Biol Trace Elem Res (2014) 159:278–287
DOI 10.1007/s12011-014-9955-1

Effect of *Physalis peruviana* L. on Cadmium-Induced Testicular Toxicity in Rats

Mohamed S. Othman · Ahmed Nada · Hassan S. Zaki · Ahmed E. Abdel Moneim

Received: 21 February 2014 / Accepted: 24 March 2014 / Published online: 13 April 2014

Abstract Cadmium (Cd) stimulates the production of reactive oxygen species and causes tissue damage. We investigated here the protective effect of *Physalis peruviana* L. (family Solanaceae) against cadmium-induced testes toxicity in rats. Twenty-eight Wistar albino rats were used. They were divided into four groups ($n=7$). Group 1 was used as control. Group 2 was intraperitoneally injected with 6.5 mg/kg body weight (bwt) of cadmium chloride for 5 days. Group 3 was orally treated with 200 mg/kg bwt of methanolic extract of physalis (MEPh). Group 4 was pretreated with MEPh before cadmium for 5 days. Changes in body and testes weights were determined. Oxidative stress markers, antioxidant enzymes, and testosterone level were measured. Histopathological changes of testes were examined, and the immunohistochemical staining for the proapoptotic (caspase-3) protein was performed. The injection of cadmium caused a significant decrease in body weight, while a significant increase in testes weight and testes weight index was observed. Pretreatment with MEPh was associated with significant reduction in the toxic effects of Cd as shown by reduced testicular levels of malondialdehyde, nitric oxide, and caspase-3 expression and increased glutathione content, and the activities of superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, and testosterone were also increased. Testicular histopathology showed that Cd produced an extensive germ cell apoptosis, and the pretreatment of MEPh in Cd-treated rats significantly reduced Cd-induced testicular damage. On the basis of the above results, it can be hypothesized that *P. peruviana* L. has a protective effect against cadmium-induced testicular oxidative stress and apoptosis in the rat.

Keywords *Physalis peruviana* L · Cadmium · Oxidative stress · Apoptosis · Testes

Abbreviations

Cas-3	Caspase-3
CAT	Catalase
Cd	Cadmium
$CdCl_2$	Cadmium chloride
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
MDA	Malondialdehyde
MEPh	Methanolic extract of physalis
NO	Nitric oxide
ROS	Reactive oxygen species
SOD	Superoxide dismutase

Introduction

Cadmium (Cd) is a toxic heavy metal in the environment. Cd is a highly accumulative toxicant with very long biological half-life of over 20 years [1]. Cd is not biodegradable and its levels in the environment are increasing due to industrial activities, and human exposures to Cd are inevitable. Acute Cd exposure produced toxicities to the lung, liver, testes, and brain, while chronic exposure to Cd often leads to renal

M. S. Othman · A. Nada · H. S. Zaki
Faculty of Biotechnology, October University for Modern Science and Arts (MSA), Giza, Egypt

A. E. Abdel Moneim
Department of Zoology and Entomology, Faculty of Science, Helwan University, Cairo, Egypt

A. E. Abdel Moneim (✉)
Department of Biochemistry and Molecular Biology, Asturias Institute of Biotechnology, University of Oviedo, Oviedo, Spain
e-mail: aest1977@hotmail.com

dysfunction, anemia, osteoporosis, and bone fractures. Cd is a potent carcinogen in a number of tissues of rodents and is classified as a human carcinogen [2, 1].

Cadmium is absorbed via the lungs in significant quantities from cigarette smoke, and it has toxic effects on the male reproductive system [3] which may account for the recent declining fertility in men in developed countries. The role of Cd in the modulation of male reproductive health stems from the observations that high levels of Cd in seminal fluid are associated with asthenozoospermia in infertile males [4]. Rodent testes are especially sensitive to the toxic effects of Cd exposure. Cd impairs reproductive capacity by causing severe testicular degeneration, seminiferous tubule damage, and necrosis in rats [5]. The adverse consequences of exposure to this heavy metal on the reproductive organs have been widely considered. After acute exposure, Cd-induced damage can be found at interstitial and tubular levels. Permeability changes in the capillary endothelium (which cause edema, hemorrhage or necrosis) seem to be implicated in the histopathological mechanism of these lesions [6, 7]. Furthermore, animal studies demonstrated a time- and dose-dependent reduction in sperm motility following Cd exposures [8]. Cd-induced toxicity to the testis is probably the result of interactions of a complex network of causes. Cd is associated with its capacity to induce oxidative stress and apoptosis of the germ cells of humans and animals. Cd has been shown to increase the expression of proapoptotic proteins p53 and Bax while reducing the expression of Bcl-2 [9].

Physalis peruviana L. of family Solanaceae is a species indigenous to South America. It is commonly used as folk medicine for treating cancer, leukemia, hepatitis, rheumatism, and other diseases [10, 11]. Major bioactive compounds of *Physalis* spp., such as physalins (B, D, and F) and glycosides (such as myricetin-3-*O*-neohesperidoside), have been shown to possess anticancer activities. Previous phytochemical studies have isolated a number of compounds from physalis, such as ticloidine, phygrine, 28-hydroxywithanolide, and 4-β-hydroxywithanolide E, withanolides, withaphysanolide, and viscosalactone [12]. Ethanol extract of physalis showed potent cytotoxic effect against Hep G2 cells, and its mechanism of action was found to relate to a mitochondria-mediated apoptotic pathway [13]. This extract also showed potent xanthine oxidase inhibition and antilipid peroxidation activities [14].

Hence, the present study was designed to examine the protective effect of *P. peruviana* L. against cadmium-induced reproductive toxicity in rats. Such investigation will explore the potential therapeutic or preventive approaches that can be developed in future studies by minimizing or blocking the destructive effects of Cd to testicular function in men.

