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RESEARCH ARTICLE

Panax ginseng ameliorate toxic effects of cadmium on germ cell apoptosis, sperm quality, and oxidative stress in male Wistar rats

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ABSTRACT

Panax ginseng is known to potentiate the immune system via its antioxidant properties. Cadmium as an environmental pollutant has destructive effects on body organs especially the reproductive system. This study investigated the effects of *P. ginseng* extract against cadmium-induced testicular toxicity in rats. In this experimental study, 50 male adult rats were randomly divided into 5 groups: A significant decrease was observed in sperm count and viability in the cadmium group versus the control group. Ginseng extract could repair these defects. Increases in apoptotic germ cells due to cadmium were significantly reduced by ginseng extract. Our results demonstrated that *P. ginseng* extract reduces cadmium-induced testicular toxicity, via anti-oxidative and anti-apoptotic processes.

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KEYWORDS
Panax ginseng; cadmium; testes; oxidative stress; apoptosis

Introduction

Cadmium poisoning induced by industrial exposure and pollution is a hazardous health problem throughout the world. Foods may also be part of the main sources of cadmium intake in the environmentally exposed population. One of the ten most important toxicants of hazardous substances from viewpoint of ATSDR (Agency for Toxic Substances and Disease Registry) is cadmium. Cadmium as a toxic heavy metal has caused irreversible damages to several vital organs such as kidney, bone, immune system, liver, and respiratory system (Godt *et al.* 2006, Fowler 2009, Siu *et al.* 2009, Skipper *et al.* 2016, Genchi *et al.* 2020). The harmful effects of cadmium on the male reproductive organs have been recognized and include germ cell loss, testicular edema, reduced sperm count, poor semen quality, and temporary or permanent sterility (Goyer *et al.* 2004, Godt *et al.* 2006, Friberg 2018, Zhu *et al.* 2020). Exposure to cadmium during fetal, neonatal, puberty, and adulthood periods can cause severe changes in Sertoli cell development and Leydig cell function (Zhu *et al.* 2020). More attention has been paid to the mechanisms of cadmium toxicity in testicular tissue in research in recent years. Cadmium disturbs the blood–testes barrier via disruption of different types of junctions between adjacent Sertoli cells (Chung and Cheng 2001, Mouro *et al.* 2020). It also causes the destruction of the endothelial cells of testicular microvessels resulting in ischemia and necrosis (Pařízek 1964). Acute cadmium toxicity causes tissue exposure to free oxygen radicals such as hydrogen peroxide (H_2O_2), nitric oxide (NO), hydroxyl (OH^-), and superoxide (O_2^-) (Birben *et al.* 2012). It does this by depletion of cellular glutathione and reaction with the sulfhydryl groups of proteins (Valko *et al.* 2005, Olaniyi *et al.* 2020). Additionally, Cadmium binds to thiol groups inside the mitochondrial membranes and interferes with the mitochondrial role in the respiratory chain reaction. This functional disorder of the mitochondria leads to ROS production (Liu *et al.* 2009, Nemmiche 2017). ROS produced by cadmium results in the peroxidation of lipid in cell membranes and also damages DNA. *Panax ginseng* has been used in traditional medicine to ameliorate various disease symptoms via antioxidant mechanisms in humans and

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Supplemental data for this article can be accessed here.

animals (Ramesh *et al.* 2012, Leung and Wong 2013, Lee *et al.* 2017). Furthermore, ginsenosides, the major bioactive components of *P. ginseng* root, have therapeutic properties in different diseases including neoplastic tumors (Li and Li 2019), diabetes mellitus (Shao *et al.* 2019), and neurological disorders (Ong *et al.* 2015). A few studies have shown the protective effect of ginseng against heavy metal poisoning (Abdel-Wahhab and Ahmed 2004, Karadeniz *et al.* 2009, Park *et al.* 2013, Wang *et al.* 2013). *Panax ginseng* reverses the effects of cadmium poisoning on liver tissue (Karadeniz *et al.* 2009, Park *et al.* 2013). To the best of our knowledge, no previously published study has examined the possible protective effects of *P. ginseng* in Cadmium-induced testicular toxicity. The present study was designed to evaluate the possible protective effects of *P. ginseng* on Cd-induced toxicity on germ cell viability, sperm quality, and testosterone levels in the rat testes.

Materials and methods

This study is a randomized experimental study. The ethical issues relating to the storage and handling of laboratory animals have been observed throughout the study, and procedures have been approved and recorded in the Ethics Committee of Kerman University of Medical Sciences (KUMS), by IR.KMU.REC.1398.266.

Panax ginseng extract preparation

The dried root of the *P. ginseng* was purchased from the local herbal market in Kerman. The plant was authenticated at the Pharmacognosy Department of Faculty of Pharmacy, KUMS. The roots and rhizomes were cleaned and powdered by an electric blender, then passed through a sieve (mesh 35). The homogeneous powder was macerated and extracted using a percolation method with 80% methanol for 72 h (1:10 w/v) as explained previously (Mandegary *et al.* 2012). Removal of the solvent was performed by a rotary evaporator at 100 rpm at 50 °C (Heidolph, Germany) and was continued with the final drying in the oven at 40 °C for 24 h. The dried extract was weighed and stored at −20 °C for subsequent experiments.

Phytochemical screening of the plant

The plant was screened phytochemically for the presence of alkaloids, saponins, flavonoids, terpenoids, and tannins (Sharififar *et al.* 2012). Total phenolic content (TPC) of the ginseng was determined with folin-ceu-calteu reagent using spectrophotometry method (Salari *et al.* 2016). The calibration curve of gallic acid was provided and the TPC of the plant was reported as gallic acid equivalent. Total flavonoid content (TFC) was measured on the basis of rutin content which was identified as the main flavonoid on the basis of thin-layer chromatography (Sarhadynejad *et al.* 2016) and was calculated from the slope of the calibration curve of rutin. For measuring the total saponin content, a double extraction gravimetric method was used (Harborne 1973). About 50 ml ethanol (20%) was added to 5 g of the powdered plant and was heated with periodic stirring in a water bath for at 55 °C (90 min). After that, the mixture was filtered through a Whatman filter paper (No. 42). The residue on paper was extracted with ethanol (20%, 50 ml) twice, was concentrated up to 40 ml, and extracted with diethyl ether. The ether phase was re-extracted with normal butanol, washed with sodium chloride (5%), evaporated to dryness and the percentage of saponin content was calculated.

Experimental design

Fifty adult male Wistar rats (purchased from the animal house of Afzalipour Kerman Medical school, Kerman, Iran) weighing 200–250 g were used in the experiment. Animals were kept in a temperature-controlled room (23 ± 1 °C) with 12 h/12 h light/dark cycles. During the study, all animals had unrestricted access to chow pellets and freshwater.

In this study, the ginseng extract was dissolved in dimethyl sulfoxide (DMSO) and distilled water (D.W.). The Cadmium chloride ($Cd/CdCl_2$) was dissolved in D.W. The animals were randomly divided into five equal groups:

Group I (control untreated rats): The animals were gavaged normal saline (equal volume to other groups) for 9 weeks. Group II (vehicle group): rats received ginseng solvent (i.e. DMSO + D.W.) daily by oral gavage for 9 weeks. Group III: rats were injected intraperitoneal (i.p.) with a single dose of Cd (1.2 mg/kg) on the first day of the study and then D.W. was administrated orally by gavage for 9 weeks (Erboga *et al.* 2016). Group IV: rats were gavaged 50 mg/kg ginseng daily for 9 weeks after receiving a single Cd injection (1.2 mg/kg, i.p.) Group V: rats were gavaged 100 mg/kg ginseng daily for 9 weeks after receiving a single Cd injection (1.2 mg/kg, i.p.). At the beginning and end of the experiment, all rats were weighed.

Sample collection

At the end of the treatment period, the rats were anesthetized by intramuscular injection of ketamine

hydrochloride (90 mg/kg BW) and xylazine hydrochloride (5 mg/kg BW). Heart blood samples (4 ml) were collected into sterile tubes without EDTA, then centrifuged at 3000 rpm for 30 min. Serum was carefully separated and stored at deep-frozen (−70 °C) for biochemical and hormone analysis. Finally, both testes and epididymis were removed and the testes were weighed, preserved for histopathological examination and sperm analysis.

