Original Research

Candesartan in a rat model of testicular toxicity: New insight on its protective mechanism

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Impact statement

Cisplatin is a commonly used drug in the treatment of solid tumors and its application is associated with testicular toxicity. The effect of candesartan in cisplatin-induced testicular toxicity and its fundamental mechanism of action were investigated. Candesartan had certainly repaired the testicular injury and ameliorated both biochemical and histopathological changes. Candesartan mitigated the gonadotoxicity induced by cisplatin via antioxidative, anti-inflammatory, and anti-apoptotic actions.

Abstract

Cisplatin (CDDP) is widely used as an effective chemotherapy; nevertheless, its use is associated with male reproductive system damage. Candesartan (Cand) is an angiotensin II receptor blocker which showed a protective effect against CDDP-induced testicular toxicity. This study was implemented to investigate further novel molecular protective effect of Cand. Animals were divided into four groups and treated for 10 days as: Group I (Normal control): received saline, Group II (Cand control): treated with Cand (10 mg/kg/day) orally, Group III (CDDP): injected with a single dose of 10 mg/kg CDDP intraperitoneally (ip), Group IV (Cand + CDDP): treated with Cand (10 mg/kg/day) orally plus a single ip dose of 10 mg/kg CDDP at day 3. Blood and testicular tissue collections were done at the end of the experiment. A marked decrease in testicular, body, and relative body weights in addition to testosterone level was observed in the CDDP group when compared with normal rats. In addition, exposure to CDDP showed a marked upregulation of testicular TNF-α mRNA level and a significant rise in testicular levels of total oxidant status, oxidative stress index (OSI) ratio, pro-apoptotic protein Bax, Bax/Bcl-2 ratio alongside with a marked reduction in testicular levels of total antioxidant status, antiapoptotic protein Bcl-2, and a significant upregulation of the testicular caspase-3 expression in comparison to normal group. Histopathological findings after CDDP injection showed apoptosis and necrosis in testicular tissues. Administration of Cand ameliorated these biochemical and histopathological findings. Cand exhibited a novel protective mechanism against CDDP induced-gonadal damage via antioxidant, anti-inflammatory, and anti-apoptotic activities.

Keywords: Cisplatin, testicular toxicity, candesartan, oxidative stress index, apoptosis

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Introduction

Various solid tumors have been treated effectively by chemotherapeutic agents including alkylating agents.[1] One of the members of the platinum-derived alkylating agent is cisplatin (cis-diamminedichloroplatinum (II), CDDP). The use of these agents is linked with serious adverse effects targeting several organs such as kidney, liver, and testis.[2] The CDDP induced testicular damage in several studies because of its deleterious effects on Leydig and Germ cells.[1,3]

The pathogenesis of CDDP-induced gonadotoxicity remains under investigations; however, the involvement of oxidative stress, inflammation, and apoptosis has been addressed in many recent studies. For that reason, amelioration of CDDP-induced organs toxicity has gained a great attention by scientists using various natural products and drugs.[1,2,4–8]

On the other hand, there are still limited experimental studies that discussed the amelioration of the angiotensin II receptor blocker (ARB) agents against the testicular damage induced in different models.[9,10] However, candesartan (Cand), a member of the ARB family, showed antioxidant, anti-inflammatory, and anti-apoptotic activities in various animal models including CDDP-induced nephrotoxicity,[11] thioacetamide-induced hepatic fibrosis,[12] and myocardium ischemia/reperfusion injury.[13]

In the literature, there is only one study of Enatsu *et al.*[14] that addressed the role of Cand in CDDP testicular toxicity. His paper reported that Cand could attenuate the CDDP gonadal damage by regulation of nephrin-podocin expressions, decreasing of the TUNEL apoptotic protein expression in testis, in addition to the restoration of the testicular superoxide dismutase (SOD) levels. Thus, our study was conducted to explore new insight into the protective mechanisms of Cand versus CDDP-induced testicular toxicity involving its impact on oxidative stress and apoptosis indices, and inflammation.

Materials and methods

Chemicals, drugs, and kits

The drugs used are: Cand which was purchased as Atacand from AstraZeneca AB, Södertälje, Sweden in addition to the Cisplatin which was purchased from Hospira Co., Warwickshire, UK, Ketamine which was purchased as Ketamax-50 from Troikaa Pharmaceuticals Ltd, Gujarat, India, and Xylazine as Xyla-Ject ® from Adwia Co., 10th of Ramadan City, Egypt.

Rat testosterone ELISA kit was purchased from Elabscience Biotechnology Co., Wuhan, China. Kits for total oxidant status (TOS) and total antioxidant status (TAS) were purchased from Rel Assay Diagnostics, Gaziantep, Turkey, while the Bcl-2 associated × protein (Bax) and B-cell lymphoma 2 (Bcl-2) kits were obtained from MyBioSource, San Diego, California, USA. Caspase-3 polyclonal rabbit antibody was obtained from Invitrogen, Thermo Fisher Scientific Inc., Carlsbad, CA, USA.

