Effects of Diallyl Sulfide and Zinc on Testicular Steroidogenesis in Cadmium-Treated Male Rats

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ABSTRACT: Cadmium (Cd) is one of the environmental pollutants that affect various tissues and organs including testis. Harmful effect of cadmium on testis is known to be germ cell degeneration and impairment of testicular steroidogenesis. In the present study, the effect of diallyl sulfide (DAS), a sulfur-containing volatile compound present in garlic, and zinc (Zn) was investigated on cadmium-induced testicular toxicity in rats. Male adult Wistar rats treated with cadmium (2.5 mg/kg body wt, five times a week for 4 weeks) showed decreased body weight, paired testicular weight, relative testicular weight, serum testosterone, luteinizing hormone, follicle-stimulating hormone, and testicular total antioxidant capacity (TAC) and protein levels. Testicular steroidogenic enzymes, such as 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD), and marker enzymes, such as sorbitol dehydrogenase (SDH), lactate dehydrogenase (LDH), acid phosphatase (ACP), alkaline phosphatase (ALP), and glucose-6-phosphate dehydrogenase (G6PD), showed a significant decrease in activities whereas that of γ-glutamyl transferase was significantly increased after cadmium exposure. The results have revealed that concurrent treatment with DAS or zinc restored key steroidogenic enzymes, SDH, LDH, and G6PD and increased testicular weight significantly. DAS restored the TAC level and increased testosterone level and relative testicular weight significantly. Zinc restored testicular protein level and body weight. It can be concluded that cadmium causes testicular toxicity and inhibits androgen production in adult male rats probably by affecting pituitary gonadotrophins and that concurrent administration of DAS or zinc provides protection against cadmium-induced testicular toxicity.


KEYWORDS: Cadmium; Testis; Diallyl Sulfide; Zinc; Marker Enzymes; Testosterone; Rats

INTRODUCTION

Cadmium (Cd) is considered as one of the most toxic transition metal elements. It is a potent industrial hazardous substance that causes severe damage to a variety of tissues and organs including the liver, kidney, lung, and testis, and there is sufficient evidence in humans to classify cadmium and cadmium compounds as carcinogenic substances [1]. This important heavy metal is widely used in batteries, metal plating, pigments, plastics, and alloys. In addition to occupational exposures, environmental cadmium exposure in humans may occur through cigarette smoking and dietary consumption [2]. Previous studies in rodents have shown that testicular tissue is particularly a sensitive target to cadmium toxicity [3]. Cadmium exposure can adversely affect male fertility and result in severe impairment of testicular functions including germ cell death and inhibition of testicular steroidogenesis [4–6]. However, the cellular pathway underlying the toxic effects of cadmium on the testis has not yet been clearly established. Cadmium toxicity is reported to be associated with oxidative damage in testicular Leydig cells through the production of reactive oxygen species (ROS) [7,8]. ROS appears to act acutely by perturbation of Leydig cell mitochondria and dissipation of mitochondrial membrane potential, resulting in the inhibition of steroid acute regulatory (StAR) protein activity [9].

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are required for quantitatively normal spermatogenesis in pubertal rats [10]. LH is a prime regulator of testicular androgenic enzyme activities [11]. In male rats, circulating LH is responsible for maintaining normal plasma testosterone concentrations [12]. Adult mammalian spermatogenesis is a testosterone-dependent process [13]. Massive testicular germ cell apoptosis is known to result directly either from exposure to Cadmium [14] or alterations of hormonal support from Leydig cells [15]. Testosterone is required for the attachment of different generations of germ cells in seminiferous tubules, and therefore a low level of
intratesticular testosterone may lead to detachment of
germ cells from seminiferous epithelium and may ini-
tiate germ cell apoptosis [16]. FSH is the key hormone
that regulates follicular growth and spermatogonial de-
velopment in normal adult rats [17].

