has been reported that CP increases ROS production, lipid peroxidation, and denaturation of structural proteins, additionally decreases the activity of the antioxidant system and promotes cell apoptosis by activating p53 (Dasari and Tchounwou 2014; Kohsaka et al. 2020). Oxidative stress is accepted as the result of the disruption of the balance between ROS production and antioxidant enzyme levels (Ekinici Akdemir et al. 2019). Oxidative stress causes testicular damage, testicular apoptosis, DNA damage, and male reproductive dysfunction, which can result in infertility (Shati 2019). Previous studies have reported that CP treatment caused the increased in level of lipid peroxidation and the decreased in level of SOD, CAT and GSH (Reddy et al. 2016; Azarbarz et al. 2020). Similarly, Afsar et al. (2017) reported that CP increased the level of oxidative stress parameters while decreased the activity of antioxidant enzymes. In line with these studies, we observed that CP increased MDA level and decreased CAT level in testicular tissue. Decreased antioxidant enzymes cause the testis to remain vulnerable to the toxic effect of CP. However, the finding of our study indicated that GA can protect the testis against oxidative damage by improving the increased MDA and decreased CAT activity in rats treated with CP. The beneficial effects of GA are mainly related to its antioxidant properties as it acts as a radical scavenger. Previous studies have reported that in doxorubicin (DOX) -induced testicular injury, GA significantly improves increased MDA and decreased antioxidant enzymes (Olusoji et al. 2016). In addition, Oyagbemi et al. (2016) reported that GA significantly improved MDA, nitrite, and H₂O₂ levels and decreased SOD, GSH cyclophosphamide-induced testicular and epididymal damage.

Apoptotic proteins classified according to their role in apoptosis are classified as pro-apoptotic and anti-apoptotic proteins (Abotaleb et al., 2018). Bax protein, a proapoptotic protein, increases under oxidative stress conditions and subsequently release the cytochrome C impairing the mitochondrial membrane permeability (Fouad et al. 2017). Cytochrome C binds to apoptotic protease-activating factor 1 (Apaf-1) and activates it. Activated Apaf-1 forms the apoptosome. Apoptosis is maintained by caspases formed in response to cell

death stimuli. The apoptotic protease-activating factor 1 (Apaf-1) controls caspase activation. As a result, activation of caspase-3, which plays a key role in the execution of apoptosis, is stimulated (Riedl et al. 2005). Since the activation of caspase-3 is an irreversible stage that induces apoptosis, it is frequently used in studies to evaluate apoptosis (Mercantepe et al. 2018; Nna et al. 2020). Proteins in the Bcl-2 families control cell death and survival by regulating the mitochondrial apoptotic pathway. Bcl-2, an anti-apoptotic protein, maintains mitochondrial membrane integrity (Fouad et al. 2017). Previous studies have reported that CP causes upregulation of caspase-3 and downregulation of Bcl-2 (Nna et al., 2020; Kohsaka et al. 2020). Similarly, Aly and Eid (2020) have reported that CP treatment increased the activity of caspase-3 and bax while decreased the activity of bcl-2 in testis. They also showed that resveratrol decreased activity of caspase-3 and bax but increased activity of bcl-2. In another study, Eren et al. (2020) reported that CP increased the caspase-3 immunoreactivity in the germinal epithelium and Leydig cells. However, they also revealed that amifostine and melatonin decreased the caspase-3 immunoreactivity in the germinal epithelium and Leydig cells. The present study revealed that CP treatment significantly increased the expressions of caspase-3 and Bax, which are important markers of the apoptotic index, while significantly decreased Bcl-2 expression in the testis. However, GA treatment significantly decreased an increase in expression of caspase-3 and Bax and increased a decrease in expression of Bcl-2. Thus, these findings of our study proved that GA could protected against CP-induced testis toxicity by regulating expressions of caspase-3, Bax and Bcl-2.