Spermiogram test

Under sterile conditions, mature sperm were collected from the caudal region of the epididymis after incubation in alpha MEM medium and 10% bovine serum albumin at 37 °C for 30 min. Sperm concentration, motility, and viability were assessed according to WHO guidelines (Menkveld 2010). For sperm counting, 10 µl of the sperm suspension was diluted with an equal volume of 10% formaldehyde fixative. Then 10 µl from this diluted solution was transferred into a Neubauer hemocytometer (Precicolor, Germany) and sperm counted by light microscopy at 400×. The concentration was expressed in millions of sperm per milliliter (Mehraein and Negahdar 2011, Zare *et al.* 2020). Assessment of sperm viability was performed based on Eosin–Nigrosin staining. Firstly, 10 µl of the fresh sperm suspension was mixed with 10 µl eosin and nigrosin (1% eosin Y and 5% nigrosine). Then a smear was prepared on a glass slide and observed under an optical microscope (400× magnification). Differential counts of 200 spermatozoa per slide were randomly performed. Live sperm cells appear white and dead sperms pink or red. The results were expressed as a percentage of live spermatozoa (Menkveld 2010, Saddein *et al.* 2019). To assess the percentage of motile sperm 10 µl of the fresh sperm suspension was placed between a clean glass slide and coverslip and examined at 400× magnification using an optical microscope (Olympus BX51, Tokyo, Japan). At least 300 spermatozoa were counted and the percentage of nonmotile and motile sperm cells (progressive or not progressive) were recorded (Menkveld 2010).

Measurement of gonadosomatic index (GSI)

The animals and the testes weights were determined by a digital balance and the percentage of gonadosomatic index (GSI) was calculated from the formula GSI = [Gonads weight (g)/Body weight (g)] × 100 (Adebayo *et al.* 2009).

Serum testosterone and liver enzyme levels assay

The serum samples from all animals were separated and used for the measurement of testosterone and liver enzymes levels, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by an enzyme-linked immunosorbent assay (ELISA) using biovendor kit (BioVendor company, Czech Republic) and Parsazmun kit (PARS AZMUN company, Iran), respectively, according to the manufacturer's instructions.

Evaluation of malondialdehyde measurement

Malondialdehyde (MDA) activity were measured based on Buege's method with minor changes as already described elsewhere (Valenzuela 1991, Worek *et al.* 1999, Paydar *et al.* 2018). In short, a mixture of serum (125 µl) and phosphoric acid (1.5 ml) was prepared in a test tube and after shaking, thiobarbituric acid (0.5 ml) was added. The test tube was placed in boiling water for 45 min after stirring. After cooling, n-butanol (1 ml) was added and the mixture centrifuged for 10 min at 100 × *g*. Finally, the pink phase was separated and absorbed at 532 nm, and the amount of malondialdehyde in the sample was calculated from the standard curve of tetraethoxypropane.

Total antioxidant capacity serum levels assay

The test used to measure the ferric-reducing ability of plasma was that described by Benzie and Strain (1996) (Benzie and Strain 1996). The Ferric-tripyridyltriazine (Fe III-TPTZ) complex is reduced to a dense blue-colored ferrous (Fe II) form at low pH by the plasma. This complex has a maximum absorbance at 593 nm and the blue color intensity is proportional to the antioxidant capacity of the sample as described elsewhere (Paydar *et al.* 2018, Abolhassani *et al.* 2019, Mortazavi *et al.* 2019). In the first step, plasma (5 µl) and 70 µl of FRAP reagent (70 µl) were mixed together. Distilled water was used as a blank. Then, the mixture was incubated at 37 °C for 5 min, and the absorbance was read at 593 nm. The FRAP values were expressed as micromolar (µM).

Testes histopathological study

After necropsy, the 50 testes from five groups were preserved in 10% neutral buffered formalin solution at the end of the 9th week. Then, the 5 µm sections were stained with Hematoxylin and Eosin. Finally, histopathological assessment of the testicular

morphology was done using a light microscope (Olympus/BX51, Japan) in five fields for each slide. About 20 seminiferous tubules per testes from 10 rats were randomly selected in each experimental group and the parameters such as the diameter of seminiferous tubules, germinal epithelium height, and the number of the germinal epithelial cells were evaluated (Murphy and Richburg 2014). Johnsen's score (spermatogenesis) was used to report the changes in the seminiferous epithelium quality (Ghanbari *et al.* 2016).

Evaluation of testicular apoptotic cells by using TUNEL assay

The Terminal deoxynucleotidyl-transferase-mediated DNA nick end-labeling (TUNEL) assay is a reliable method for detection and quantification of apoptosis at the single-cell level, based on labeling of DNA strand breaks. TUNEL staining was performed using the *in situ* cellular death detection kit, POD (Roche-11684817910 version 15, Germany). Firstly, testicular sections were dewaxed and rehydrated by heating at 60 °C for 30 min followed by washing in xylene and rehydration through a graded series of ethanol and double-distilled water. Next, tissue sections were incubated for 30 min at 37 °C with Proteinase K working solution and rinsed twice with phosphate-buffered saline (PBS). Then, the slides were incubated in a TUNEL reaction mixture for 60 min at 37 °C in the dark. After washing twice in PBS and adding 50 µl POD (Anti-fluorescein antibody, Fab fragment from sheep, conjugated with horse-radish peroxidase), the samples were incubated in a humidified chamber for 30 min at 37 °C. The slides were rinsed 3 times with PBS and 50–100 µl 3,3′-Diaminobenzidine (DAB) substrate was added to the slides. These were incubated for 10 min at 15–25 °C. Finally, after washing with PBS, the slides were re-washed carefully with distilled water and the sections were stained with hematoxylin. The slides were mounted using entellan and examined under a light microscope (Hakemi *et al.* 2019). The apoptotic index was calculated as follows: mean number of total TUNEL positive spermatogonia and primary spermatocyte cells/mean number of total spermatogonia and primary spermatocytes.

Statistical analysis

Statistical analysis of the obtained data was performed using SPSS version 21 (Chicago, USA). All of the data with normal distribution are presented as the mean- ± standard error of the mean. The One-Way ANOVA and Tukey's tests were used for the analysis of parametric data and Kruskal–Wallis test for non-parametric data. Statistically significant differences were accepted as $p < 0.05$.

Results

Phytochemical study, TPC, and TFC content of ginseng

A phytochemical study of the plant indicated the presence of alkaloids, flavonoids, terpenoids, and saponins. TPC of ginseng was estimated 143.26 ± 3.16 mg gallic acid equivalent/100 g plant extract and TFC which was calculated using rutin standard curve ($R^2 = 0.9956$), was 93.86 mg rutin equivalent/100g plant extract. The total saponin content of ginseng was assessed 14.51 ± 1.59 mg/100g plant extract.

Gonadosomatic index (GSI) and gross morphological assessments

No rats died or became ill during the 9 weeks of treatment. There were no significant differences in the rat body weight between all groups ($p > 0.05$). Right and left testes weights were decreased significantly in the Cd, Cd + Gi50, and Cd + Gi100 groups compared to the control and vehicle groups ($p < 0.05$). Further, GSI of the Cd group decreased significantly ($p < 0.05$), compared to the control and vehicle groups, while it increased significantly ($p < 0.05$) in the Cd + Gi100 group, compared to the Cd group. The right and left testes' short diameters and the right testes' long diameter were decreased significantly in the Cd, Cd + Gi50, and Cd + Gi100 groups compared to the other groups ($p < 0.05$). The left testes' long diameter was decreased significantly in the Cd, Cd + Gi50 ($p < 0.05$) (Table 1).

Spermiogram findings

The effects of Cd and Cd + Gi on the total epididymal sperm count are shown in Figure 1. The Cd significantly decreased sperm count (2.1 ± 0.11, $p = 0.000$). Co-treatment of Cd with Gi could significantly change the sperm count in comparison to the Cd group in a dose-dependent manner.

Sperm viability in the Cd group was significantly lower than that of the control group ($p = 0.000$). The percentage of viable sperms was comparable between the Cd group and two Gi-treated groups (50 and 100 mg/kg, $p = 0.000$). Administration of Gi at a dose of 100 mg/kg caused an increase in alive sperm as compared to Cd + Gi50 group ($p = 0.000$) (Figure 1).