Garlic (Allium sativum) has been used worldwide
not only as a flavoring agent but also as a tradi-
tional medicine since ancient times. According to folk
medicine, there is a cherished belief that garlic helps in
maintaining good health [18]. Garlic is particularly rich
in organosulfur compounds that have been reported in
animals or in cell lines to have diverse cellular events,
involving xenobiotic-detoxifying metabolism, apoptosis,
gene expression, and antioxidant effect [19]. Of
sulfur compounds, naturally occurring in garlic, diallyl
sulfide (DAS) is one of the most abundant compounds.
DAS is formed from the natural precursor allicin when
a garlic glove is crushed. The concentration of allicin in
garlic is 5 mg/g, and it has been estimated that a 70-kg
individual eating 1.4 g of garlic per day would receive
0.1 mg/kg body wt of sulfides [19,20]. Recently, DAS
was found to effectively check the mutations induced by
environmental toxicants through inducing the expres-
sion of heme oxygenase-1 (HO-1), which plays a
critical role in cell defense system against oxidative
stress [21,22]. DAS was found to have more potent
inhibitory effects than diallyl disulfide on chemical tox-
icity, carcinogenesis, and mutagenesis in the upregu-
ation of cytochrome P4502B1 (CYP2B1) [19]. Despite
the fact that numerous studies have been performed on
garlic and its organosulfur compounds, no detailed
in vivo studies have been performed on the effect of
DAS on cadmium-induced impairment of testicular
functions.

Zinc (Zn) is an essential trace element and has
important biological functions that control many cell
processes including normal growth, reproduction, and
wound healing [23]. It is present in calf’s liver, mush-
rooms, spinach, thyme, and shrimp. Zinc is needed for
the functions of more than 300 enzymes. It is essential
for DNA, RNA, and protein synthesis and, as such, is
important for cell division [24]. In addition, zinc has
antioxidant potential effects that could be related to
several mechanisms including being a cofactor of
superoxide dismutase (SOD) and formation of zinc
metallothionein (MT) complexes [25,26]. By preventing
proteins from oxidation, zinc could contribute to the
SH group stabilization and thus might influence tran-
scription factors such as p53 [27]. Cadmium has been
reported to compete and displace zinc from its nor-
mal localization, thus altering zinc homeostasis and its
physiological function [28,29]. Zinc deficiency caused
by cadmium was demonstrated to result in reduction of
spermatogenesis, testosterone production, and induc-
tion of apoptosis [7,30]. Recently, it has been shown that
zinc inhibited caspase-3 and prevented Cd-induced ef-
fects on the expression levels of p53 and p21 [31]. The
aim of the present study was to assess whether cotreat-
ment with DAS and zinc could improve the detrimen-
tal effect of chronic cadmium exposure on testicular
steroidogenesis in rats.

**MATERIALS AND METHODS**

**Drugs and Chemicals**

Cadmium chloride (CdCl₂·2H₂O), DAS, tetra-
sodium pyrophosphate, glycerol, and tripyridyltriazine were purchased from Sigma-Aldrich Chemicals
Co. (St. Louis, MO). All other chemicals were of pure
analytical grade.

**Experimental Design**

Adult male albino rats of Wistar strain weighing
150 ± 10 g (10–12 weeks old) were used in the
present study. They had free access to water and
food throughout the experimental period. The local
committee approved the design of the experiments,
and the protocol conformed to the guidelines of the
National Institutes of Health. After an adaptation pe-
riod of 5 days, the animals were randomly divided into
four groups as follows: Group I (control) received nor-
mal saline throughout the course of the study. Group II
(cadmium) received intraperitoneal injections of
cadmium (2.5 mg/kg body wt) five times a week for a
period of 4 weeks [32]. Group III (DAS) received
oral DAS (70 mg/kg body wt) [33] suspended in 0.5%
xanthan gum along with intraperitoneal injections of
cadmium (2.5 mg/kg body wt) five times a week for a
period of 4 weeks. Group IV (zinc) received ZnCl₂
in drinking water in a dose of 114 mg/L [34] along
with intraperitoneal injections of cadmium (2.5 mg/kg
body wt) five times a week for a period of 4 weeks.
Treatment with either DAS or zinc was started 1 week
before cadmium administration.