The CDDP-induced testicular damage

Forty adult Male Sprague–Dawley rats (8–10 weeks old) with an average weight of 200–250 g were utilized in this experiment and kept under controlled conditions (25°C, regular cycle 12-h light/12-h dark) with food and water allowance ad libitum. The animals were purchased from Mansoura Experimental Research Center, Faculty of Medicine, Mansoura University, housed as four rats per one ventilated cage, and acclimatized for seven days before the study. All animal experiments were conducted in accordance with the Guide for Care and Use of Laboratory Animals and approved by the local ethical committee, Faculty of Medicine, Mansoura University.

Rats were allocated into four groups as following ($n = 10$/group):

Group I (Normal control): injected intraperitoneally (ip) with (5 mL/kg) normal saline.

Group II (Cand control): received Cand (10 mg/kg/day) by oral gavage for 10 days.

Group III (CDDP): injected ip with a single dose of 10 mg/kg CDDP to cause testicular damage.

Group VI (Cand + CDDP): received Cand (10 mg/kg/day) for 10 days by oral gavage plus 10 mg/kg CDDP ip single dose on the third day.

The CDDP dose used was reported in the literature to induce testicular damage,[4,5] while the Cand dose was selected based on other studies that showed its pharmacological effect.[11,15] Finally, all animals were weighed, and then sacrificed after anesthesia using a mixture of ketamine 60 mg/kg/ip and xylazine 10 mg/kg/ip.[16]

Blood samples were collected from rats' heart and allowed to clot for 15 min at room temperature, and then centrifuged at 3000 r/min for 10 min to separate the serum for testosterone determination. Testes were removed rapidly, one was weighed then fixed in 10% formaldehyde solution for 24 h and the other testis was frozen immediately in liquid nitrogen for further biochemical examinations.

Testicular tissue homogenate was made by homogenization of testis in phosphate buffer saline solution (PH7.4) and centrifugation at 4°C for 10 min, and then the supernatant was frozen at −80°C for further biochemical determination.

Estimation of the testis index

At the end of the study, all rats were weighed as well as the removed testis and the relative testis weight was estimated as (testis weight/body weight) × 100.[17]

Assay of serum testosterone

Serum testosterone level was measured utilizing a rat testosterone ELISA kit in accordance with the manufacturer's instructions.

Determination of oxidative stress index

The TOS and TAS were analyzed in testicular tissue homogenates by using commercially obtainable kits. The TOS was determined as described by Erel[18] and the results were presented as μmol H_2O_2 equivalent/mg protein, while the TAS was estimated as described by Erel 2004[19] and the results were presented as mmol Trolox equivalent/mg protein. The oxidative stress index (OSI) was described as the ratio of TOS to the TAS levels. The OSI is considered as an indicator of oxidative stress degree.[20] OSI (Arbitrary Unit; AU) = TOS (μmol H_2O_2 equivalent/mg protein)/TAS (mmol Trolox equivalent/mg protein) × 100.[21]

Detection of inflammation

To detect the gene expression of the inflammatory marker tumor necrosis factor alpha (TNF-α) in rats' testis, quantitative real-time polymerase chain reaction (qPCR) was used. In brief, total RNA was separated from testicular tissue by using RNeasy Mini Kit (Qiagen, Valencia, CA, USA). To analyze the isolated RNA quantity and quality (A260/A280), the NanoDrop One Spectrophotometer was used. After that, the total RNA was reversely transcribed to cDNA by using High Capacity cDNA Reverse Transcriptase kit (Applied Biosystems, Foster City, CA, USA). The amplification of the cDNA was done by using SYBR Green PCR Master Mix Kit (Applied Biosystems) by the Step One Plus real-time PCR system. The housekeeping gene used is glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The TNF-α relative mRNA expression was determined according to the $2^{-\Delta\Delta Ct}$ method.[22] Primers used for TNF-α were: Forward: 5′-AACTCGAGTGAC AAGCCCGTAG-3′ and Reverse: 5′-GTACCACCAGTTG

GTTGTCTTTGA-3′ (gene bank accession number XM_008772775.1) and for GAPDH were: Forward 5′-ATC CCGCTAACATCAAATGG-3′ and Reverse: 5′-GTGG TTCACACCCATCACAA-3′ (gene bank accession number NM_017008.4).