Morphometric examination of the testis is very important to determine male reproductive injury by CP-induced. Based on histological semi-quantitative evaluation, Jahan et al. (2018) reported that CP decreased sperm production, seminiferous tubule diameter, number of spermatogonia, spermatocytes and spermatids compared to control group. They also showed that rutin co-treatment could ameliorated a decreases in sperm production, cell number and tubule diameter. Similarly, Azarbarz et al. (2020) revealed that CP treatment significantly decreased the seminiferous tubules diameter, germinal epithelium thickness, the number of spermatogonia, spermatocyte, and Sertoli cells. In another study, semiquantitative data of Afsar et al. (2017) revealed that CP caused a reducing of the number of Leydig cells inducing the degradation in Leydig cells. They also showed that *Acacia hydaspica* extract ameliorated the degradation in Leydig cells. The quantitative findings of our study revealed that CP decreased the total number of spermatogonia, Sertoli, and Leydig cells in the morphometric examination performed by unbiased stereological methods. This decrease in testicular cells may be a result of apoptosis induced by increased ROS production caused by CP (Köroğlu et al. 2019). Also, quantitative data of our study obtained using stereological methods demonstrated that total volume of testis, total volume of seminiferous tubule and germinal epithelial height significantly decreased in animals that received CP compare to control groups. We estimate that the decrease in these parameters of our study may have been due to the decrease in the total number of testicular cells caused by CP. These changes in testicular morphometric parameters confirmed that CP treatment causes toxicity of testicular tissue. However, GA treatment inhibited the total volume of testis, total volume of seminiferous tubule and germinal epithelial height.

According to histopathological examinations of previous studies, Köroğlu et al. (2019) reported that CP caused the atrophy in seminiferous tubules, an decrease in spermatogenic cell lines and an increase in number of abnormal spermatozoa. They also showed that Apocynin ameliorate the these pathological changes due to CP. Shati (2019) reported that CP increased abnormal sperm number, decreased the sperm

count and motility. Besides, they demonstrated that Resveratrol alleviated these structural distortions. Azarbarz et al. (2020) revealed that CP caused a significantly decrease in Johnsen's testicular score. In accordance with these studies, The histopathological findings of our study proved that CP treatment caused the atrophy in the seminiferous tubules, a decreased sperm cells in tubulus lumen, and a decrease in the number of germinal epithelial layers, and a significant decrease in the Johnsen testicular score. Additionally, CP caused the reduced mature sperm count in epididymis. However, GA significantly improved these structural deteriorations caused by CP in testis. It is estimated that the decrease in sperm count in the epididymis may be related to the deterioration in spermatogenesis, as a consequence of the decrease in the number of spermatogenic cells caused by CP. In our study, the decreases in the total number of testicular cells, the germinal epithelium height, the Johnsen score, and the increase in the number of caspase-3 and Bax immunopositive cells confirm each other.

The previous studies reported that CP causes DNA damage by causing cross-linking of DNA double strands of proliferating cells especially in the testis (Mohammadnejad et al., 2012). 8-OHdG, an important biomarker of DNA damage and oxidative stress, is widely used in experimental studies to determine DNA damage (Köroğlu et al. 2019). Mitochondrial and nuclear DNA damage caused by ROS can be detected with 8-OHdG (Mercantepe et al. 2018).

Köroğlu et al. (2019) found that CP treatment induced a higher levels of 8-OHdG compared to control group in testis of rats. But, they also showed that Apocynin alleviated the DNA damage reducing the levels of 8-OHdG. In another study, Eren et al. (2020) reported that the 8-OHdG was increased in testis of CP-received rats in the germinal epithelium and Leydig cells. However, they also revealed that amifostine and melatonin decreased the levels of 8-OHdG in the germinal epithelium and Leydig cells. In our study, CP significantly increased 8-OHdG expression, which is a marker of DNA damage and oxidative injury. These findings of our study are consistent with previous studies (Amin et al. 2012; Mohammadnejad et al. 2012). However, GA treatment significantly reduced 8-OHdG expression in testis of CP-applied rats.