At the end of the fourth week, the animals were sac-
cificed by decapitation. Blood samples were collected
and centrifuged to separate serum, which was kept at
−30°C until testosterone, LH, and FSH were assayed.
Both testes were excised, dissected free of surrounding
connective tissue, and rinsed in ice-cold saline. Testes
were weighed and homogenized in ice-cold double-
distilled water to give a 20% homogenate. Aliquots of
the tissue homogenate were suitably processed for the
assessment of the following biochemical parameters.

**Testicular Marker Enzymes**

A suitable aliquot of homogenate was mixed with
Tris–HCl buffer (0.01 mol/L, pH 7.4) to make 10%
homogenate and ultracentrifuged at 10,000 × g at 4°C for 30 min using Dupont Sorvall (Wilmington, DE) ultracentrifuge to obtain the fraction used for the estimation of marker enzymes. Sorbitol dehydrogenase (SDH) activity was assayed by using the method of Chauncey et al. [35]. Lactate dehydrogenase (LDH) activity was measured by using commercially available kit provided by Stanbio (San Antonio, TX), according to the method of Buhl and Jackson [36]. The SDH assay is based on interconversion of D-sorbitol and D-fructose, and the LDH assay is based on the interconversion of pyruvate and lactate. γ-Glutamyl transferase (γ-GT) was measured by using the method of Orlowski and Meister [37] with the help of the kit supplied by Scalvo Diagnostics (Siena, Italy). γ-GT assay depends on the rate of formation of p-nitroaniline at 405 nm, resulting from catalyzing the transfer of γ-glutamyl group from the substrate γ-glutamyl-4-nitroanilide to glycyl-glycine.

Acid phosphatase (ACP) and alkaline phosphatase (ALP) were estimated by using the kits supplied by Quimica Clinica Aplicada (Amposta, Tarragona, Spain) and Biolabo SA (Maizy, France) according to the methods described in [38,39]. The assay of ALP and ACP depends on the hydrolysis of p-nitrophenylphosphate in alkali and acid medium, respectively. The liberated p-nitrophenol is quantified spectrophotometrically at 420 nm. Glucose-6-phosphate dehydrogenase (G6PD) activity was determined according to the method of Kornberg and Horecker [40]. G6PD assay depends on the rate of reduction of NADP at 340 nm, utilizing glucose-6-phosphate as a substrate.

Ferric Reducing Antioxidant Power Assay

The total antioxidant capacity (TAC) of testicular tissue was measured by using the ferric reducing antioxidant power (FRAP) assay method [41]. It measures the ferric reducing ability of antioxidants in various samples. At low pH, the ferric-tripyridyltriazine complex (FeIII–TPTZ) is reduced to the ferrous (FeII) form for the samples. At low pH, the ferric–tripyridyltriazine complex was measured by using the ferric reducing antioxidant power (FRAP) assay method [41]. It measures the antioxidant power (FRAP) assay method [41]. It measures the total antioxidant capacity (TAC) of testicular tissue.

Determination of 3β-Hydroxysteroid Dehydrogenaseβ and 17β-Hydroxysteroid Dehydrogenaseβ Activities

Testicular 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD) activities were assayed following the methods described in [42,43]. Testicular tissue homogenate (20%) was mixed with a homogenizing fluid containing 20% spectroscopic-grade glycerol, 5 mM potassium phosphate, and 1 mM EDTA at a tissue concentration of 25 mg/mL homogenizing mixture and centrifuged at 10,000 × g for 30 min in an ultracentrifuge at 4°C. One-milliliter of aliquot of the supernatant was mixed with 100 μmol sodium pyrophosphate buffer (pH 8.9), 0.9 mL double distilled water, and 30 μg dehydroepiandrosterone in ethanol making up the incubation mixture to a total of 3 mL. 3β-HSD activity was measured after the addition of 0.5 μmol of NAD+ to the tissue supernatant mixture at 340 nm. The same supernatant collected for the testicular assay of 3β-HSD was used for assaying of 17β-HSD activity. One-milliliter aliquot of the supernatant was mixed with 440 μmol sodium pyrophosphate buffer (pH 10.2) and 0.3 μmol 4-androst-ene-17β-ol-3-one (testosterone) in ethanol, bringing the incubation mixture to a total of 3 mL. Enzyme activity was measured after the addition of NADP (1.1 μmol) to the supernatant mixture at 340 nm. Protein content was determined by using the method of Lowry et al. [44].