Evaluation of apoptosis

The Bax and Bcl-2 levels in the testicular tissue homogenate of all studied groups of experimental animals were determined using Bax and Bcl-2 ELISA kits according to the manufacturer's instructions. The results of Bax and Bcl-2 were showed as ng/mg protein. The ratio of Bax/Bcl-2 levels was considered as an apoptosis index.[23,24]

Examination of histopathology and injury scoring

The testis was fixed in 10% formalin solution, and then dehydrated in ethanol and fixed in paraffin. Two sets of 5 um thickness paraffin sections were cut. The first set was stained with hematoxylin and eosin (H&E) and examined using a light microscopy. The thickness of the germinal cell layer and the diameter of the seminiferous tubules were measured to detect the spermatogenic cell density. Seminiferous tubules were scored from 1 to 10. The detailed criteria of histological scores as previously described by Johnsen[25] were: Score 1: No seminiferous epithelium; Score 2: No germinal cells, sertoli cells only; Score 3: Spermatogonia only; Score 4: No spermatozoa or spermatids, few spermatocytes; Score 5: No spermatozoa or spermatids, many spermatocytes; Score 6: No spermatozoa, no late spermatids, few early spermatids; Score 7: No spermatozoa, no late spermatids, many early spermatids; Score 8: Less than five spermatozoa per tubule, few late spermatids; Score 9: Slightly impaired spermatogenesis, many late spermatids, disorganized epithelium.; and Score 10: Full spermatogenesis. Moreover, the testicular insult was evaluated by a semi-quantitative analysis for the damage in the seminiferous epithelium, tubular necrosis, interstitial edema, and congestion using a scale graded from 0 to 3, where 0 used for no abnormal findings, and 3 for severe abnormal findings.[26]

Immunohistochemistry (IHC) and scoring

The second set of paraffin sections was used for IHC. After deparaffinized and rehydration in ethanol, blocking the endogenous peroxidase activity was performed by 3% hydrogen peroxide (H_2O_2) for 5 min at room temperature. The immunohistochemical staining was done as stated in the manufacturer's instructions using a caspase-3 polyclonal rabbit antibody (1:200). The level of IHC staining intensity was scored 0, negative; 1, weak; 2, moderate and 3, strong staining.[27] All readings were blindly performed by a pathologist.

Statistical analysis

Statistical analysis was done by computer software SPSS version 20 (Chicago, IL, USA). The results were expressed as mean ± SD. The one-way analysis of variance (ANOVA) followed by a *post hoc* Bonferroni test was used to assess the differences between groups, while the statistical analysis of histopathological scores including Johnsen score, injury scores, and IHC staining scores was performed by Kruskal–Wallis test, followed by Dunn's test. The P value ≤ 0.05 was considered a statistically significant.

Results

Effect on body and testis weight

The injection of CDDP showed a notable reduction in testicular and body weights ($P < 0.001$) in addition to the relative testis weight ($P < 0.05$) when compared with normal control group. The Cand administration exhibited a marked rise in testicular and body weights in comparison to CDDP-injected rats ($P < 0.05$) (Table 1).

Effect on serum testosterone

In Figure 1, a significant decline in serum testosterone level by 72.3% was demonstrated in rats treated with CDDP injection (group III) when compared with normal control rats (group I). A significant increase in serum testosterone level by 1.2 folds was seen after Cand treatment in group IV rats compared to CDDP-treated rats (group III) ($P < 0.001$).

Effect on oxidative stress index

Figure 2 demonstrated a significant increase in testicular levels of TOS (by 130.9%) and OSI (by 6 folds) with a significant decrease in testicular TAS levels (by 65.5%) after

Table 1. Effect of cisplatin (CDDP, 10 mg/kg single dose ip) alone and its combination with candesartan (Cand, 10 mg/kg/day orally for 10 days) on testis and body weights, and relative testis weight in the experimental groups.

Group	Testis weight (g)	Body weight (g)	Relative testis weight
Normal control	1.66 ± 0.06	241.6 ± 4.3	0.68 ± 0.03
Cand control	1.64 ± 0.11	244.8 ± 3.9	0.67 ± 0.04
CDDP	1.15 ± 0.1[a]	214.8 ± 4[a]	0.53 ± 0.04[b]
Cand + CDDP	1.4 ± 0.15[c]	225.4 ± 4.6[c]	0.62 ± 0.07

Note: All results were presented as Mean ± SD.
[a]Significance difference from normal control group $P < 0.001$.
[b]Significance difference from normal control group $P < 0.05$.
[c]Significance difference from CDDP group $P < 0.05$.

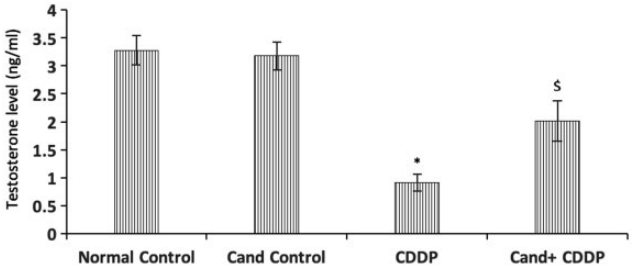

Figure 1. Effect of cisplatin (CDDP, 10 mg/kg single dose ip) alone and its combination with candesartan (cand, 10 mg/kg/day orally for 10 days) on serum testosterone levels in the experimental groups. All results were presented as Mean ± SD. * Significance difference from normal control group $P < 0.001$. [$]significance difference from CDDP group $P < 0.001$.