Table 1. Effects of cadmium (Cd) and different doses of ginseng (50 and 100 mg/kg) on testes gross morphological parameters.

	Control	Vehicle	Cadmium	Cd + Gi50	Cd + Gi100
Right testes weight (mg)	429.6 ± 12.22	432.6 ± 10.11	403.3 ± 12.77[ab]	405.8 ± 13.52[ab]	413.6 ± 13.41[ab]
Right testes long diameter (mm)	17.17 ± 0.31	17.32 ± 0.73	13.27 ± 0.564[ab]	13.05 ± 0.34[ab]	14.14 ± 0.41[ab]
Right testes short diameter (mm)	11.22 ± 0.16	11.2 ± 0.42	8.45 ± 0.29[ab]	8 ± 0.15ab	8.18 ± 0.35[ab]
Left testes weight (mg)	555.2 ± 7.86	548.6 ± 7.13	324.8 ± 12.95[ab]	365.2 ± 10.48[ab]	397.3 ± 14.71[ab]
Left testes long diameter (mm)	16.7 ± 0.42	15.4 ± 0.24	14.19 ± 0.64[a]	13.87 ± 0.37[a]	14.16 ± 0.35[a]
Left testes short diameter (mm)	10.9 ± 0.31	10.8 ± 0.37	8.3 ± 0.27[ab]	7.77 ± 0.19[ab]	8.17 ± 0.32[ab]
Gonadosomatic index	0.39 ± 0.02	0.33 ± 0.01	0.26 ± 0.0[ab]	0.26 ± 0.01[ab]	0.32 ± 0.02[c]

Results are expressed as mean ± SEM. Significant differences ($p < 0.05$) are indicated by[a]; vs. the control group in the same row,[b]; vs. the vehicle group in the same row,[c]; vs. the Cd group in the same row,[d]; vs. the Cd + Gi 50 group in the same row,[e]; vs. the Cd + Gi 100 group in the same row.

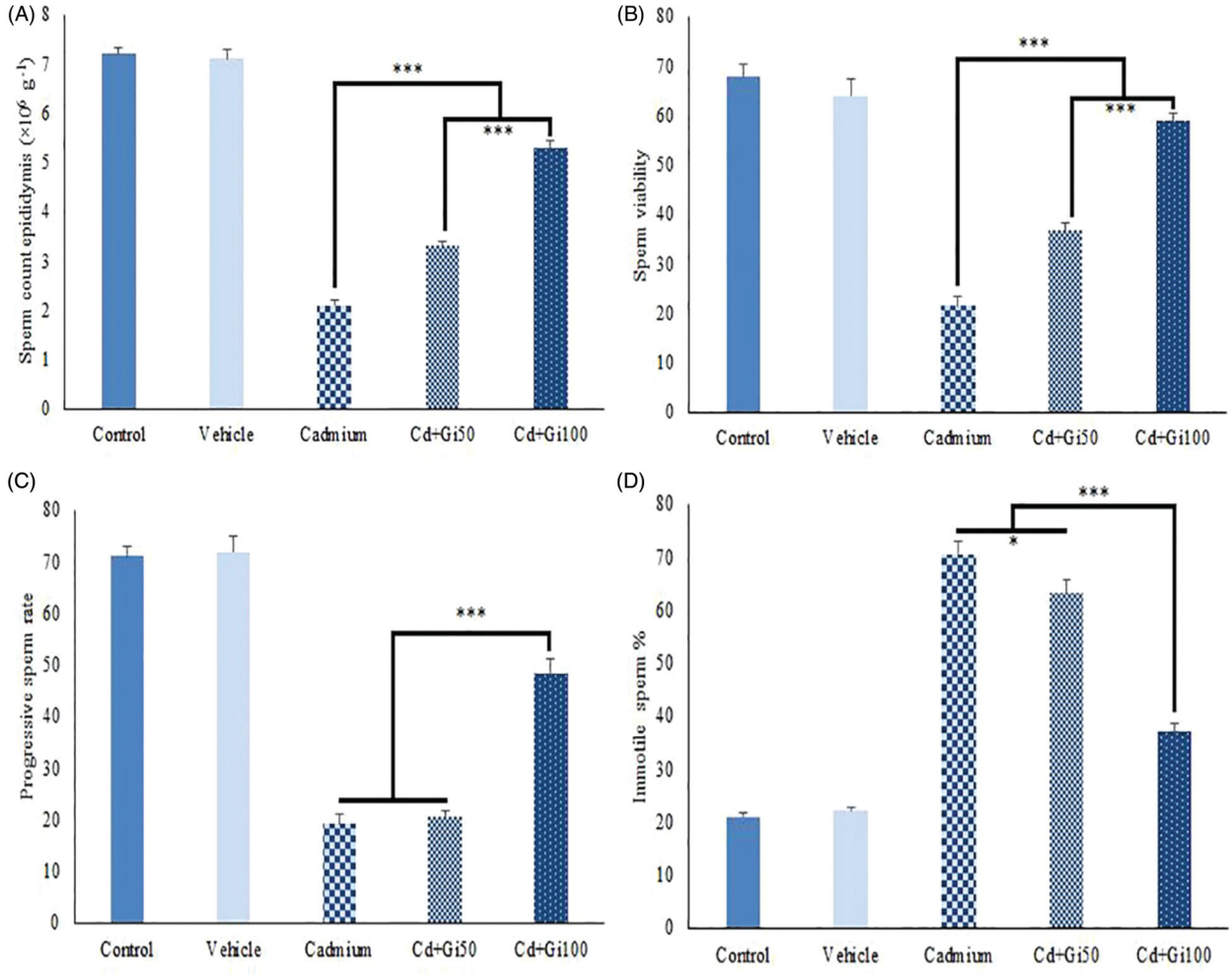

Figure 1. Effect of *P. ginseng* treatment after acute cadmium toxicity on sperm quality parameters. Ginseng therapy effectively improved epididymal sperm count, decreased percentage of immotile sperm, and increased percentage of sperm progressive motility and viability. Values are expressed as mean ± SEM. Significant differences are expressed as $^{*}p < 0.05$; $^{**}p < 0.01$, and $^{***}p < 0.001$.

Figure 1 illustrates the sperm motility and progression in the different groups. Sperm progressive motility rate in the Cd and Cd + Gi50 groups was significantly lower ($p < 0.001$) than those of the other groups. Oral administration of Gi at a dose of 100 mg/kg could prevent this effect. The rate of immotile sperms was dose-dependently decreased in the 50 and 100 Gi mg/kg groups in comparison to the Cd group ($p < 0.001$) (Figure 1).

Evaluation of oxidative stress biomarkers

The results of oxidative stress biomarkers measurements including MDA; the final product of lipid peroxidation and total antioxidant capacity (TAC) in the serum of the rats are shown in Figure 2. Administration of Cd significantly increased ($p = 0.000$) MDA level in the serum. In contrast, treatment by Gi at a dose of 100 mg/kg resulted in significantly

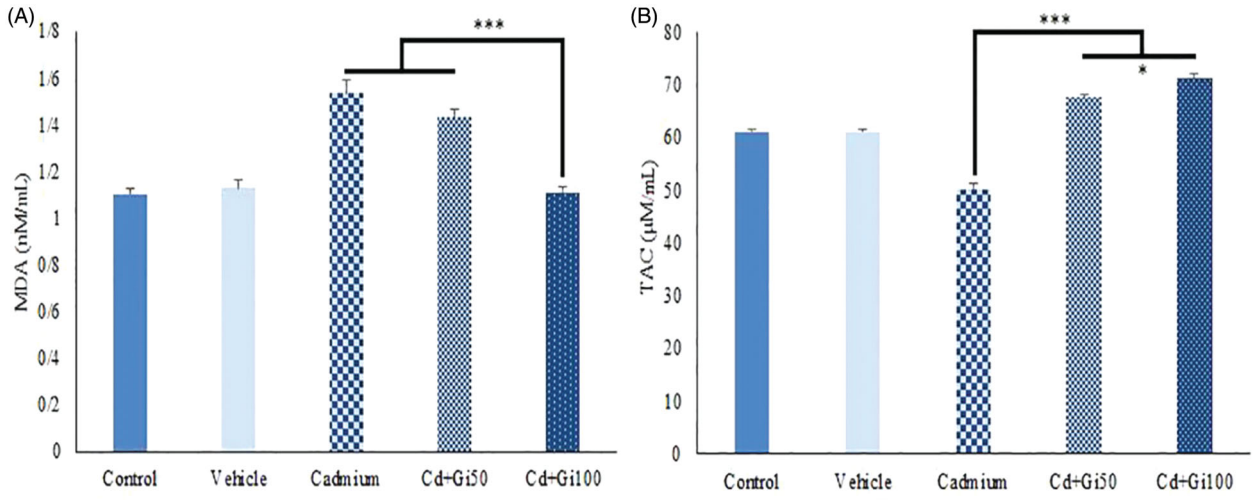

Figure 2. Serum MDA and TAC levels at 9 weeks after Cd toxicity. Values represent mean ± SEM in each group. Significant differences are expressed as $^{*}p < 0.05$; $^{**}p < 0.01$, and $^{***}p < 0.001$.

decreased Malondialdehyde levels. However, treatment of rats with Gi significantly prevented changes in lipid peroxidation. Moreover, total antioxidant capacity significantly decreased in the Cd group versus the other groups ($p = 0.000$), while Gi treatment significantly increased TAC levels ($p = 0.000$). TAC in Cd + Gi100 was significantly higher than Cd + Gi50 group ($p = 0.02$).