Assay of Serum Testosterone, Luteinizing Hormone, and Follicle-Stimulating Hormone Levels

Levels of serum testosterone, LH, and FSH were measured by using automated chemiluminescence’s immunoassay systems (ADVIA Centaur; Bayer Vital, Fernwald, Germany).

Statistical Analysis

The values are expressed as mean ± standard error of the mean (SEM) for six animals. Differences between groups were assessed by one-way analysis of variance (ANOVA). Tukey–Kramer test was performed for intergroup comparisons. A p-value < 0.05 was considered significant.

RESULTS

Body Weight, Testicular Weight, and Relative Testicular Weight

Rats exposed to CdCl2 (group II) showed significant reduction in the body weight, testicular weight, and relative testicular weight (Table 1). Concurrent treatment with DAS or zinc (groups III and IV) caused significant increase in testicular weight. Zinc was able to restore body weight; however, no significant change was observed in relative testicular weight. On the other hand, DAS showed no significant increase in body weight.
TABLE 1. Effect of cadmium, DAS, and zinc on Body Weight, Testicular Weight, and Relative Testicular Weight

<table>
<thead>
<tr>
<th></th>
<th>Group I (Control)</th>
<th>Group II (Cd)</th>
<th>Group III (Cd and DAS)</th>
<th>Group IV (Cd and Zn)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>149.2 ± 2.39</td>
<td>152.5 ± 3.3</td>
<td>150 ± 3.4</td>
<td>153.3 ± 2.1</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>192.5 ± 4.8</td>
<td>146.6 ± 6.6</td>
<td>162.5 ± 9.37</td>
<td>192.5 ± 7.6</td>
</tr>
<tr>
<td>Testicular weight (g)</td>
<td>2.7 ± 0.08</td>
<td>0.4 ± 0.04</td>
<td>1.0 ± 0.099</td>
<td>0.8 ± 0.04</td>
</tr>
<tr>
<td>Relative testicular weight (g %)</td>
<td>1.4 ± 0.03</td>
<td>0.27 ± 0.03</td>
<td>0.56 ± 0.07</td>
<td>0.38 ± 0.01</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM for six rats. Comparisons are made as follows: * with group I; † with group II. Values are statistically significant at p < 0.05.

TABLE 2. Effect of cadmium, DAS, and zinc on Activities of Testicular Steroidogenic Enzymes and Levels of Serum Testosterone, LH, and FSH

<table>
<thead>
<tr>
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<th>Group IV (Cd and Zn)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3β-HSD (nmol/mg protein)</td>
<td>3.1 ± 0.35</td>
<td>0.66 ± 0.08</td>
<td>2.36 ± 0.28</td>
<td>2.24 ± 0.5</td>
</tr>
<tr>
<td>17β-HSD (nmol/mg protein)</td>
<td>4.2 ± 0.3</td>
<td>0.109 ± 0.01</td>
<td>4.8 ± 0.69</td>
<td>5.7 ± 1.3</td>
</tr>
<tr>
<td>Testosterone (ng/mL)</td>
<td>5.84 ± 1.99</td>
<td>0.5 ± 0.06</td>
<td>2.65 ± 0.34</td>
<td>0.78 ± 0.04</td>
</tr>
<tr>
<td>LH (mU/mL)</td>
<td>0.58 ± 0.02</td>
<td>0.25 ± 0.018</td>
<td>0.29 ± 0.03</td>
<td>0.24 ± 0.028</td>
</tr>
<tr>
<td>FSH (mU/mL)</td>
<td>0.14 ± 0.016</td>
<td>0.07 ± 0.002</td>
<td>0.072 ± 0.002</td>
<td>0.069 ± 0.003</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM for six rats. Comparisons are made as follows: * with group I; † with group II. Values are statistically significant at p < 0.05.

weight whereas a significant increase in relative testicular weight was observed as compared to the cadmium-treated group.