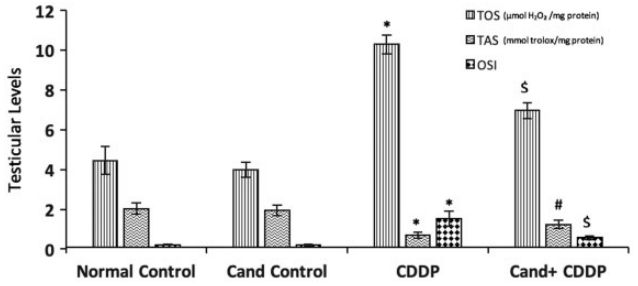

Figure 2. Effect of cisplatin (CDDP, 10 mg/kg single dose ip) alone and its combination with candesartan (Cand, 10 mg/kg/day orally for 10 days) on the total oxidant status (TOS), total antioxidant status (TAS) levels in testicular tissue, and oxidative stress index (OSI) in the experimental groups. All results were presented as Mean ± SD. *Significance difference from normal control group $P < 0.001$. #Significance difference from CDDP group $P < 0.05$. $Significance difference from CDDP group $P < 0.001$.

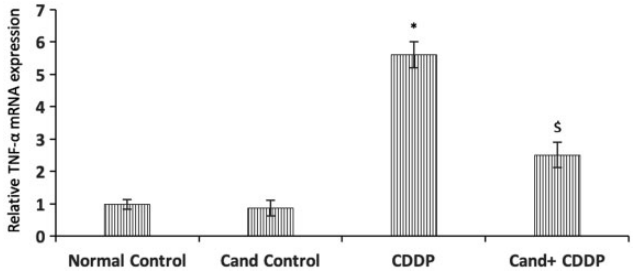

Figure 3. Effect of cisplatin (CDDP, 10 mg/kg single dose ip) alone and its combination with candesartan (cand, 10 mg/kg/day orally for 10 days) on the testicular TNF-α mRNA expression in the experimental groups. All results were presented as Mean ± SD. *Significance difference from normal control group $P < 0.001$. $Significance difference from CDDP group $P < 0.001$.

CDDP administration in group III when compared with normal control rats in group I ($P < 0.001$). After Cand administration, a marked decrease in testicular levels of TOS (by 32.3%) and OSI (by 62.9%) ($P < 0.001$) with a marked rise in testicular TAS levels (by 77.1%) ($P < 0.05$) was evident when compared with CDDP-treated rats.

Effect on inflammation

The exposure to CDDP exhibited a marked upregulation of testicular TNF-α mRNA expression by 4.7 folds in comparison to normal control rats ($P < 0.001$), while a significant downregulation of testicular TNF-α mRNA expression by 55.4% was observed after Cand treatment when compared with CDDP-injected rats ($P < 0.001$) (Figure 3).

Effect on apoptosis

Figure 4 illustrated the Bax and Bcl-2 protein levels in testicular tissue in addition to the Bax/Bcl-2 ratio of all experimental groups. Injection of CDDP showed a marked elevation in testicular Bax protein levels (by 2 folds) and Bax/Bcl-2 ratio (by 2.8 folds) with a significant decline in testicular Bcl-2 protein levels (by 52.9%) when compared with normal control group ($P < 0.001$). The Cand treatment showed a significant decrease in testicular Bax protein levels (by 39.6%) and Bax/Bcl-2 ratio (by 62.7%) in addition

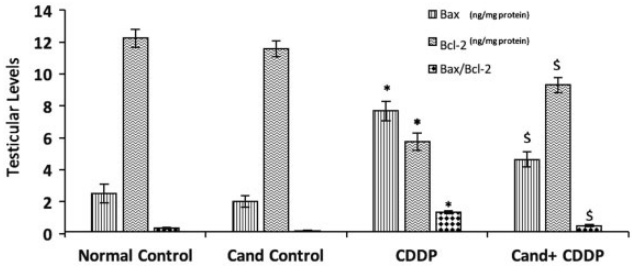

Figure 4. Effect of cisplatin (CDDP, 10 mg/kg single dose ip) alone and its combination with candesartan (Cand, 10 mg/kg/day orally for 10 days) on testicular Bax and Bcl-2 protein levels and Bax/Bcl-2 ratio in the experimental groups. All results were presented as Mean ± SD. *Significance difference from normal control group $P < 0.001$. $Significance difference from CDDP group $P < 0.001$.

to a marked rise in testicular Bcl-2 protein levels (by 61.6%) in comparison to CDDP group ($P < 0.001$).

Effect on histopathological examination

Testes of control rats showed normal seminiferous tubules with full spermatogenesis and normal interstitial cells of Leydig (Figure 5(a) to (c)). In CDDP exposed rats, testis displayed an obvious deformity of seminiferous tubules, vacuolization, necrosis and desquamation of tubular epithelium with diminished spermatogenesis, marked edema, and congestion of interstitium. Apoptotic interstitial cells of Leydig were observed also in untreated cisplatin group (Figure 5(d) to (f)). Testis of Cand-treated rats showed a preserved histology of tubular epithelium with mild edema and congestion of interstitium. Normal interstitial cells of Leydig were seen in treated rats (Figure 5(g) to (i)).