Histopathological findings

Orchiectomy sections from control and vehicle groups were easily differentiated and all seminiferous tubules showed normal spermatogenesis composed of spermatogonia, primary and secondary spermatocytes, spermatids, and spermatozoa. Also interspersed Sertoli cells were present. Interstitial Leydig cells were normal in number. The tunica albuginea had a normal thickness. In contrast, the examination of the testes in the Cd group revealed degenerative changes in the spermatogonia cells. Some of the seminiferous tubules showed cell sloughing and were disorganized; Spermatocytic arrest was observed. Most of the tubules showed a "Sertoli cell only" pattern and few of them were hyalinized, calcified, and necrotic. Leydig cells were hyperplastic in the interstitial areas and the tunica albuginea was thickened by fibrosis. No evidence for hypertensive or vasculitic changes was noticed (Figure 3). However, morphological examination of the testes in the Cd + Gi50 and Cd + Gi100 groups showed almost normal spermatogenesis. The tunica albuginea had a normal appearance. The diameters of the seminiferous tubules and germinal epithelium height in the rats of the Cd group were significantly lower than those of other groups (Table 2). Figure 4 displays Johnsen's score and the changes in a spermatogenic cell line in the different groups, respectively.

Germ cell apoptosis assessment

In the present study, to examine the possible involvement of apoptosis in the mechanism of Cd-induced atrophy of the gonad and the possible ameliorative effects of ginseng, apoptotic germ cells were distinguished by TUNEL staining (Figure 5). TUNEL positive cells significantly increased in Cd exposed rats compared with the controls ($p = 0.000$). Ginseng administration significantly diminished Cd-induced germ cell apoptosis in the seminiferous tubules ($p = 0.000$). This decline was markedly higher in the Cd + Gi100 group compared to the Cd + Gi50 group ($p < 0.001$) consistent with a dose-related response.

Serum testosterone and liver enzymes assay

There was no significant difference in serum levels of testosterone among different groups. Analysis of the liver enzyme levels showed that ALT enzyme level did not vary significantly between groups but AST level was significantly elevated in the Cd + Gi50 group compared to the Cd group ($p < 0.05$). There were no significant changes between the control, vehicle, Cd, and Cd + Gi100 groups (data not shown).

Discussion

The findings of the present study indicated that administration of a single dose of Cadmium (1.2 mg/kg, i.p.) to Wistar rats causes considerable damage to spermatogenesis confirmed by the epididymal

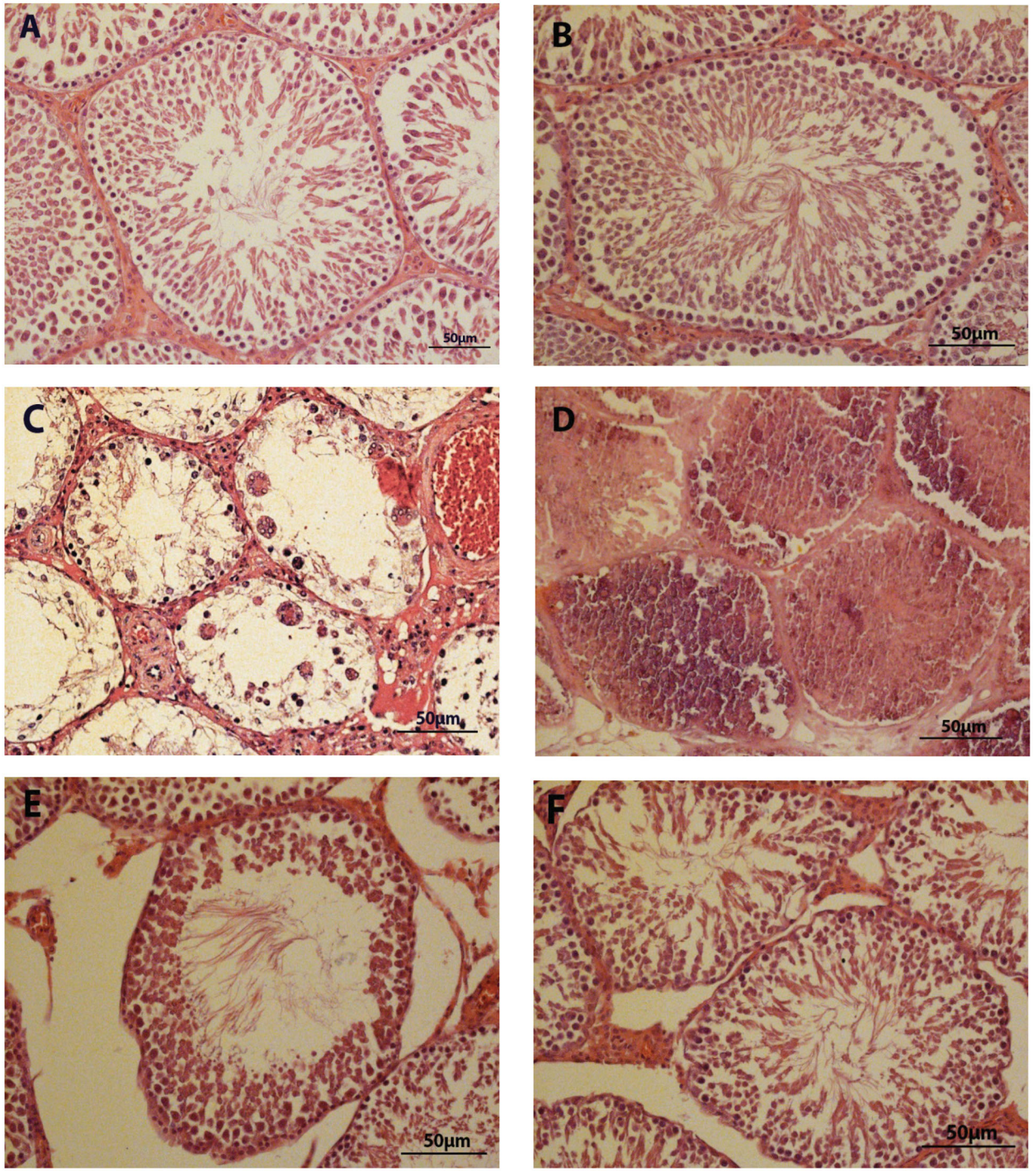

Figure 3. Light micrographs of testicular sections stained with Hematoxylin & Eosin after 9 weeks (×400). (A) Control and (B) vehicle groups show the normal architecture of testicular tissue and active spermatogenesis in seminiferous tubules. (C) Cadmium group showing destruction the most of spermatogonia and primary spermatocytes cells, marked reduction in spermatogenesis, tissue destruction and (D) complete calcification in few of the seminiferous tubules. (E) Photomicrographs of rat testis that received cadmium and ginseng extract (50 mg/kg) showing improvement in structure of seminiferous tubules and (F) 100 mg/kg ginseng extract showing regular spermatogenesis in the tubular epithelium.

spermography and testicular histological findings. Moreover, an increase in apoptotic spermatogonia and primary spermatocyte cells were found in seminiferous tubules of Cd-treated rats. *Panax* ginseng extract at two doses of 50 and 100 mg/kg on Cd-treated rats improved the adverse effects of Cd on testicular parameters. These results are in agreement with the findings of previous studies showing toxic effects of Cd in rat testes including harmful changes in the seminiferous tubules and sperm parameters along with

Table 2. Effects of cadmium (Cd) and different doses of ginseng (50 and 100 mg/kg) on testicular parameters.