**Effect on Testicular Steroidogenic Enzyme Activities and Serum Levels of Testosterone, LH, and FSH**

Testicular 3β-HSD and 17β-HSD are the key enzymes for testosterone biosynthesis. There was a significant decrease in testicular 3β-HSD and 17β-HSD activities in cadmium-treated rats as compared with the control group (Table 2). A significant decrease was also shown in serum levels of testosterone, LH, and FSH in the cadmium-treated group. The decreased testicular 3β-HSD and 17β-HSD activities resulting from cadmium treatment were reversed in animal groups treated with either DAS or zinc. Only concurrent treatment with DAS was able to cause a significant increase in the serum testosterone level as compared with the cadmium-treated group. Treatment with either DAS or zinc did not show any significant change in serum LH and FSH levels.

**Effect on Activities of Testicular Marker Enzymes and the TAC Level**

Compared with the control group, testicular SDH, LDH, ACP, ALP, and G6PD activities showed a significant decrease in the cadmium-treated group (group II) whereas that of γ-GT showed a significant increase. In addition, the significant reduction was demonstrated in the levels of testicular TAC and protein. Cotreatment with DAS or zinc effectively increased the activities of reduced SDH, LDH, and G6PD enzymes toward normalcy (Table 3), whereas no significant change was detected in the activities of γ-GT, ACP, and ALP as compared with the cadmium-treated group. Normalization of testicular TAC was only achieved in the DAS-treated group, whereas no significant change was observed in the zinc-treated group as compared with the cadmium-administered group. In contrast, normalization of testicular protein level was revealed in the zinc-treated group whereas no significant change was found in the DAS-treated group.

**DISCUSSION**

The present study shows that chronic exposure of a known environmental toxicant and industrial pollutant, cadmium, to male rats can alter reproductive functions by decreasing paired testicular weight, inhibiting testicular androgenesis, and decreasing concentrations of serum testosterone and gonadotrophins. Testicular weight, a valuable index of reproductive toxicity in male animals, showed a significant decrease that is consistent with a previous study [45]. In addition, the significant decrease in body weight and the testicular protein level demonstrated in the cadmium-treated group indicates that the general metabolic functions of the animals are not in the normal range and of the impending toxicity as suggested previously [46] (Tables 1 and 2).
Testicular steroidogenic events, 3β-HSD and 17β-HSD, play a key regulatory role as these are the prime enzymes in testicular androgenesis. The diminution of these enzymes by cadmium treatment in this study is consistent with a recent finding that cadmium caused a decrease in cytochrome P450 side chain cleavage complex (P450scc) and a gradual decrease in 3β-HSD mRNA levels [5]. Cadmium being a divalent heavy metal has a strong affinity for the thiol group of proteins and enzymes and can cause conformational changes that interfere with their function [47]. The decrease in serum testosterone level in cadmium-treated rats may be due to the inhibition of these testicular androgenic enzyme activities, because these enzymes are responsible for the regulation of testosterone biosynthesis [5]. Moreover, the inhibition of testicular androgenic enzymes in cadmium-treated rats may be the result of low-serum level of LH, which is a prime regulator of testicular androgenic enzyme activities [11]. Cadmium was found to decrease the testosterone production through the reduction of testicular luteinizing hormone receptor, messenger ribonucleic acid, and cyclic adenosine monophosphate level in rats [6,48]. Cadmium administration was reported to affect steroidogenesis (reduced testosterone synthesis) even at concentrations that do not cause any testicular necrosis, indicating specific disruptive mechanisms in male rats [49].