Materials and Methods

Chemicals

Cadmium chloride ($CdCl_2$; CAS Number 10043-52-4), nitroblue tetrazolium, *N*-(1-naphthyl)ethylenediamine, and Tris-HCl were purchased from Sigma (St. Louis, MO, USA). Thiobarbituric acid and trichloroacetic acid were purchased from Merck. All other chemicals and reagents used in this study were of analytical grade. Double-distilled water was used as the solvent.

Animals

Twenty-eight adult male Wistar albino rats weighing 200–250 g (10–12 weeks) were obtained from The Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). The animals were kept in wire-bottomed cages in a room under standard condition of illumination with a 12-h light–dark cycle at 25±1 °C for one week until the beginning of treatment. They were provided with tap water and balanced diet ad libitum. All animals have received human care in compliance with the state authorities following the Egyptian rules of animal protection.

Plant Material

P. peruviana L. fresh fruits were collected from market of East Cairo, Egypt in the months of February–March, 2012. The plant material was authenticated in Botany Department, Faculty of Science, Helwan University, Cairo, Egypt, on the basis of taxonomic characters and by direct comparison with the herbarium specimens that are available at the herbarium of the Botany Department.

Physalis Extract Preparation

The fresh fruits of *P. peruviana* L. (10 kg) were separated from their calyxes and homogenized. The pulp was consecutively macerated for 1 day in petroleum ether, ethyl acetate, chloroform, and methanol, respectively. On basis of the preliminary phytochemical tests conducted, the methanol extract was found to be rich in terms of chemical constituents and therefore was selected for the experiment. The methanol was removed under reduced pressure to obtain a semisolid mass of methanolic extract of physalis (MEPh). The MEPh was then stored in −20 °C until used.

Experimental Protocol

To study the protective effects of physalis on cadmium-mediated reproductive toxicity, Adult male rats were randomly allocated to four groups of seven rats each. Group I (Con)

served as control and received 300 μL of saline by oral administration route every day for 5 days. Group II (Cd) received daily 300 μL of saline by oral administration, and after 1 hour, they received intraperitoneal injection of 6.5 mg $CdCl_2$/kg body weight (bwt) for 5 days. Group III (MEPh) received daily oral administration of 200 mg MEPh/kg bwt for 5 days, and the animals of group IV (MEPh + Cd) were administrated with 200 mg MEPh/kg bwt 1 hour before cadmium injection and daily for 5 days. After 24 h of the last injection of $CdCl_2$, the animals of all groups were sacrificed by cervical dislocation under diethyl ether anesthesia and blood samples were collected for serum analysis. Right testis was excised, weighed, and homogenized immediately to give 50 % (*w*/*v*) homogenate in ice-cold medium containing 50 mM Tris-HCl, pH 7.4. The homogenate was centrifuged at 3,000 rpm for 10 min at 4 °C. The supernatant (10 %) was used for the various biochemical determinations.

Testes Index

Relative weight of testes was calculated as left testis weight/body weight×100.

Cadmium Concentration

Cadmium concentration in the testis was determined by the method of Nürnberg [15]. The testes were digested with a mixture of nitric acid, perchloric acid, and sulfuric acid in the ratio 6:1:1. Cadmium concentration was measured by atomic spectrophotometry at 228.8 nm (Perkin-Elmer model 5000). The estimated limit of Cd detection by this method was 0.001 ppm.

Biochemical Estimations

Oxidative Stress

Nitrite/nitrate (NO) and malondialdehyde (MDA) were assayed colorimetrically in testes homogenates according to the methods of Green et al. [16] and Ohkawa et al. [17], respectively, where MDA was determined by using 1 mL of trichloroacetic acid 10 % and 1 mL of thiobarbituric acid 0.67 % which were then heated in a boiling water bath for 30 min. Thiobarbituric acid-reactive substances (TBARS) were determined by the absorbance at 535 nm and expressed as MDA formed. Nitric oxide was determined by optimized acid reduction method at 540 nm.

In addition, testicular glutathione (GSH) was determined by the methods of Ellman [18]. This method is based on the reduction of Ellman's reagent (5,5′ dithiobis (2-nitrobenzoic acid) "DTNB") with GSH to produce a yellow compound. The reduced chromogen was directly proportional to GSH concentration and its absorbance can be measured at 405 nm.

Enzymatic Antioxidant Status

The activities of testicular antioxidant enzymes as catalase (CAT) were assayed by the method of Aebi [19]. As a result, CAT reacts with a known quantity of H_2O_2. The reaction is stopped exactly after 1 min with catalase inhibitor. In the presence of peroxidase (HRP), the remaining H_2O_2 reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity that is inversely proportional to the amount of CAT in the original sample. The resulting chromophore had a bright rose color which can be measured at 510 nm. Testicular superoxide dismutase (SOD) activity was assayed by the method of Nishikimi et al. [20]. This assay relies on the ability of the enzyme to inhibit the phenazine methosulfate-mediated reduction of nitroblue tetrazolium dye. Glutathione-*S*-transferase (GST) activity in the testes was assayed by the method of Habig et al. [21]. The total GST activity was done by measuring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione. The conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST activity in the sample. Testicular glutathione peroxidase (GPx) activity was measured by the method of Paglia and Valentine [22], and the assay was an indirect measurement of the activity of GPx. Oxidized glutathione, produced upon reduction of organic peroxide by GPx, was recycled to its reduced state by the enzyme glutathione reductase (GR). The reaction was initiated by the addition of hydrogen peroxide, and the oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm. Finally, glutathione reductase activity of the testes was assayed by the method of Factor et al. [23]. GR catalyzes the reduction of glutathione in the presence of NADPH, which is oxidized to $NADPH^+$. As a result, the decrease in absorbance at 340 nm was measured.

Estimation of Serum Testosterone

Quantitative measurement of serum testosterone was carried out adopting ELISA technique using kits specific for rats purchased from BioVendor (Gunma, Japan) according to the protocol provided with each kit.

Histological Examination

The testes tissues were collected and immediately fixed with 10 % buffered formalin and embedded in paraffin. Sections (5–7 μm) were prepared and then stained with hematoxylin and eosin dye for photomicroscopic observations.