	Control	Vehicle	Cadmium	Cd + Gi50	Cd + Gi100
Spermatogonia cell number	63.2 ± 2.03	60 ± 3.16	21.9 ± 5.2^{ab}	52.5 ± 7.35^{cd}	73.5 ± 5.96^{c}
Primary spermatocyte cell number	142 ± 5.92	150 ± 7.07	34.2 ± 8.06^{ab}	63.2 ± 10.38^{ab}	96 ± 8.84^{abcd}
Leydig cell number	5.4 ± 0.34	6 ± 0.44	4 ± 0.66	3.7 ± 0.26^{ab}	4.8 ± 0.29
Seminiferous tubules diameters (μm)	437.6 ± 10.21	455.2 ± 13.32	259.3 ± 12.55^{ab}	361.2 ± 18.64^{abc}	420.4 ± 12.66^{cd}
Germinal epithelium height (μm)	161.2 ± 9.41	167 ± 12.8	60.3 ± 4.34^{ab}	116.7 ± 7.7^{abc}	123.6 ± 4.9^{abc}

Results are expressed as mean ± SEM. Significant differences ($p < 0.05$) are indicated by [a]; vs. the control group in the same row, [b]; vs. the vehicle group in the same row, [c]; vs. the Cd group in the same row, [d]; vs. the Cd + Gi 50 group in the same row, [e]; vs. the Cd + Gi 100 group in the same row.

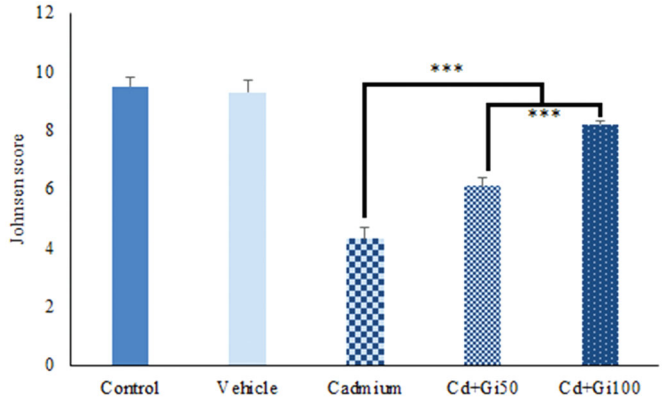

Figure 4. Semi-quantitative indicator of spermatogenesis (Johnson's score) in testes of different experimental groups. Ginseng therapy preserved germ cells order inside seminiferous tubules in cadmium treated groups. Significant differences are expressed as $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

pro-apoptotic of Cd potential in rodent testicular germ cells. (Erboga *et al.* 2016, Rinaldi *et al.* 2017, Wu *et al.* 2017, Chemek *et al.* 2018).

Testicular oxidative stress, by overproduction of oxygen radicals and dysfunction of the antioxidant defense systems, has repeatedly been reported as the main mechanism of cadmium's toxic effects leading to male infertility (Cuypers *et al.* 2010, Nemmiche 2017, Wang *et al.* 2017, He *et al.* 2018). In this study, Cd significantly diminished the total antioxidant capacity and increased lipid peroxidation (LPO). Therefore, the primary antioxidant system is defeated against free radicals. Our findings, especially heightened LPO and decreased TAC levels in the Cd-challenged rats, are indicative of oxidative stress and are compatible with the reports of some investigators (Casalino *et al.* 2002, Ognjanovic *et al.* 2003, Park *et al.* 2013, Rajeshkumar *et al.* 2013, Hakemi *et al.* 2019). The marked increase in the LPO in the Cd group can damage the cellular membrane and cytoplasmic membrane structures. Also, Cd may cause an increase in oxidative stress by binding to sulfhydryl groups of proteins and critical molecules in mitochondria. Such changes like damaging polyunsaturated fatty acids in sperm plasma membrane by reactive oxygen species (ROS) might result in decreased sperm motility (Acharya *et al.* 2008, Ma *et al.* 2013). Cd could decrease adenosine triphosphate (ATP) production and sperm motility via promoting tyrosine phosphorylation of dihydrolipoamide dehydrogenase (DLD) and inhibit its dehydrogenase activity (Zhao *et al.* 2017). In the present study, sperm count and viability decreased significantly in Cd-exposed rats. Also, immotile sperm was higher in rats exposed to cadmium in the other groups. In agreement with our data, Adamkovicova *et al.* reported that sperm characteristics markedly affected after exposure to Cd when compared to control (Adamkovicova *et al.* 2016). Leydig cell number and testosterone level were not significantly changed in the cadmium group in comparison to the control group, but cadmium can induce oxidative stress as reflected by increased lipid peroxidation and reduced total antioxidant capacity. This may explain the testicular damage seen in our study. The results of the different oxidative stress indicators in this study support this hypothesis.

The results of the present study, in accordance with other studies (Ji *et al.* 2012, Ji *et al.* 2013, Zhang *et al.* 2019), showed that Cd induces disorganization of the seminiferous epithelium and increases programmed cell death in rat germ cells. Apoptosis rate, determined by TUNEL staining, increased within the seminiferous tubules of Cd-treated rats. Apoptosis was confined to the spermatogonia and primary spermatocytes suggesting that there was higher sensitivity to Cd in mentioned cells rather than other epithelial cells. This cytotoxic agent could induce germ cell apoptosis afterward direct Cd-induced damage to the germ cells by increase gene expression and release of apoptosis-