Testosterone, which plays a prominent role in the initiation and maintenance of spermatogenesis, is secreted by Leydig cells. Previous studies demonstrated that CP decreased the testosterone but, rutin increased the decrease in testosterone caused by CP (Jahan et al. 2018). Similarly, Salem et al. (2012) reported that CP treatment caused the decrease in the testosterone level and Selenium and Lycopene attenuated the decrease in testosterone levels induced by CP in the Wistar rat model. In addition, Salah Azab et al. (2019) found that CP treatment significantly decreased the testosterone levels but taurine significantly increased the testosterone levels in cisplatin plus taurine group compared to cisplatin group. In our study, serum testosterone levels significantly decreased in rats applied CP. According to findings of our study, it is estimated that the decrease in serum testosterone level caused by CP application, may be related to the decrease in Leydig cell number as a result of increased apoptosis in Leydig cells. Because testosterone secretion is directly related to Leydig cell function and number (Gholami Jourabi et al. 2021). Decreased testosterone level caused by may cause disruption of the spermatogenesis process and thus may cause male infertility.

Conclusion

Our findings demonstrated that CP can cause male reproductive damage through various mechanisms, such as oxidative stress, DNA damage, apoptosis, structural and functional defects. Furthermore, our findings revealed that GA can prevent CP-induced male reproductive toxicity by inhibiting oxidative stress, DNA damage, apoptosis, and restoring the structural and functional defects in testis and epididymis. According to the findings of this study, it is suggested that GA can be used as a protective agent against the side effects of CP treatment in testis and epididymis

Declarations

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Availability of data The datasets used in the current study are available from the corresponding author

Author Contribution **Fikret Altındağ**: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition. **İsmet Meydan**: Validation, Formal analysis (biochemical parameters), Resources, Data Curation, Funding acquisition, Review & Editing.

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Consent to participate All of the authors consented to participate in the drafting of this manuscript