Immunohistochemistry for Detection of Caspase-3

For immunohistochemistry detection, testes sections (4 μm) were deparaffinized and then boiled to unmask antigen sites; the endogenous activity of peroxidase was quenched with 0.03 % H_2O_2 in absolute methanol. Testes sections were incubated overnight at 4 °C with a 1:200 dilution of a goat polyclonal caspase-3 antibody (Santa Cruz CA, USA) in phosphate-buffered saline (PBS). After removal of the unbound primary antibodies by rinsing with PBS, slides were incubated with a 1:500 dilution of biotinylated antigoat secondary antibody. Bound antibodies were detected with avidin-biotinylated peroxidase complex ABC-kit Vectastain, and the chromogen 3,3′-diaminobenzidine tetrachloride (DAB) is used as substrate. After appropriate washing in PBS, slides were counterstained with hematoxylin. All sections were incubated under the same conditions with the same concentration of antibodies and at the same time; so, the immunostaining was comparable among the different experimental groups.

Real-Time PCR

Total RNA was isolated from the testes tissue using an RNeasy Plus Minikit (Qiagen, Valencia, CA). One microgram total RNA and random primers was used for complementary DNA (cDNA) synthesis using the RevertAid™ H Minus Reverse Transcriptase (Fermentas, Thermo Fisher Scientific Inc., Canada). For real-time PCR analysis, the cDNA samples are run in triplicate and β-actin is used as reference gene. Each PCR amplification includes non-template controls containing all reagents except cDNA. Real-time PCRs were performed using Power SYBR® Green (Life Technologies, CA) and were conducted on the Applied Biosystems 7500 instrument. The typical thermal profile is 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s and 56 °C for 30 s. After PCR amplification, the ΔCt is calculated by subtraction of the β-actin Ct from each sample Ct. The method of Pfaffl was used for data analysis. The PCR primers for *SOD2* and *GPx* genes were synthesized by Jena Bioscience GmbH (Jena, Germany). Primers were designed using Primer-Blast program from NCBI. The PCR primer sequences are BLAST searched to insure for specificity to this particular gene. The primer sets used are as follows:

SOD2 (S): 5′-AGCTGCACCACAGCAAGCAC-3′
SOD2 (AS): 5′-TCCACCACCCTTAGGGCTCA-3′
GPx (S): 5′-CGGTTTCCCGTGCAATCAGT-3′
GPx (AS): 5′-ACACCGGGGACCAAATGATG-3′
β-Actin (S): 5′-GGCATCCTGACCCTGAAGTA-3′
β-Actin (AS): GGGGTGTTGAAGGTCTCAAA-3′

Statistical Analysis

Results were expressed as the mean±standard error of the mean (SEM). Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as a post hoc test according to the statistical package program (SPSS version 17.0) and figures were drawn with Origin (version 8). All p values are two-tailed and $p<0.05$ was considered as significant for all statistical analysis in this study.

Results

Treatment of rats with cadmium for 5 days significantly increased the Cd content of the testes. On the other hand, testicular cadmium concentration was decreased significantly ($p<0.05$) with physalis treatment when compared with cadmium-treated rats (Table 1).

The toxicity of $CdCl_2$ on testes weight and the relative testes weight are presented in Fig. 1. The treatment of rats with $CdCl_2$ caused significant increases in testes weight (1.93±0.09 g, $p<0.05$) and relative testes weight by 166.67 % comparing to the control group. However, the simultaneous administration of physalis before $CdCl_2$ caused significant increases in both testes weight and relative testes weight (1.27±0.08 g and 64.91 %, respectively), but when compared with $CdCl_2$, physalis administration caused a significant decrease in these parameters. Physalis extract per se did not change the testes weight (0.82±0.05 g) and relative testes weight as compared with those in the control group.

Cadmium exposure resulted in significant increases in MDA and NO levels in the testicular tissues when compared with the control (Table 2, $p<0.05$). As observed in the study, Cd injection led to modulation of several parameters of oxidative stress relative to the control animals. Cadmium injection induced significant reduction in GSH level and SOD, CAT, GPx, and GR activities relative to the control ($p<0.05$). However, simultaneous MEPh treatment significantly suppressed lipid peroxidation and decreased the

Table 1 Effect of physalis on testicular content of cadmium in cadmium-treated albino rats

Treatment	Testicular cadmium content (ppm)
Control	Nil
Cd	58.73±2.56[a]
MEPh	Nil
MEPh + Cd	18.52±1.93[ab]

Values are means±SEM ($n=7$)

[a] $p<0.05$, significant change with respect to control group (Con)

[b] $p<0.05$, significant change with respect to cadmium group (Cd)

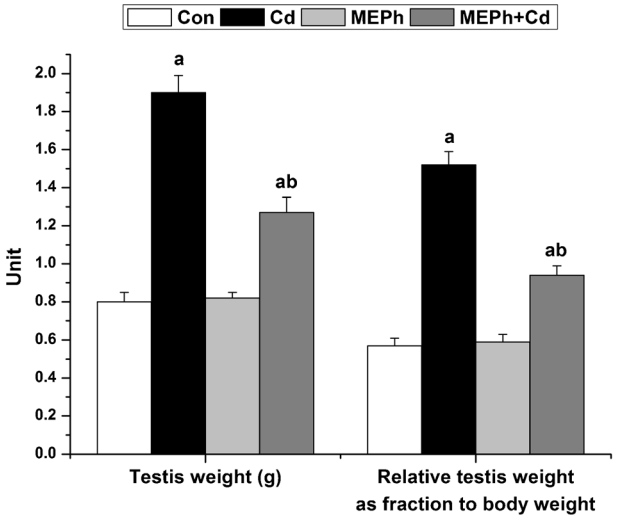

Fig. 1 Effects of physalis extract (MEPh) preadministration on testes weight and the relative testes weight in rats exposed to cadmium (Cd). Values are expressed as mean±SEM. [a]$p<0.05$, significant change with respect to control group (Con); [b]$p<0.05$, significant change with respect to cadmium group (Cd)