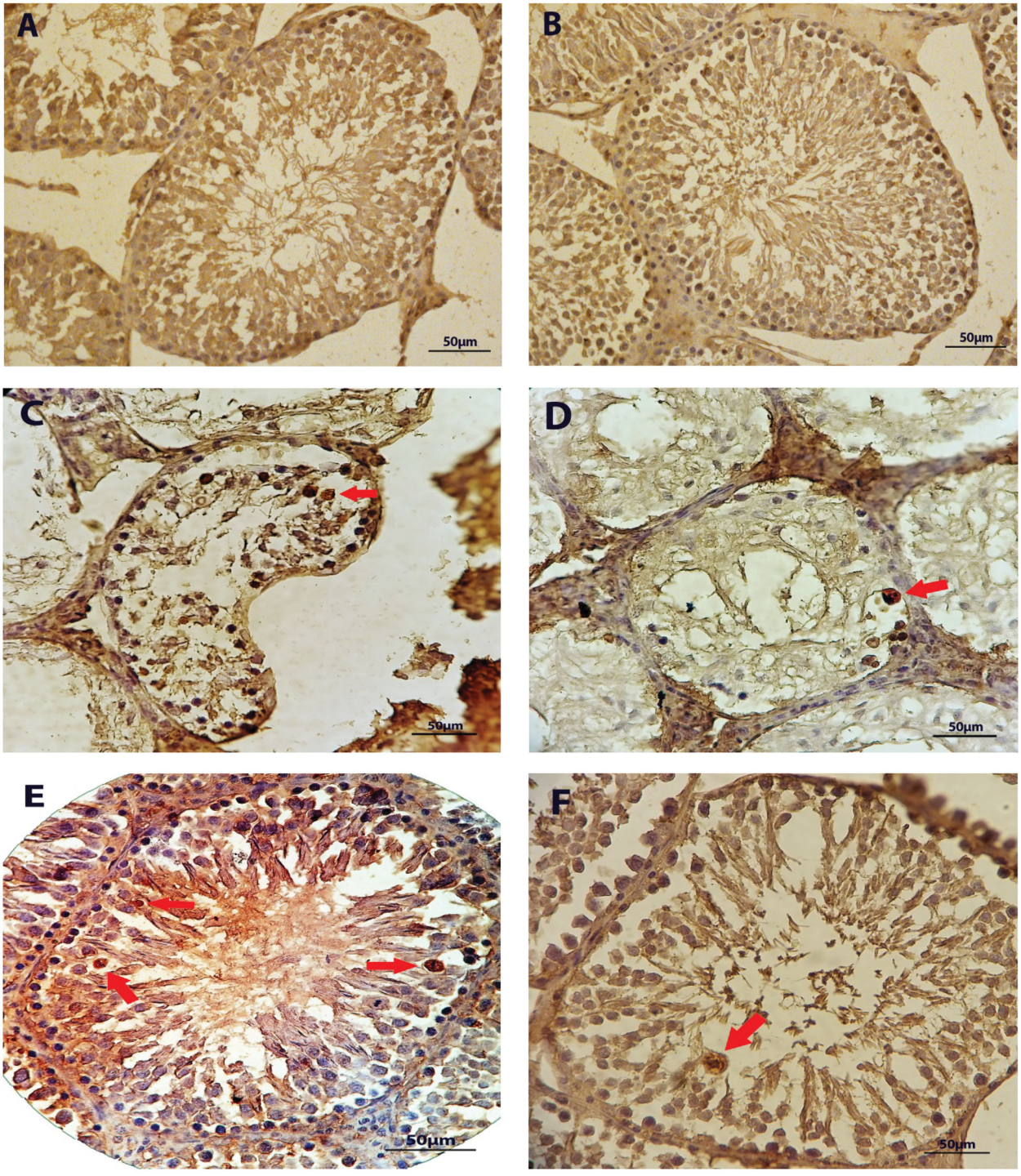

Figure 5. Immunohistochemical staining in rat testis sections after 9 weeks of treatment (×400). Photomicrograph of TUNEL-stained rat testicular sections in control (A) and vehicle (B) groups showing absence of positively stained cells. The brown cells are broken DNA stained with diaminobenzidine. (C, D) The most of testicular germ cells is undergoing apoptosis in cadmium testes. (E) Marked decrease in apoptotic testicular germ cells was seen in rat testes which treated with ginseng extract at dose of 50 mg/kg, and (F) 100 mg/kg. TUNEL: terminal dUTP nick-end labeling.

inducing factor (AIF), which triggers cell death in the testes from Cd-exposed rats (Zongping 2013, Cupertino *et al.* 2017). Furthermore, Cd group exhibited necrosis and atrophy of testicular tissue which can be related to vascular failure in seminiferous tubules (Niknafs *et al.* 2015). These histopathological changes were parallel with a decline in total antioxidant capacity and increased malondialdehyde as a marker of lipid peroxidation. As the spermatozoa plasma membrane contains a high content of

polyunsaturated fatty acids, sperms are predominantly susceptible to oxidative damage. Therefore, Cd-induced oxidative stress might be a reason for germ cell death, sperm quality reduction, and finally infertility (Benoff *et al.* 2009, Mouro *et al.* 2019).

Nowadays, male infertility is a growing problem in the world and one of the main reasons is testicular oxidative stress, which may be induced by different pathophysiological conditions. Heavy metals including Cd, an inducer of oxidative stress, greatly diminish the testicular antioxidant enzymes (Siu *et al.* 2009, Farjad and Momeni 2018). Several studies reported the ameliorating effect of diverse plant extracts on Cd-induced testes toxicity (Ola-Mudathir *et al.* 2008, El-Shahat *et al.* 2009, Rajendar *et al.* 2011, Mitra *et al.* 2012, Khafaga *et al.* 2019) . The theory is that antioxidants compounds help cells to ward off damage from free radicals and minimize the impact of cadmium toxicity. In this study, for the first time, we examined the effects of different doses of ginseng in Cd-treated animals. In the present study, we found that in two Gi + Cd groups, ginseng could reverse the adverse effects of cadmium chloride on lipid peroxidation and total antioxidant capacity in the serum. *Panax* ginseng is one of the most valuable herbs in traditional Chinese medicine. *Panax* ginseng as a potent antioxidant was shown to scavenge free radicals such as hydroxyl and superoxide in *in vitro* study (Kim *et al.* 2002) and increase the capacity of the antioxidant defense system in the cells and tissues (Lee *et al.* 2017). *Panax* ginseng root extract contains saponin compounds (generally known as ginsenosides) such as triterpene and sugar moieties and nonsaponin compounds including polysaccharides at different concentrations. Among flavonoids component present in *P.* ginseng, rutin, catechin, gentisic, kaempferol, and quercetin have powerful antioxidant activities (Kim 2016). Ok *et al.* reported that when TM3 Leydig and TM4 Sertoli cells were treated with cultivated wild ginseng extract, activity and expression of dismutase 1 (SOD1), catalase (CAT), and glutathione peroxidase (GPx1/2) were induced (Ok *et al.* 2016).

In the present study, administration of *P.* ginseng 50 and 100 mg/kg could modulate Cd-induced imbalanced antioxidant enzyme and therefore could repair the sperm and testicular damages. Ramesh *et al.* (2012) reported that *P.* ginseng treatment (200 mg/kg/day for 4 months) in aged rats could improve sperm maturation and testicular functions by reducing MDA and increasing enzymatic and nonenzymatic antioxidants levels (Ramesh *et al.* 2012). They suggested that many antioxidants in *P.* ginseng including ginsenosides, trace elements, and vitamins play key roles in these alterations. Eskandari *et al.* (Eskandari *et al.* 2016) reported that *P.* ginseng treatment could improve the percentage of sperm cells with normal morphology and motility, as well as reduce the apoptotic indexes of spermatogenic cells in epididymo-orchitis rats. In our study, total sperm count, percentage of total motile, and progressively motile sperm dramatically decreased 9 weeks after the acute challenge with cadmium chloride. Logically, it seems that damage to testicular tissue and spermatogenesis eventually leads to a drastic reduction in the sperm count in the tail of epididym. These results are a line with previous studies that reported the deleterious effect of acute and chronic cadmium toxicity on sperm production and quality (El-Demerdash *et al.* 2004, Li *et al.* 2010, Meeker *et al.* 2008, Oliveira *et al.* 2009, Tvrdá *et al.* 2013). Conversely, good preservation of testicular tissue in case of encountering cadmium toxicity could reverse this condition. It has been shown that nutrients that have antioxidant properties such as vitamin E, vitamin C, zinc, and magnesium could maintain sperm quality of toxic animals with cadmium as good as healthy pair (Acharya *et al.* 2008, Babaknejad *et al.* 2018). *Panax* ginseng has very strong antioxidant effects that have been proven in numerous animal and human studies (Kim *et al.* 2005, Zhang *et al.* 2008, Kim *et al.* 2011, Chen and Huang 2019) and effectively, supported the spermatogenesis and production of normal motile sperm in ours study.

In the current study, no changes in the liver enzyme levels were seen following the administration of different doses of ginseng. Also, no significant difference was observed in the serum ALT and AST levels between the animals in different groups. However, Park *et al.* showed that pretreatment with *P.* ginseng significantly improved liver damage due to cadmium exposure in rats (Park *et al.* 2013). In the present study, no significant difference was detected in total testosterone levels among all animals. In contrast, Liu reported that the level of circulating testosterone is decreased in cadmium-exposed rats against control animals (Liu *et al.* 2013).

In conclusion, the result of this study indicates that administration of different doses of *P.* ginseng water extract could ameliorate the testes structure and sperm parameters and reverse the levels of MDA and total antioxidant capacity in $CdCl_2$-induced testicular toxicity in Wistar rats. Although the exact mechanism of action of *P.* ginseng in cells cannot be understood, it could partially be due to anti-oxidative and anti-apoptotic actions in gonado-protection by *P.* ginseng.

Thus, *P. ginseng* may have a role in the treatment of some patients suffering from male infertility.

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References

Abdel-Wahhab, M.A. and Ahmed, H.H., 2004. Protective effect of Korean *Panax ginseng* against chromium VI toxicity and free radicals generation in rats. *Journal of ginseng research*, 28, 11–17.

Abolhassani, M., *et al.*, 2019. Organochlorine and organophosphorous pesticides may induce colorectal cancer; a case-control study. *Ecotoxicology and environmental safety*, 178, 168–177.

Acharya, U.R., *et al.*, 2008. Effect of vitamins C and E on spermatogenesis in mice exposed to cadmium. *Reproductive toxicology*, 25 (1), 84–88.

Adamkovicova, M., *et al.*, 2016. Sperm motility and morphology changes in rats exposed to cadmium and diazinon. *Reproductive biology and endocrinology*, 14 (1), 42.