Consent for publication Not applicable

Competing interests The authors declare that they have no competing interests

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Figures

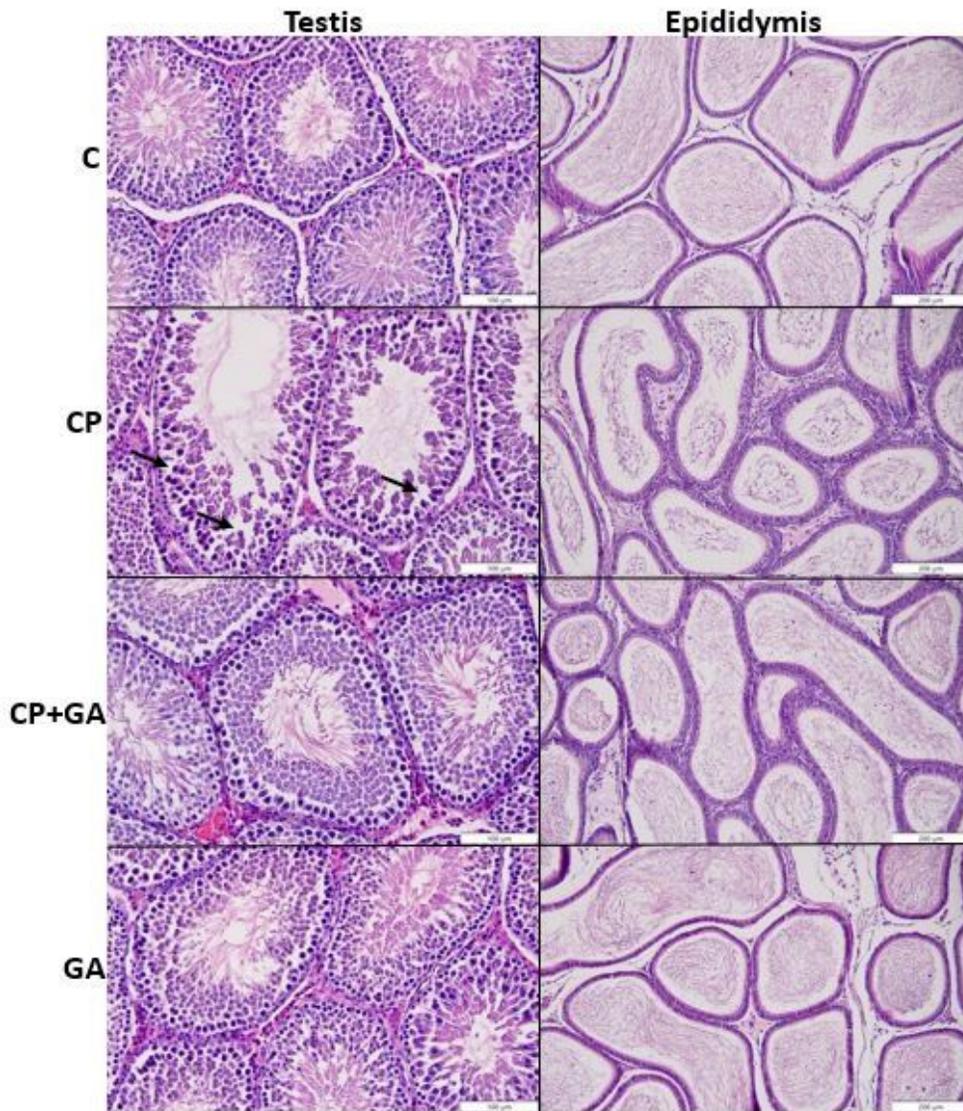


Figure 1

Light microscope images of testicular and epididymal tissues stained by hematoxylin and eosin (H-E) staining. Testis and epididymis have normal histological architecture in control and GA groups. In the CP group, atrophy (arrow) in the seminiferous tubules, loss of germ cells and a decrease in sperm count in the lumen of the testis and the ductus epididymis were observed. GA improved testicular and epididymal structural deterioration caused by CP.