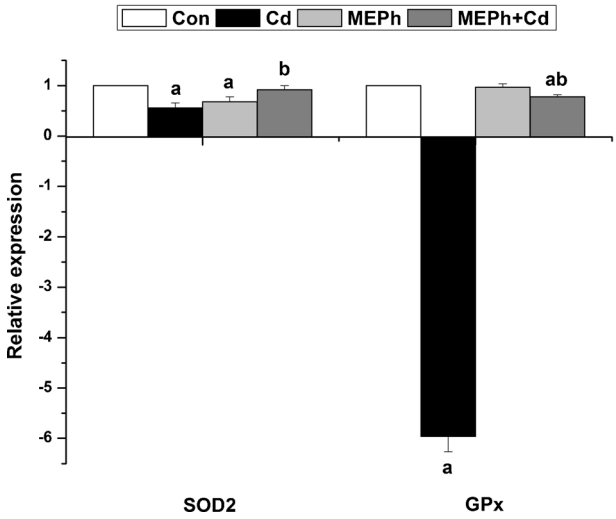

Fig. 2 Effects of physalis extract (MEPh) preadministration on mRNA expression of *SOD* and *GPx* genes in the testes of rats exposed to cadmium (Cd). Values are expressed as mean±SEM. [a]$p<0.05$, significant change with respect to control group (Con); [b]$p<0.05$, significant change with respect to cadmium group (Cd)

production of NO when compared with the cadmium-treated rats. Meanwhile, physalis treatment effectively restored GSH level and SOD, CAT, GPx, and GR activities against cadmium-induced injury ($p<0.05$).

The decreases in SOD and GPx were confirmed by results of real-time PCR which showed a decrease in mRNA expression of SOD2 and GPx after cadmium treatment for 5 days. Treatment with physalis was able to significantly ameliorate these enzymes after 5 days of treatment concurrently with cadmium (Fig. 2). An obvious significant decrease ($p<0.05$) in expression of SOD2 was noticed in rats treated alone with physalis (group III).

The intraperitoneal injection of cadmium caused a significant decrease in testosterone level (Fig. 3, $p<0.05$). On the other hand, MEPh preadministration to cadmium caused a significant increase in testosterone level as compared to the cadmium group. However, the testosterone level in MEph + Cd group showed even a significant decrease when compared to the control group.

Figure 4a, c shows the normal spermatogenesis of the control and physalis-treated rats. The seminiferous tubules shown in this figure display a clear lumen, and all cell types are represented including Sertoli cells, spermatogonia, spermatocytes, and round and elongated spermatids. Tail structures associated with spermatids are clearly seen, as are residual bodies. The tubule basement membrane is seen with the interstitium containing Leydig cells, blood vessels, and sparse fibrous elements. On the contrary to the control, cadmium chloride treatment caused complete destruction of the seminiferous tubules. The epithelial elements were totally necrotic, and the tubular lumen was filled with debris of broken and fragmented spermatozoa and containing scattered residue of basophilic chromatin material (Fig. 4b). Most tubules displayed abnormal structure with vacuolar degeneration.

Table 2 Effect of physalis on testicular content of oxidative stress marker levels and antioxidant enzyme activities in cadmium-treated albino rats

Parameters	Con	Cd	MEPh	MEPh + Cd
LPO (nmol/mg protein)	0.94±0.04	1.53±0.04[a]	0.97±0.11	1.02±0.05[b]
NO (μmol/mg protein)	1.49±0.09	2.38±0.13[a]	1.53±0.10	2.01±0.10[ab]
GSH (mmol/mg protein)	0.26±0.01	0.17±0.01[a]	0.27±0.02	0.27±0.01[b]
SOD (U/mg protein)	1279.11±49.60	1030.11±31.42[a]	1213.93±23.01	1311.14±49.91[b]
CAT (U/mg protein)	0.24±0.02	0.16±0.01[a]	0.27±0.03	0.23±0.02[b]
GPx (U/mg protein)	1.63±0.07	0.82±0.03[a]	1.81±0.19	1.44±0.05[b]
GR (μmol/mg protein)	3.67±0.19	2.09±0.16[a]	3.35±0.35	3.17±0.16[b]

Values are means±SEM ($n=7$)

[a] $p<0.05$, significant change with respect to control group (Con)

[b] $p<0.05$, significant change with respect to cadmium group (Cd)

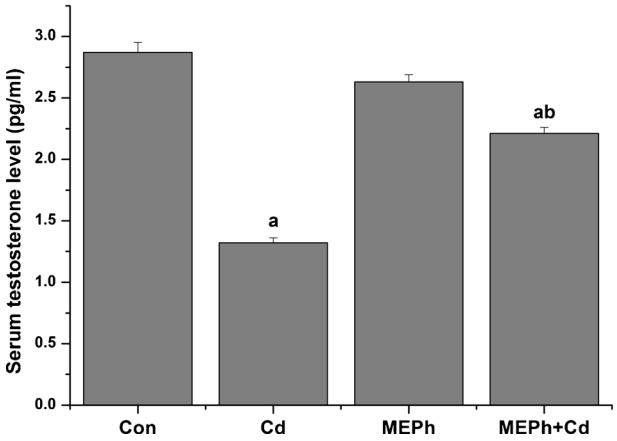

Fig. 3 Effects of physalis extract (MEPh) preadministration on serum testosterone level in rats exposed to cadmium (Cd). Values are expressed as mean±SEM. [a]$p<0.05$, significant change with respect to control group (Con); [b]$p<0.05$, significant change with respect to cadmium group (Cd)

These degenerative changes caused by cadmium chloride were significantly reduced when rats were pretreated with physalis (Fig. 4d).

The immunohistochemistry results showed that cadmium significantly upregulated Cas-3 (proapoptotic protein) expression (Fig. 5b). Treatment with MEPh alone non-significantly changed Cas-3 expression when compared with that of the control. In combination with cadmium, MEPh markedly downregulated cadmium-induced increase in Cas-3 expression (Fig. 6, $p<0.05$).