Effect on spermatogenic cell density and the testicular injury and spermatogenesis scores

Statistical analysis of the thickness of germ cell layers, the diameter of seminiferous tubules (Figure 6(a)), and the spermatogenic cell density (Figure 6(b)) in the experimental groups demonstrated a considerable decrease in CDDP group by 39.04% ($P < 0.001$), 17.2% ($P < 0.05$), and 31.03% ($P < 0.001$), respectively, when compared with controls. However, Cand-treated group (Cand + CDDP) exhibited a marked rise in the thickness of germ cell layers, the diameter of seminiferous tubules, and the spermatogenic cell density in comparison to CDDP-injected rats by 55.2% ($P < 0.001$), 13.7% ($P < 0.05$), and 45% ($P < 0.001$), respectively.

Moreover, CDDP produced a marked elevation in the testicular injury score (Figure 6(c)) with a marked reduction in the spermatogenesis score (Figure 6(d)) when compared with the control group, while Cand administration significantly reduced the testicular injury scores and increased the spermatogenesis score when compared with CDDP group ($P < 0.05$).

Effect on immunohistochemistry and scoring

Figure 7 showed the impact of CDDP injection and Cand administration on the apoptotic marker caspase-3. Our results showed a negative staining against caspase-3 in the control group (Figure 7(a)). An increased intensity of

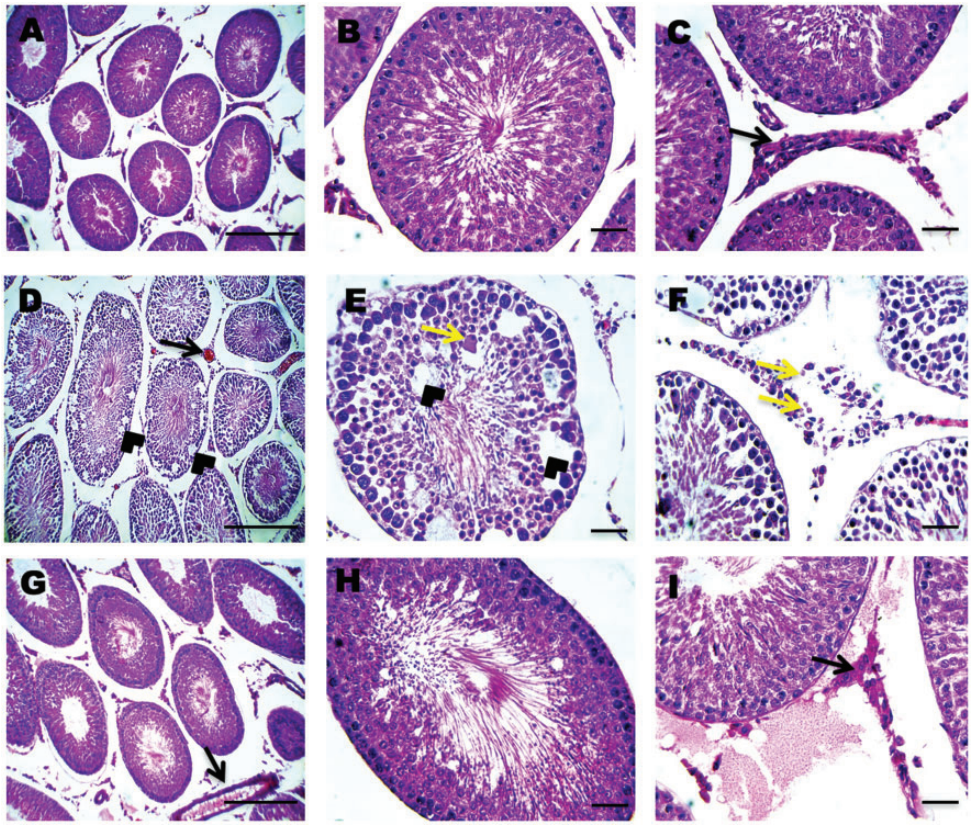

Figure 5. Cross sections of seminiferous tubules in the experimental groups from H&E stain. Control rats (a and b) showing normal seminiferous tubules with full spermatogenesis and normal interstitial cells of Leydig (black arrows) (c), testis of untreated cisplatin exposed rats showing vacuolization (arrowheads), necrosis (yellow arrow) of tubular epithelium (d and e) and congestion of interstitium (black arrow) (d) with apoptotic interstitial cells of Leydig (yellow arrows) (f). Testis of candesartan treated rats showing preserved histology of tubular epithelium (g and h) and congestion of interstitium (black arrow) (g) with normal interstitial cells of Leydig (black arrow) (i). (a, d, g scale bar 100, ×100) (b, c, e, f, h, i scale bar 50, ×400). (A color version of this figure is available in the online journal.)