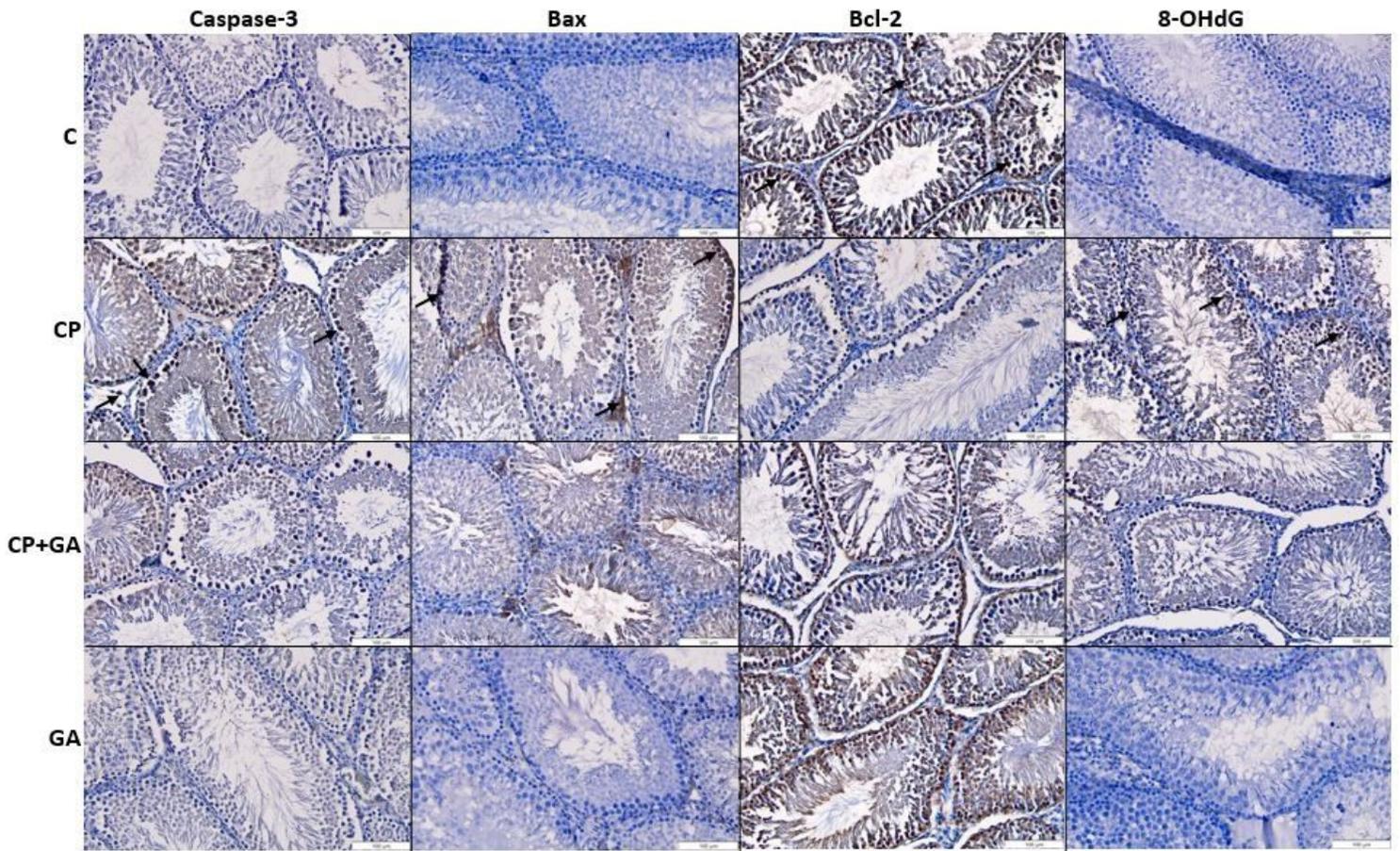


Figure 2

Light microscopic images of caspase-3, Bax, Bcl-2, and 8-OHdG expressions in the testis. Arrows indicate immunopositive cells in groups. CP triggers upregulation of caspase-3, Bax, and 8-OHdG and downregulation of Bcl-2. GA improves upregulation of caspase-3, Bax, and 8-OHdG and downregulation of Bcl-2 triggered by CP.

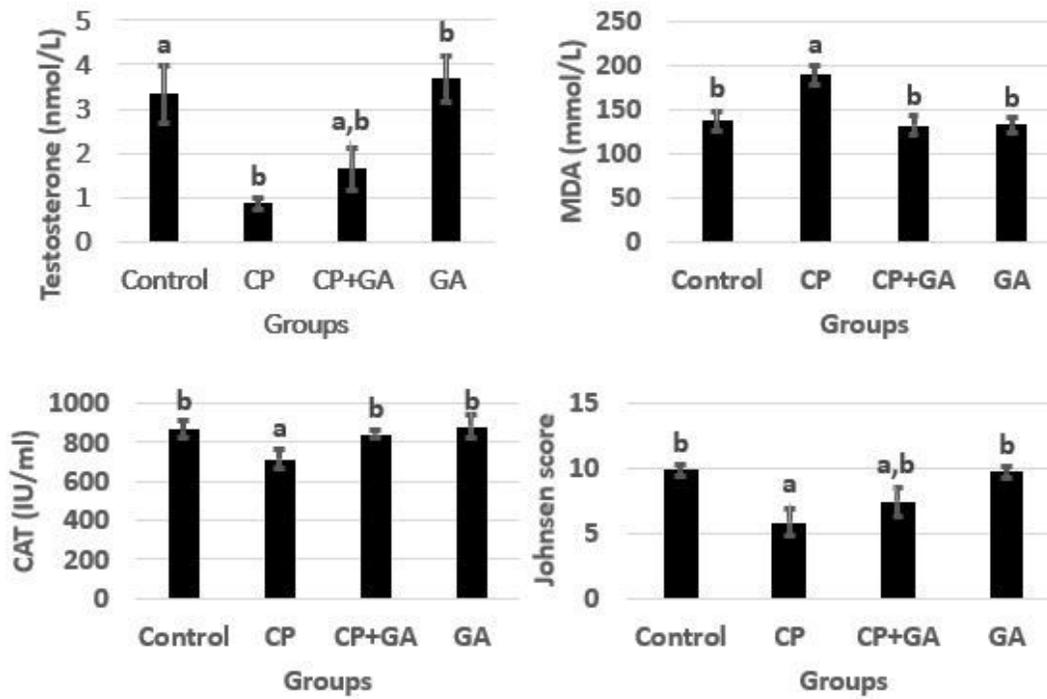


Figure 3

Effects of GA on testosterone, MDA, CAT, and Johnsen score in CP-induced rats. Values are expressed as means \pm SD. Significant differences as compared with the Control group at aP < 0.05, Significant differences as compared with the CP group at bP < 0.05.