Discussion

The potential effects of Cd exposure on male reproductive system and fertility have attracted public attention in recent years. Exposure to Cd could induce a large range of health problems including dysfunction of blood–testis barrier, decrease in the secretion of gonadotropic hormone (GnRH), infertility, congenital malformations, prenatal death, intrauterine dysplasia, sexual dysfunction, abnormal embryonic development, etc. [24, 25].

Our results showed that Cd injection ($CdCl_2$, 6.5 mg/kg) increased the relative weights of the testes in accordance with previous data [26, 25]. Our investigation also demonstrated that acute exposure to Cd decreased the testosterone concentrations in plasma, indicating interference with spermatogenesis and steroidogenesis. Pretreatment with MEPh abolished these histopathological alterations. We found that histopathological alterations of the testes of rats in the MEPh + Cd-treated group were alleviated compared to the Cd-treated group. These results confirmed that MEPh can ameliorate Cd-induced testicular damage.

The exact mechanism(s) for Cd transport is not well understood. Studies have indicated that Cd may pass through cell membranes by diffusion, carrier-mediated transport, or ion channels. Cd is not an essential element for organisms. Therefore, transport of Cd into cells by carrier or channel may rely on routes utilized by other similar elements [27].

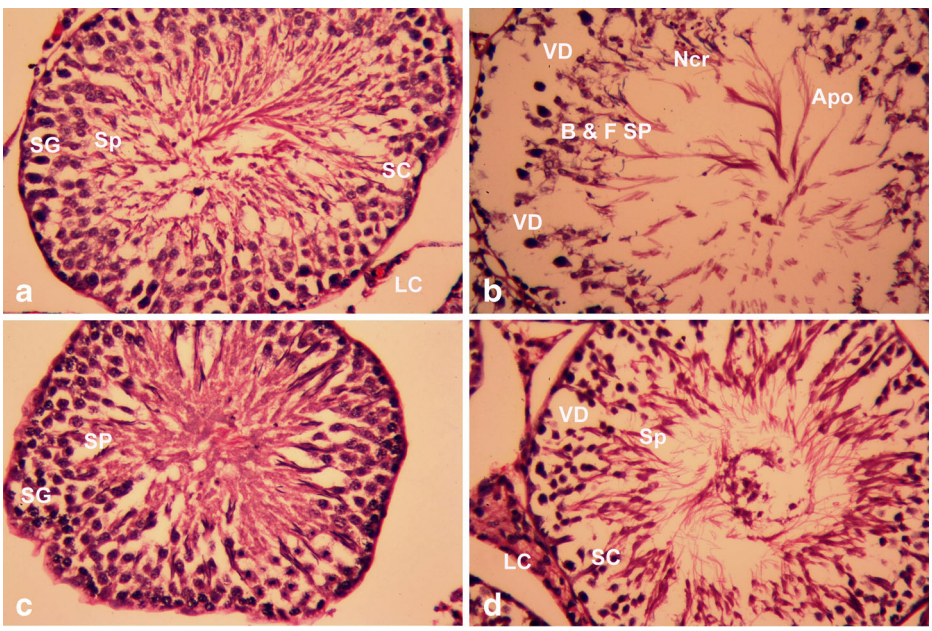

Fig. 4 Morphological changes of rat seminiferous epithelium after oral administration of cadmium and physalis for 5 days. **a** Normal histological structure of seminiferous tubules with well-developed spermatozoa in control group. **b** Degenerative seminiferous tubules with broken and fragmented spermatozoa, extensive vacuolation, and absence of late-stage germ cells in cadmium-treated group. **c** Normal histological structure of seminiferous tubules with well-developed spermatozoa in physalis group. **d** Normal histological structure of seminiferous tubules with well-developed spermatozoa in group of physalis preadministered to cadmium. However, some vacuolated areas were seen. *SC* Sertoli cells, *SG* spermatogonia, *Sp* spermatids, *LC* Leydig cells, *VD* vacuolar degeneration, *B & F SP* broken and fragmented spermatozoa, *Ncr* necrosis, *Apo* apoptosis

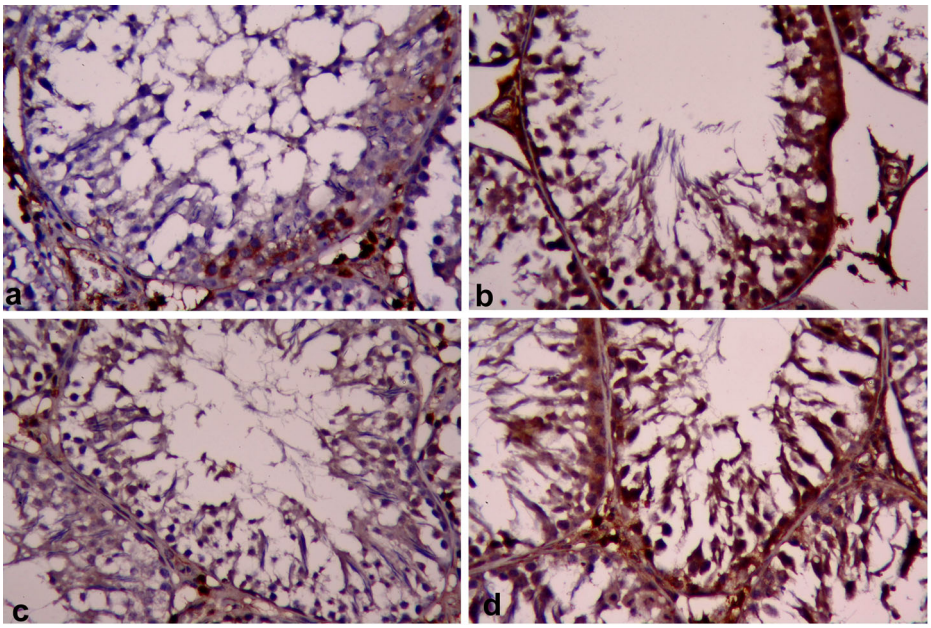

Fig. 5 Changes of caspase-3 expression of rat germ cells in seminiferous epithelium after oral administration of cadmium and physalis for 5 days. **a**–**d** Seminiferous epithelium morphology of the control, cadmium-, physalis-, and physalis + cadmium-treated rats, respectively. (×400)

For example, Cd can be transported into cells via the calcium channel because of the similarity in radius [28].