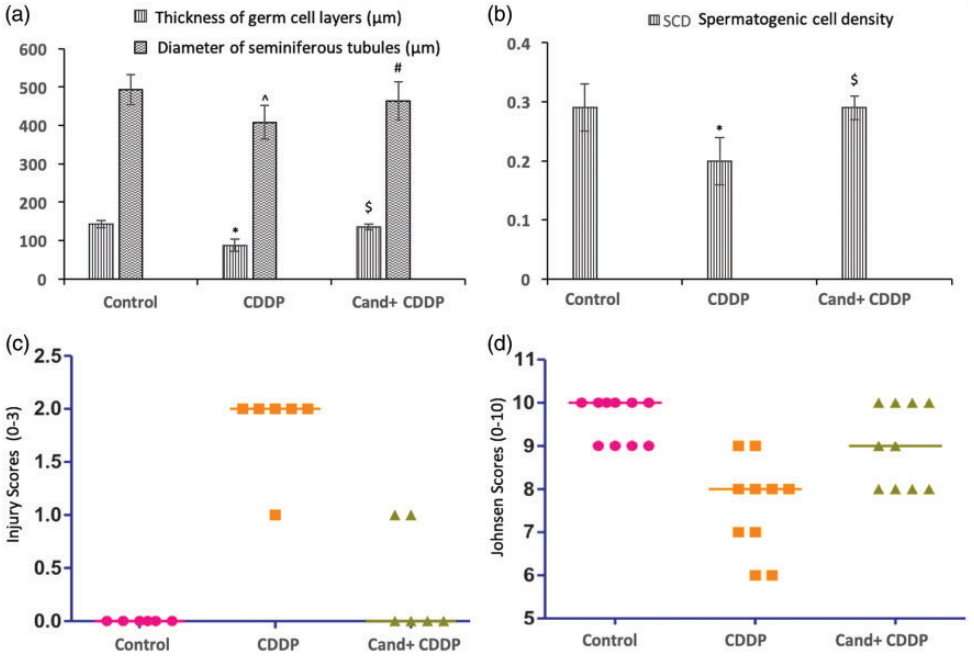

Figure 6. Effect of cisplatin (CDDP, 10 mg/kg single dose ip) alone and its combination with candesartan (Cand, 10 mg/kg/day orally for 10 days) on the thickness of germ cell layers, diameter of seminiferous tubules (a), spermatogenic cell density (b), injury scores (c), and Johnsen score (d) in the testes of the experimental groups. All results were presented as Mean ± SD. ^Significance difference from normal control group $P < 0.001$. Significance difference from normal control group $P < 0.05$. $^{\#}$Significance difference from CDDP group $P < 0.05$. $^{\$}$Significance difference from CDDP group $P < 0.001$. (A color version of this figure is available in the online journal.)

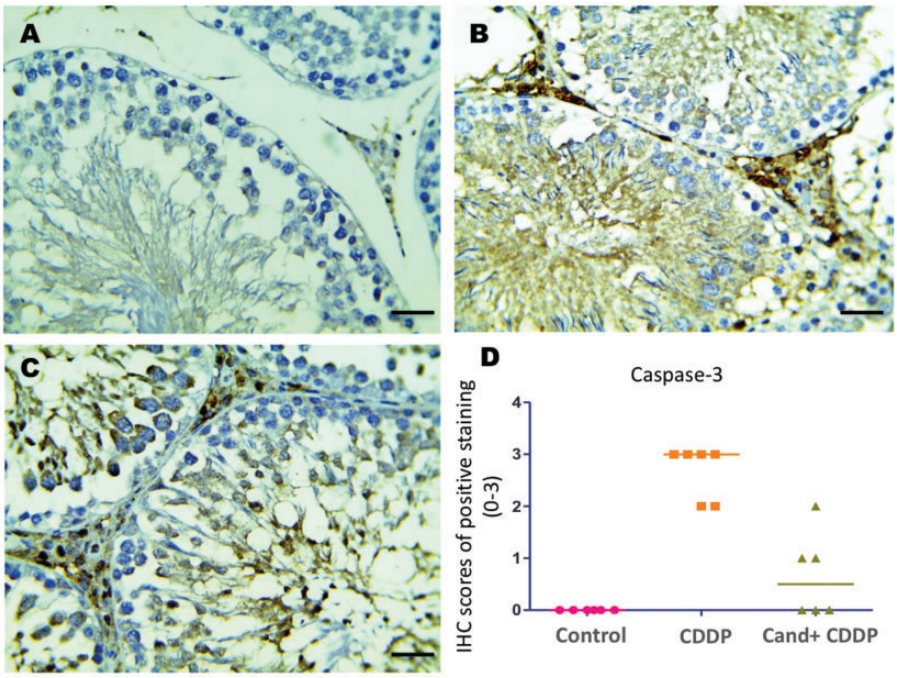

Figure 7. Effect of cisplatin (CDDP, 10 mg/kg single dose ip) alone and its combination with candesartan (Cand, 10 mg/kg/day orally for 10 days) on immunostaining of caspase-3 in the experimental groups (a-c, scale bar 50, ×400) and the IHC scores of positive staining cells (d). Cross sections of seminiferous tubules for IHC showed a negative staining in control groups (a), increased intensity of staining in CDDP group (b), and marked decrease in positive cells in Cand+ CDDP group (c). (A color version of this figure is available in the online journal.)

staining in lining epithelium of seminiferous tubules and interstitial cells of Leydig was seen in untreated CDDP exposed rats (Figure 7(b)). Meanwhile, testis from Cand-treated rats showed a marked decrease in positive signal in both lining epithelium of seminiferous tubules and interstitial cells (Figure 7(c)).