Adebayo, A., Oke, B., and Akinloye, A., 2009. Characterizing the gonadosomatic index and its relationship with age in greater cane rat (*Thryonomys swinderianus*, Temminck). *Journal of veterinary anatomy*, 2 (2), 53–59.

Babaknejad, N., *et al.*, 2018. Cadmium testicular toxicity in male Wistar rats: protective roles of zinc and magnesium. *Biological trace element research*, 185 (1), 106–115.

Benoff, S., *et al.*, 2009. Cadmium concentrations in blood and seminal plasma: correlations with sperm number and motility in three male populations (infertility patients, artificial insemination donors, and unselected volunteers). *Molecular medicine*, 15 (7–8), 248–262.

Benzie, I.F. and Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, 239 (1), 70–76.

Birben, E., *et al.*, 2012. Oxidative stress and antioxidant defense. *World allergy organization journal*, 5, 9–19.

Casalino, E., *et al.*, 2002. Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium. *Toxicology*, 179 (1–2), 37–50.

Chemek, M., *et al.*, 2018. Involvement of testicular DAAM1 expression in zinc protection against cadmium-induced male rat reproductive toxicity. *Journal of cellular physiology*, 233 (1), 630–640.

Chen, F. and Huang, G., 2019. Antioxidant activity of polysaccharides from different sources of ginseng. *International journal of biological macromolecules*, 125, 906–908.

Chung, N.P. and Cheng, C.Y., 2001. Is cadmium chloride-induced inter-Sertoli tight junction permeability barrier disruption a suitable in vitro model to study the events of junction disassembly during spermatogenesis in the rat testis? *Endocrinology*, 142 (5), 1878–1888.

Cupertino, M.C., *et al.*, 2017. Differential susceptibility of germ and leydig cells to cadmium-mediated toxicity: impact on testis structure, adiponectin levels, and steroidogenesis. *Oxidative medicine and cellular longevity*, 2017, 1–11.

Cuypers, A., *et al.*, 2010. Cadmium stress: an oxidative challenge. *Biometals: an international journal on the role of metal ions in biology, biochemistry, and medicine*, 23 (5), 927–940.

El-Demerdash, F.M., *et al.*, 2004. Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and β-carotene. *Food and chemical toxicology*, 42 (10), 1563–1571.

El-Shahat, A.E.-R., *et al.*, 2009. Altered testicular morphology and oxidative stress induced by cadmium in experimental rats and protective effect of simultaneous green tea extract. *International journal of morphology*, 27 (3), 757–764.

Erboga, M., *et al.*, 2016. Anti-apoptotic and anti-oxidant effects of caffeic acid phenethyl ester on cadmium-induced testicular toxicity in rats. *Biological trace element research*, 171 (1), 176–184.

Eskandari, M., *et al.*, 2016. Ameliorating effect of ginseng on epididymo-orchitis inducing alterations in sperm quality and spermatogenic cells apoptosis following infection by uropathogenic Escherichia coli in rats. *Cell journal*, 18, 446.

Farjad, E. and Momeni, H.R., 2018. Silymarin ameliorates oxidative stress and enhances antioxidant defense system capacity in cadmium-treated mice. *Cell journal*, 20, 422.

Fowler, B.A., 2009. Monitoring of human populations for early markers of cadmium toxicity: a review. *Toxicology and applied pharmacology*, 238 (3), 294–300.

Friberg, L., 2018. *Cadmium in the environment*. Boca Raton (FL): CRC press.

Genchi, G., *et al.*, 2020. The effects of cadmium toxicity. *International journal of environmental research and public health*, 17 (11), 3782.

Ghanbari, E., Nejati, V., and Khazaei, M., 2016. Antioxidant and protective effects of Royal jelly on histopathological

changes in testis of diabetic rats. *International journal of reproductive biomedicine*, 14, 519.

Godt, J., *et al.*, 2006. The toxicity of cadmium and resulting hazards for human health. *Journal of occupational medicine and toxicology*, 1, 22.

Goyer, R.A., Liu, J., and Waalkes, M.P., 2004. Cadmium and cancer of prostate and testis. *Biometals: an international journal on the role of metal ions in biology, biochemistry, and medicine*, 17 (5), 555–558.

Hakemi, S.G., *et al.*, 2019. The effects of olive leaf extract on the testis, sperm quality and testicular germ cell apoptosis in male rats exposed to busulfan. *International journal of fertility & sterility*, 13, 57.

Harborne, J., 1973. *Textbook of phytochemical methods*. 1st ed. London, UK: Champraan and Hall Ltd., 110–113.

He, L., *et al.*, 2018. Protective effects of proanthocyanidins against cadmium-induced testicular injury through the modification of Nrf2-Keap1 signal path in rats. *Environmental toxicology and pharmacology*, 57, 1–8.

Ji, Y.L., *et al.*, 2012. Melatonin alleviates cadmium-induced cellular stress and germ cell apoptosis in testes. *Journal of pineal research*, 52 (1), 71–79.

Ji, Y.-L., *et al.*, 2013. N-acetylcysteine protects against cadmium-induced germ cell apoptosis by inhibiting endoplasmic reticulum stress in testes. *Asian Journal of andrology*, 15 (2), 290–296.

Karadeniz, A., Cemek, M., and Simsek, N., 2009. The effects of *Panax ginseng* and Spirulina platensis on hepatotoxicity induced by cadmium in rats. *Ecotoxicology and environmental safety*, 72 (1), 231–235.

Khafaga, A.F., *et al.*, 2019. The potential modulatory role of herbal additives against Cd toxicity in human, animal, and poultry: a review. *Environmental science and pollution research international*, 26 (5), 4588–4604.

Kim, J.-S., 2016. Investigation of phenolic, flavonoid, and vitamin contents in different parts of Korean Ginseng (*Panax ginseng* CA Meyer). *Preventive nutrition and food science*, 21 (3), 263–270.

Kim, Y.K., Guo, Q., and Packer, L., 2002. Free radical scavenging activity of red ginseng aqueous extracts. *Toxicology*, 172 (2), 149–156.

Kim, S., *et al.*, 2005. Effects of *Panax ginseng* extract on exercise-induced oxidative stress. *Journal of sports medicine and physical fitness*, 45, 178.

Kim, H.-G., *et al.*, 2011. Antioxidant effects of *Panax ginseng* CA Meyer in healthy subjects: a randomized, placebo-controlled clinical trial. *Food and chemical toxicology*, 49 (9), 2229–2235.

Lee, Y.-M., *et al.*, 2017. Implications of red *Panax ginseng* in oxidative stress associated chronic diseases. *Journal of ginseng research*, 41 (2), 113–119.

Leung, K.W. and Wong, A.S., 2013. Ginseng and male reproductive function. *Spermatogenesis*, 3 (3), e26391.

Li, H. and Li, H., 2019. Ginsenoside-Rg5 inhibits growth and metastasis of ovarian carcinoma via suppressing expression of fibroblast growth factor-8b (FGF8b). *Journal of King Saud University-science*, 32 (1), 1162–1167.

Liu, Q., *et al.*, 2013. Effect of cadmium on rat Leydig cell testosterone production and DNA integrity in vitro. *Biomedical and environmental sciences*, 26, 769.

Liu, J., Qu, W., and Kadiiska, M.B., 2009. Role of oxidative stress in cadmium toxicity and carcinogenesis. *Toxicology and applied pharmacology*, 238 (3), 209–214.

Li, X.-H., *et al.*, 2010. Effects of cadmium on testis spermatocyte apoptosis and epididymis mature spermatozoa quality of mice. *Chinese journal of applied ecology*, 4, 987–992.

Ma, D., *et al.*, 2013. Oxidative damages and ultrastructural changes in the sperm of freshwater crab Sinopotamon henanense exposed to cadmium. *Ecotoxicology and environmental safety*, 98, 244–249.

Mandegary, A., *et al.*, 2012. Anticonvulsant activity of the essential oil and methanolic extract of *Bunium persicum* (Boiss). B. Fedtsch. *Journal of ethnopharmacology*, 140 (2), 447–451.

Meeker, J.D., *et al.*, 2008. Cadmium, lead, and other metals in relation to semen quality: human evidence for molybdenum as a male reproductive toxicant. *Environmental health perspectives*, 116 (11), 1473–1479.