It has been proposed that Cd may initiate oxidative stress through the following three pathways: (1) depletion of antioxidant enzymes [29]; (2) enhancing production of reactive oxygen species, both in vitro and in vivo [30, 31]; and (3) binding to –SH groups from cell membrane proteins, cytoplasmic proteins, and enzymes.

Amara et al. [32] showed that subchronic exposure to Cd decreases the activity of antioxidant enzymes and increases MDA and DNA oxidation in the livers and kidneys of rats. Moreover, Muller reported that single-dose cadmium administration increased MDA and decreased glutathione in the liver [33]. This effect was related to induction of oxidative stress. Our results showed that the GPx, CAT, and SOD activities were distinctly lower in the testes of Cd-exposed rats. Therefore, the increase in testicular MDA, a biomarker of lipid peroxidation, level observed in the present study could be due to the concomitant increase in generation of free radicals, such as H_2O_2 and $OH^{\cdot}$, in the testes of the Cd-treated rats.

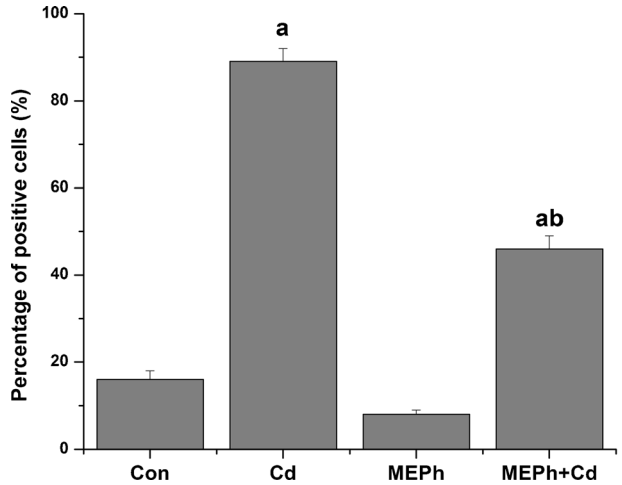

Fig. 6 Changes of caspase-3-positive germ cells (percentage) after oral administration of physalis and cadmium for 5 days. The germ cells include spermatogonia, spermatocytes, and round spermatids. Cadmium 6.5 mg/kg and physalis 200 mg/kg. Values are expressed as mean±SEM. [a] $p<0.05$, significant change with respect to control group (Con); [b] $p<0.05$, significant change with respect to cadmium group (Cd)

It was also observed that exposure to Cd caused an increase in NO production. NO is a highly reactive endogenous chemical produced by activated macrophages and serves as a mediator for expressing cytotoxic activity [34]. Macrophages, neutrophils, and neural cells generate superoxide and NO which can combine to form peroxynitrite anion. This highly reactive oxygenated species is known to induce membrane lipid peroxidation [35]. Thus, the induction of NO synthesis and superoxide production in the testes may contribute to the cytotoxicity of Cd, resulting in membrane damage. Misra et al. [36] suggested that Cd induces the production of NO and in the presence of oxygen, NO forms intermediates that displace Cd from metallothionein. The free Cd may subsequently induce DNA damage, resulting in cellular growth arrest [37]. This elevation in NO was significantly decreased with the preadministration of physalis. This may be a potential mechanism by which physalis extract can act as antiinflammatory [38] and thus protect the testes. But further study is needed for its exact mechanism.

The interaction between Cd and essential trace elements could be one of the reasons for decreased antioxidant enzymes in the rat testes. Bauer et al. [39] reported that Cd^{3+} was able to occupy the site of Zn in the CuZnSOD molecule and create an inactive form of the enzyme ($CuCd^{3+}SOD$). Moreover, there

is increasing evidence that Cd interacts with selenium (Se) to form Se–Cd complex that disrupts GPx activity [40]. Wasowicz et al. [41] found that Cd exposure decreased the Se concentration and GPx activity in both the erythrocytes and plasma of exposed workers.

Furthermore, it was revealed that physalis extract recovered the activities of the antioxidant enzymes such as SOD, CAT, GPx, and GR in $CdCl_2$-treated rats. The protective effects of physalis extract in maintaining the GSH level towards control have increased the capacity of endogenous antioxidant defense and increased the steady state of GSH and/or its rate of synthesis that confers enhanced protection against oxidative stress. It has been noticed that many of the plants which are rich in phenolic compounds and flavonoids are widely used as antioxidant and antimutagenic [42]. Various chemical compounds like 28-hydroxywithanolide, withanolides, phygrine, kaempferol, and quercetin di- and tri-glycosides are reported to be present in physalis [43].

In agreement with the results of El-Shahat et al. [44], the current study has shown marked histological changes in the testes tissue in the form of degeneration of spermatogenic cells, edema, hemorrhage, congestion, and multifocal areas of ischemic necrosis. Moreover, Blanco et al. [6] have claimed that even low doses of $CdCl_2$ (1 mg/kg bwt for 1 month) induced lack of spermatogenesis and severe necrosis of the testes of rats. Also, Santos et al. [45] have reported that endothelial damage of the small blood vessels, edema, and hemorrhage of the rat testes can be demonstrated by using just a single parenteral dose of $CdCl_2$ at 2–4 mg/kg bwt. Several studies focusing on Cd-related changes in testicular histopathology have implicated testicular blood vessel damage, followed by the degeneration of spermatopoietic epithelial, as the main cause of Cd toxicity [46, 47]. The nature and degree of testicular damage is Cd concentration dependent. More than 10 μmol/kg dose of Cd causes severe testicular lesions, such as hemorrhagic necrosis in rats and mice and interstitial cell tumor in rats [48].