Statistical analysis of the IHC scores in the experimental groups showed a significant variation between control and CDDP groups and between CDDP and Cand-treated groups ($P < 0.05$) (Figure 7(d)).

Discussion

Gonadotoxicity is one of the major deleterious side effects that occurred while using the antineoplastic drug CDDP.[1,8] The CDDP is a potent chemotherapy causing disintegration in the testicles, damaging of the Leydig cells responsible for the production of testosterone, apoptosis in the germ cells, and atrophy in the seminiferous tubules.[1,4,28] The involvement of an angiotensin-mediated system has been reported to maintain the testicular architecture and function in various animal models[9,10]; therefore, it was concluded that ARB could exhibit a gonado-protective potential against testicular injury induced by various toxic stimuli.[14]

In the current study, CDDP produced a marked decline in the testicular, body, and relative testis weights, and testosterone level when compared to normal control rats. Similar results were reported previously in many studies.[8,17,28–30] Moreover, our study showed a marked decrease in the thickness of germ cell layers, the seminiferous tubules diameter, and the spermatogenic cell density after CDDP injection. These results are in agreement with prior studies that documented the same finding confirming the gonadotoxicity of CDDP.[1,4,5,28]

The histopathological examination of the testicular tissue after CDDP injection showed irregularities in seminiferous tubules, apoptosis, and necrosis in interstitial cells of Leydig, and degenerative changes in germ cells. These findings were observed also in other studies[1,3,4,16,29] confirming the cytotoxic effect of CDDP.

After Cand treatment, a significant increase in testicular, body, and relative testis weights in addition to a significant rise in testosterone level was noticed. On contrary, Enatsu *et al.*[14] did not observe any significant change in the testicular weight after 5 mg/kg Cand administration; however, a marked improvement in the sperm analysis was determined when compared with CDDP. Moreover, it was reported that another ARB agent telmisartan (10 mg/kg, orally) significantly increased the testosterone level in a model of cadmium-induced testicular toxicity.[10]

Furthermore, the histopathological study of the testis of Cand administered group showed for the first-time amelioration in the testicular architecture with regular seminiferous tubules and normal spermatogenic cell density. Similarly, telmisartan restored the testicular damage induced by diabetes.[9] This finding confirms the testicular protective effect of Cand in CDDP-induced gonadotoxicity.

The participation of inflammation in the pathogenesis of CDDP-induced gonadotoxicity has been recognized.[3,17,31] Exposure to CDDP produced a significant upregulation of testicular TNF-α mRNA expressions and this was reported before by other investigators.[5,8,17] The CDDP activated the

transcription factor nuclear factor-kappa B (NF-kB) which promotes the inflammatory expressions like TNF-α and IL-1β.[31]

On the other hand, it has been known that angiotensin II (Ag II) was capable to generate reactive oxygen species which results in tissue oxidative stress damage and consequently to inflammation, and apoptosis.[10,32] Therefore, blocking the Ag II receptor by ARB agents could lead to the antioxidant, anti-inflammatory, and anti-apoptotic activities. This study showed that Cand administration reduced significantly the TNF-α testicular mRNA expression in comparison to the CDDP group. Furthermore, a marked reduction in renal TNF-α protein levels after treatment with Cand was documented in CDDP-induced nephrotoxicity model.[11] Also, Cand showed a significant downregulation of hepatic TNF-α mRNA expression in a model of thioacetamide-induced hepatotoxicity and these findings confirming the anti-inflammatory effect of Cand.[12]

Oxidative stress is one of the major contributors in the mechanism underlying testicular damage induced by CDDP.[6,8] It occurred when a status of disequilibrium between the production of oxidants and elimination by antioxidants defense mechanism happened.[33] This study determined the oxidative stress parameters TOS, TAS, and OSI in testicular tissue homogenates in all studied groups to assess the overall oxidative stress status. The TOS level is evaluated as a sensitive indicator for the overall oxidative stress and lipid peroxidation, while the TAS level measured is considered as the total antioxidants defense system which scavenges the ROS released protecting body tissue from oxidative damage.[34,35] Therefore, OSI ratio was used as a sign for the oxidative stress degree in addition to treatment follow-up with TOS and TAS.[34]

A significant increase in testicular TOS level and OSI together with a marked reduction in TAS testicular level was noticed after exposure to CDDP. This could be explained by the fact that CDDP could induce the ROS production, suppress the antioxidant testicular defense activity, and this lead to a disturbance in the balance of oxidants/antioxidants resulting in male testicular injury.[36] A recent study of Koyuncu *et al.*[37] showed a similar finding in renal, hepatic, and serum levels in rats injected with CDDP compared to normal controls. On the other side, Aldemir *et al.*[38] showed a non signifcant difference in TOS and TAS serum levels between CDDP-treated and normal groups.