Mehraein, F. and Negahdar, F., 2011. Morphometric evaluation of seminiferous tubules in aged mice testes after melatonin administration. *Cell journal*, 13, 1.

Menkveld, R., 2010. Clinical significance of the low normal sperm morphology value as proposed in the fifth edition of the WHO Laboratory Manual for the examination and processing of human semen. *Asian journal of andrology*, 12 (1), 47–58.

Mitra, E., *et al.*, 2012. Protective effect of aqueous curry leaf (*Murraya koenigii*) extract against cadmium-induced oxidative stress in rat heart. *Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association*, 50 (5), 1340–1353.

Mortazavi, N., *et al.*, 2019. Organochlorine and organophosphorus pesticides and bladder cancer: a case-control study. *Journal of cellular biochemistry*, 120 (9), 14847–14859.

Mouro, V.G., *et al.*, 2019. Subacute testicular toxicity to cadmium exposure Intraperitoneally and orally. *Oxidative medicine and cellular longevity*, 2019, 1–14.

Mouro, V.G.S., *et al.*, 2020. Cadmium-induced testicular toxicity in mice: subacute and subchronic route-dependent effects. *Biological trace element research*, 193 (2), 466–482.

Murphy, C.J. and Richburg, J.H., 2014. Implications of Sertoli cell induced germ cell apoptosis to testicular pathology. *Spermatogenesis*, 4 (2), e979110.

Nemmiche, S., 2017. Oxidative signaling response to cadmium exposure. *Toxicological sciences*, 156 (1), 4–10.

Niknafs, B., Salehnia, M., and Kamkar, M., 2015. Induction and determination of apoptotic and necrotic cell death by cadmium chloride in testis tissue of mouse. *Journal of reproduction & infertility*, 16 (1), 24–29.

Ognjanovic, B., *et al.*, 2003. Protective influence of vitamin E on antioxidant defense system in the blood of rats treated with cadmium. *Physiological research*, 52, 563–570.

Ok, S., Kang, J.S., and Kim, K.M., 2016. Testicular antioxidant mechanism of cultivated wild ginseng extracts. *Molecular & cellular toxicology*, 12 (2), 149–158.

Ola-Mudathir, K.F., *et al.*, 2008. Protective roles of onion and garlic extracts on cadmium-induced changes in sperm characteristics and testicular oxidative damage in rats. *Food and chemical toxicology*, 46 (12), 3604–3611.

Olaniyi, K.S., *et al.*, 2020. Protective role of glutamine against cadmium-induced testicular dysfunction in Wistar rats: involvement of G6PD activity. *Life sciences*, 242, 117250.

Oliveira, H., *et al.*, 2009. Adverse effects of cadmium exposure on mouse sperm. *Reproductive toxicology*, 28 (4), 550–555.

Ong, W.-Y., *et al.*, 2015. Protective effects of ginseng on neurological disorders. *Frontiers in aging neuroscience*, 7, 129.

Pařízek, J., 1964. Vascular changes at sites of oestrogen biosynthesis produced by parenteral injection of cadmium salts: the destruction of placenta by cadmium salts. *Reproduction*, 7, 263–265.

Park, S.J., *et al.*, 2013. Protective effects of Korean red ginseng extract on cadmium-induced hepatic toxicity in rats. *Journal of ginseng research* , 37 (1), 37–44.

Paydar, P., *et al.*, 2018. The role of acetylcholinesterase, paraoxonase, and oxidative stress in breast tumors. *International journal of cancer management*, 11 (11), e83370.

Rajendar, B., *et al.*, 2011. Protective effect of an aphrodisiac herb Tribulus terrestris Linn on cadmium-induced testicular damage. *Indian journal of pharmacology*, 43 (5), 568–573.

Rajeshkumar, S., Mini, J., and Munuswamy, N., 2013. Effects of heavy metals on antioxidants and expression of HSP70 in different tissues of Milk fish (Chanos chanos) of Kaattuppalli Island, Chennai, India. *Ecotoxicology and environmental safety*, 98, 8–18.

Ramesh, T., *et al.*, 2012. Panax ginseng reduces oxidative stress and restores antioxidant capacity in aged rats. *Nutrition research*, 32 (9), 718–726.

Rinaldi, M., *et al.*, 2017. Cadmium, organ toxicity and therapeutic approaches: a review on brain, kidney and testis damage. *Current medicinal chemistry*, 24 (35), 3879–3893.

Saddein, E., *et al.*, 2019. Preventative effects of vitamin E on testicular damage and sperm parameters in the first-generation mice pups due to pre-and postnatal mancozeb exposure. *Journal of toxicology*, 2019, 4763684.

Salari, S., *et al.*, 2016. Evaluation of antifungal activity of standardized extract of *Salvia rhytidea* Benth. (Lamiaceae) against various *Candida* isolates. *Journal de mycologie medicale*, 26 (4), 323–330.

Sarhadynejad, Z., *et al.*, 2016. Pharmacological safety evaluation of a traditional herbal medicine "Zereshk-e-Saghir" and assessment of its hepatoprotective effects on carbon tetrachloride induced hepatic damage in rats. *Journal of ethnopharmacology*, 190, 387–395.

Shao, J.-W., *et al.*, 2019. Therapeutic potential of ginsenosides on diabetes: from hypoglycemic mechanism to clinical trials. *Journal of functional foods*, 64, 103630.

Sharififar, F., *et al.*, 2012. Essential oil and methanolic extract of *Zataria multiflora* Boiss with anticholinesterase effect. *Pakistan journal of biological sciences*, 15 (1), 49–53.

Siu, E.R., *et al.*, 2009. Cadmium-induced testicular injury. *Toxicology and applied pharmacology*, 238 (3), 240–249.

Skipper, A., *et al.*, 2016. Cadmium chloride induces DNA damage and apoptosis of human liver carcinoma cells via oxidative stress. *International journal of environmental research and public health*, 13 (1), 88.

Tvrdá, E., *et al.*, 2013. The impact of lead and cadmium on selected motility, prooxidant and antioxidant parameters of bovine seminal plasma and spermatozoa. *Journal of environmental science and health, part A*, 48 (10), 1292–1300.

Valenzuela, A., 1991. The biological significance of malondialdehyde determination in the assessment of tissue oxidative stress. *Life sciences*, 48 (4), 301–309.

Valko, M., Morris, H., and Cronin, M., 2005. Metals, toxicity and oxidative stress. *Current medicinal chemistry*, 12 (10), 1161–1208.

Wang, B., *et al.*, 2013. Ginsenoside Rd maintains adult neural stem cell proliferation during lead-impaired neurogenesis. *Neurological sciences*, 34 (7), 1181–1188.

Wang, Y., *et al.*, 2017. Role of autophagy in cadmium-induced testicular injury. *Human & experimental toxicology*, 36 (10), 1039–1048.

Worek, F., *et al.*, 1999. Improved determination of acetylcholinesterase activity in human whole blood. *Clinica chimica acta*, 288 (1–2), 73–90.

Wu, X., *et al.*, 2017. A brief exposure to cadmium impairs Leydig cell regeneration in the adult rat testis. *Scientific reports*, 7, 1–11.

Zare, M., *et al.*, 2020. The prophylactic effect of date palm (*Phoenix dactylifera* L.) fruit extract on testicular toxicity induced by formaldehyde: an experimental study. *International journal of reproductive biomedicine*, 18, 275.

Zhang, Y., Li, S., and Li, S., 2019. Relationship between cadmium content in semen and male infertility: a meta-analysis. *Environmental science and pollution research*, 26 (2), 1947–1953.

Zhang, Q.H., *et al.*, 2008. Protective effects of total saponins from stem and leaf of *Panax ginseng* against cyclophosphamide-induced genotoxicity and apoptosis in mouse bone marrow cells and peripheral lymphocyte cells. *Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association*, 46 (1), 293–302.

Zhao, L.-L., *et al.*, 2017. Reproductive effects of cadmium on sperm function and early embryonic development in vitro. *PLoS one*, 12 (11): e0186727.

Zhu, Q., Li, X., and Ge, R.-S., 2020. Toxicological effects of cadmium on mammalian testis. *Frontiers in genetics*, 11, 527.

Zongping, L., 2013. Oxidative stress and mitogen-activated protein kinase pathways involved in cadmium-induced BRL 3A cell apoptosis. *Oxidative medicine and cellular longevity*, 2013, 516051.