Oxidative stress has been implicated as a key event in Cd toxicity. Indeed, acute Cd reproductive toxicity is associated with apoptosis induction, although a few studies have shown the induction of apoptotic cell death following exposure to acute Cd alone via different cell death pathways [49]. In mammals, procaspase-3 exists within the cytosol as inactive dimers. Once activated, it can cleave vital intracellular proteins and control various signal transduction pathways. After exposure to Cd, immunohistochemistry analysis showed that the intensity of apoptotic cells in testes was significantly elevated. It indicates that Cd can activate caspase-3. Indeed, physalis protection against the reproductive toxicity of Cd appears to be related to suppression of apoptotic pathways [11]. Thus, the protective effect of physalis against Cd toxicity could also be due, at least in part, to direct suppression of cell death pathways leading to apoptosis and necrosis. Indeed, physalis was effective in decreasing Cd-induced apoptosis and suppressed Cd-induced caspase-3 expression in the testes of rats. However, further tests are needed to explore the exact mechanism of action at the molecular level and to know the actual constituents responsible for this antiapoptotic activity.

The administration of physalis in our study caused significant decrease in superoxide dismutase; this reduction may be due to the interference of physalis active ingredients with SOD. However, polyphenols are known to be able to modulate the transcription and expression of proteins related to the endogenous antioxidant defense by interacting with antioxidant response elements in gene promoter regions of genes encoding proteins related to oxidative injury management. It is not clear, however, if the effects of physalis juice intake result from polyphenols' interference with enzymes and genes or if it is a consequence of a broader and more unspecific action connected to their antioxidant potential. Moreover, many factors need to be taken into account when examining the present results, as the degree of oxidative stress, the polyphenol class, and the concentration as well as the biological system studied may all introduce other elements of variability in the response to antioxidant ingestion [50]. However, further studies are encouraged to isolate the active phytochemical constituent for exploring exact mechanism of this inhibition.

On the basis of the above results, it can be hypothesized that the methanolic extract of *P. peruviana* L. has a protective effect against cadmium-induced testicular oxidative stress and apoptosis in the rats. Therefore, these results indicated that *P. peruviana* L. consumed by human and animals may attenuate the toxicity of environmental cadmium on reproduction. However, further investigations are needed in clarifying the exact mechanisms of *P. peruviana* L. in improving cadmium-induced reproductive toxicity.

References

1. Yang Z, Yang S, Qian SY et al (2007) Cadmium-induced toxicity in rat primary mid-brain neuroglia cultures: role of oxidative stress from microglia. Toxicol Sci 98(2):488–494
2. Waalkes MP (2003) Cadmium carcinogenesis. Mutat Res 533(1–2): 107–120
3. Foote RH (1999) Cadmium affects testes and semen of rabbits exposed before and after puberty. Reprod Toxicol 13(4):269–277
4. Al-Azemi M, Omu FE, Kehinde EO, Anim JT, Oriowo MA, Omu AE (2010) Lithium protects against toxic effects of cadmium in the rat testes. J Assist Reprod Genet 27(8):469–476
5. Burukoglu D, Baycu C (2008) Protective effects of zinc on testes of cadmium-treated rats. Bull Environ Contam Toxicol 81(6): 521–524
6. Blanco A, Moyano R, Vivo J et al (2007) Quantitative changes in the testicular structure in mice exposed to low doses of cadmium. Environ Toxicol Pharmacol 23(1):96–101