Protection against CDDP-induced gonadal damage involves the use of antioxidant agents that suppressed the oxidative stress.[8,36] Improvement of oxidative stress parameters measured in testicular tissue was observed in Cand-treated group when compared with the CDDP group. Previous studies reported that ARB agents possess antioxidant activity in various animal models including; Cand ameliorated the cyclosporine-induced nephrotoxicity, losartan attenuated the CDDP-induced nephrotoxicity, and telmisartan alleviated cadmium-induced testicular toxicity and their effect was through the attenuation of tissue oxidative damage.[10,39,40] Meanwhile, losartan could alleviate the diabetes mellitus (DM)-induced oxidative stress through a marked reduction in TOS level along with a significant rise in TAS level in the corpus cavernosum when compared to untreated DM rats.[41]

Growing evidence advocates that the apoptosis plays a central role in the pathogenesis of CDDP-induced male gonadal toxicity.[16] Exposure to CDDP exhibited a significant rise in testicular protein levels of Bax and Bax/Bcl-2 ratio and a marked decline in Bcl-2 testicular levels. Similar findings reported previously in which Bax/Bcl-2 showed their highest in the CDDP group and lowest in the control group.[16] Recent studies documented that a single injection of CDDP demonstrated a significant elevation in the pro-apoptotic marker Bax either protein or mRNA expressions with a marked decrease in the anti-apoptotic marker Bcl-2 in testicular tissue whenever determined by ELISA or q-PCR or immunohistochemically.[3,16,42] The balance between the pro-apoptotic and anti-apoptotic proteins was crucial for the cell to see if it will undergo apoptosis or not.[43] The CDDP treatment shifts this balance towards the pro-apoptotic signaling pathway via Bax activation and Bcl-2 degradation.[44]

Immunohistochemical analysis exhibited a significant upregulation of caspase-3 protein expression in CDDP injection when compared with normal controls. These findings were in coupe with other studies that showed a marked increase in immunostaining intensity of caspase-3 in CDDP group and decreased intensity in control group.[16,45] The CDDP induces the Bax translocation to mitochondria releasing ROS and cytochrome c which then stimulates caspases 3,8,9 triggering apoptosis.[44] Caspase-3 is the executive caspase that induces apoptosis and its activation by CDDP confirmed the CDDP apoptotic damage in the testicular tissue.[1]

Administration of Cand demonstrated a marked decrease in Bax level and Bax/Bcl-2 ratio with a marked rise in Bcl-2 level in testicular tissue in addition to the downregulation of caspase-3 testicular expression compared to CDDP treatment. Lower Bax protein expression in renal tissue was found after Cand administration in CDDP-induced nephrotoxicity rat model[11] indicating its anti-apoptotic activity. Additionally, telmisartan showed a reduction in testicular and renal cells death by a marked

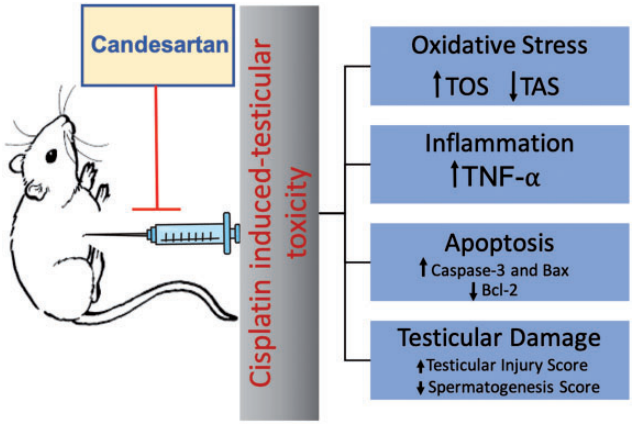

Figure 8. A diagram summarizing the mechanism of the testicular toxicity induced by cisplatin and the protective effect of candesartan. (A color version of this figure is available in the online journal.)

downregulation of caspase-3 protein expression when compared with either untreated STZ-induced germ cell toxicity group[9] or CDDP-induced nephrotoxicity.[44]

Conclusion

This study presented a new insight into the protective mechanism of Cand versus CDDP-induced testicular toxicity (Figure 8). Cand could be used as an adjuvant therapy with CDDP as it possessed: (1) antioxidant activity through the partial restoration of oxidants/antioxidants balance. (2) anti-inflammatory action via downregulation of TNF-α mRNA testicular expression. (3) anti-apoptotic properties by reduction of pro-apoptotic protein (Bax) and elevation of anti-apoptotic protein (Bcl-2) testicular levels restoring the balance between them in addition to downregulation of caspase-3 testicular expression. Future studies are encouraged to be applied in clinical trials and to explore further protective mechanisms of Cand.

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