7. de Souza PF, Diamante MA, Dolder H (2010) Testis response to low doses of cadmium in Wistar rats. Int J Exp Pathol 91(2):125–131
8. Xu LC, Wang SY, Yang XF, Wang XR (2001) Effects of cadmium on rat sperm motility evaluated with computer assisted sperm analysis. Biomed Environ Sci 14(4):312–317
9. Zhou T, Zhou G, Song W et al (1999) Cadmium-induced apoptosis and changes in expression of p53, c-jun and mt-i genes in testes and ventral prostate of rats. Toxicology 142(1):1–13
10. Chang JC, Lin CC, Wu SJ et al (2008) Antioxidative and hepatoprotective effects of *Physalis peruviana* extract against acetaminophen-induced liver injury in rats. Pharm Biol 46(10–1):724–731
11. Al-Olayan E, Elkhadragy MF, Othman MS, Aref A, Kassab R, Abdel Moneim AE (2014) The potential protective effect of *Physalis peruviana* L. Against carbon tetrachloride-induced hepatotoxicity in rats is mediated by suppression of oxidative stress and downregulation of mmp-9 expression. Oxid Med Cell Longev
12. El-Gengaihi SE, Hassan EE, Hamed MA, Zahran HG, Mohammed MA (2013) Chemical composition and biological evaluation of *Physalis peruviana* root as hepato-renal protective agent. J Diet Suppl 10(1):39–53
13. Wu SJ, Ng LT, Lin DL, Huang SN, Wang SS, Lin CC (2004) *Physalis peruviana* extract induces apoptosis in human Hep G2 cells through CD95/CD95l system and the mitochondrial signaling transduction pathway. Cancer Lett 215(2):199–208
14. Wu SJ, Ng LT, Huang YM et al (2005) Antioxidant activities of *Physalis peruviana*. Biol Pharm Bull 28(6):963–966
15. Nürnberg HW (1983) Processing biological samples for metal analysis. In: Brown SS, Savory J (eds) International Union of Pure and Applied Chemistry. Academic, New York, pp 31–44
16. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR (1982) Analysis of nitrate, nitrite, and [15n]nitrate in biological fluids. Anal Biochem 126(1):131–138
17. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95(2): 351–358
18. Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82(1):70–77
19. Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126
20. Nishikimi M, Appaji N, Yagi K (1972) The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun 46(2):849–854
21. Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 249(22):7130–7139
22. Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70(1):158–169
23. Factor VM, Kiss A, Woitach JT, Wirth PJ, Thorgeirsson SS (1998) Disruption of redox homeostasis in the transforming growth factor-alpha/c-myc transgenic mouse model of accelerated hepatocarcinogenesis. J Biol Chem 273(25):15846–15853
24. Ates I, Suzen HS, Aydin A, Karakaya A (2004) The oxidative DNA base damage in testes of rats after intraperitoneal cadmium injection. Biometals 17(4):371–377
25. Amara S, Abdelmelek H, Garrel C et al (2008) Preventive effect of zinc against cadmium-induced oxidative stress in the rat testis. J Reprod Dev 54(2):129–134
26. Saygi S, Deniz G, Kutsal O, Vural N (1991) Chronic effects of cadmium on kidney, liver, testis, and fertility of male rats. Biol Trace Elem Res 31(3):209–214
27. Foulkes EC (2000) Transport of toxic heavy metals across cell membranes. Proc Soc Exp Biol Med 223(3):234–240
28. Yang PM, Chiu SJ, Lin LY (2005) Differential effects of salen and manganese–salen complex (euk-8) on the regulation of cellular cadmium uptake and toxicity. Toxicol Sci 85(1):551–559
29. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160(1):1–40
30. Watjen W, Beyersmann D (2004) Cadmium-induced apoptosis in c6 glioma cells: influence of oxidative stress. Biometals 17(1):65–78
31. Filipic M, Hei TK (2004) Mutagenicity of cadmium in mammalian cells: implication of oxidative DNA damage. Mutat Res 546(1–2): 81–91
32. Amara S, Abdelmelek H, Garrel C et al (2006) Influence of static magnetic field on cadmium toxicity: study of oxidative stress and DNA damage in rat tissues. J Trace Elem Med Biol 20(4):263–269
33. Muller L (1986) Consequences of cadmium toxicity in rat hepatocytes: mitochondrial dysfunction and lipid peroxidation. Toxicology 40(3):285–295
34. Hibbs JB Jr, Taintor RR, Vavrin Z, Rachlin EM (1988) Nitric oxide: a cytotoxic activated macrophage effector molecule. Biochem Biophys Res Commun 157(1):87–94
35. Abdel Moneim AE (2012) Evaluating the potential role of pomegranate peel in aluminum-induced oxidative stress and histopathological alterations in brain of female rats. Biol Trace Elem Res 150(1–3):328–336
36. Misra RR, Hochadel JF, Smith GT, Cook JC, Waalkes MP, Wink DA (1996) Evidence that nitric oxide enhances cadmium toxicity by displacing the metal from metallothionein. Chem Res Toxicol 9(1): 326–332
37. Hassoun EA, Stohs SJ (1996) Cadmium-induced production of superoxide anion and nitric oxide, DNA single strand breaks and lactate dehydrogenase leakage in J774A.1 cell cultures. Toxicology 112(3): 219–226
38. Wu SJ, Tsai JY, Chang SP et al (2006) Supercritical carbon dioxide extract exhibits enhanced antioxidant and anti-inflammatory activities of *Physalis peruviana*. J Ethnopharmacol 108(3):407–413
39. Bauer R, Demeter I, Hasemann V, Johansen JT (1980) Structural properties of the zinc site in Cu, Zn-superoxide dismutase; perturbed angular correlation of gamma ray spectroscopy on the Cu, ^{111}Cd-superoxide dismutase derivative. Biochem Biophys Res Commun 94(4):1296–1302
40. Trabelsi H, Azzouz I, Ferchichi S, Tebourbi O, Sakly M, Abdelmelek H (2013) Nanotoxicological evaluation of oxidative responses in rat nephrocytes induced by cadmium. Int J Nanomedicine 8:3447–3453
41. Wasowicz W, Gromadzinska J, Rydzynski K (2001) Blood concentration of essential trace elements and heavy metals in workers exposed to lead and cadmium. Int J Occup Med Environ Health 14(3):223–229
42. Lee HZ, Liu WZ, Hsieh WT, Tang FY, Chung JG, Leung HW (2009) Oxidative stress involvement in *Physalis angulata*-induced apoptosis in human oral cancer cells. Food Chem Toxicol 47(3):561–570
43. Abdel Moneim AE, El-Deib KM (2012) The possible protective effects of *Physalis peruviana* on carbon tetrachloride-induced nephrotoxicity in male albino rats. Life Sci J 9(3):1038–1052
44. El-Shahat AE-R, Gabr A, Meki A-R, Mehana E-S (2009) Altered testicular morphology and oxidative stress induced by cadmium in experimental rats and protective effect of simultaneous green tea extract. Int J Morphol 27:757–764
45. Santos FW, Oro T, Zeni G, Rocha JB, Do Nascimento PC, Nogueira CW (2004) Cadmium induced testicular damage and its response to administration of succimer and diphenyl diselenide in mice. Toxicol Lett 152(3):255–263
46. Messaoudi I, Banni M, Said L, Said K, Kerkeni A (2010) Evaluation of involvement of testicular metallothionein gene expression in the protective effect of zinc against cadmium-induced testicular pathophysiology in rat. Reprod Toxicol 29(3):339–345
47. Thompson J, Bannigan J (2008) Cadmium: toxic effects on the reproductive system and the embryo. Reprod Toxicol 25(3):304–315

48. Waalkes MP (2000) Cadmium carcinogenesis in review. J Inorg Biochem 79(1–4):241–244
49. Wang B, Li Y, Tan Y, Miao X, Liu XD, Shao C, Yang XH, Turdi S, Ma LJ, Ren J, Cai L (2012) Low-dose Cd induces hepatic gene hypermethylation, along with the persistent reduction of cell death and increase of cell proliferation in rats and mice. PLoS One 7(3): e33853
50. Abdel Moneim AE, Othman MS, Mohmoud SM, El-Deib KM (2013) Pomegranate peel attenuates aluminum-induced hepatorenal toxicity. Toxicol Mech Methods 23(8):624–633