The low-serum level of gonadotrophins in the present study may be due to elevated secretion of corticosterone via activation of the stress signal pathway and the hypophysial-adenocortical axis. The elevated corticosterone level may suppress the sensitivity of gonadotrophic cells to gonadotrophin-releasing hormone and therefore, may prevent gonadotrophin secretion as postulated in arsenic-treated rats by Jana et al. [50].

Testis and sperm function are particularly vulnerable to the injury produced by ROS [51,52]. It has been reported that hydrogen peroxide, a potent oxidant, inhibits steroidogenesis in Leydig cells [9]. An increase in oxidative stress causes ROS-induced damage to macro-molecules such as DNA, protein, and key enzymes involved in testicular steroidogenesis and spermatogenesis [5]. To test this notion, the total testicular antioxidant capacity level was measured (Table 3). The data showed that the administration of cadmium decreased the testicular TAC level significantly. This decrease of the testicular TAC level can indicate deficiency of testicular ascorbic acid level because ascorbic acid contributes a major part to TAC in the FRAP assay. Ascorbic acid is present in the spermatogenic chamber and Leydig cells [53] and has been shown to play an important role in preventing oxidative damage to the spermatozoa and steroidogenesis [54]. Ascorbic acid was also reported to ameliorate ROS-related testicular toxicity in lead-exposed animals and cadmium-induced thyroid dysfunction [55]. Furthermore, the decrease in the activities of key steroidogenic enzymes is found to be consistent with a previous report that relates such reduction to the reduced ascorbic acid level in the testes of fish [56].

The reduction of serum LH and FSH levels and consequent reduction in testosterone production may, therefore, be responsible for the cadmium-induced changes manifested by alteration in the marker enzymes. Activities of testicular marker enzymes such as SDH, LDH, γ-GT, ACP, and ALP are considered functional indicators of spermatogenesis. In the present study, SDH, LDH, ACP, and ALP showed a significant decrease whereas γ-GT exhibited a significant increase. LDH and SDH are associated with the maturation of germinal epithelial layer of seminiferous tubules. SDH is primarily associated with pachytene spermatocyte maturation of germinal epithelium. Its activity is related to the function of germ cells and decreases during the depletion of germ cells [57]. SDH is also responsible for providing energy to sperm cells by converting sorbitol to fructose. The decreased activity of this enzyme in cadmium-treated rats suggests an altered cellular physiology of the germinal elements in seminiferous tubules. Since the activity of LDH is closely associated with spermatogenesis and male testicular

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</tr>
</thead>
<tbody>
<tr>
<td>SDH (mU/mg protein)</td>
<td>3.66 ± 0.3</td>
<td>1.18 ± 0.13</td>
<td>4.9 ± 0.5</td>
<td>3.98 ± 0.6</td>
</tr>
<tr>
<td>LDH (mU/mg protein)</td>
<td>2.36 ± 0.1</td>
<td>0.74 ± 0.66</td>
<td>1.96 ± 0.2</td>
<td>2.08 ± 0.3</td>
</tr>
<tr>
<td>γ-GT (nmol/mg protein)</td>
<td>13.07 ± 0.79</td>
<td>81.2 ± 6.007</td>
<td>94.3 ± 7.3</td>
<td>71.5 ± 6.9</td>
</tr>
<tr>
<td>ACP (U/mg protein)</td>
<td>152.5 ± 8.4</td>
<td>67.28 ± 5.4</td>
<td>87.95 ± 4.07</td>
<td>86.7 ± 8.25</td>
</tr>
<tr>
<td>ALP (U/mg protein)</td>
<td>160.4 ± 3.0</td>
<td>84.05 ± 11.2</td>
<td>100.86 ± 6.5</td>
<td>97.29 ± 7.9</td>
</tr>
<tr>
<td>G6PD (nmol/mg protein)</td>
<td>6.48 ± 0.48</td>
<td>2.39 ± 0.25</td>
<td>5.63 ± 1.69</td>
<td>6.05 ± 0.4</td>
</tr>
<tr>
<td>TAC (μmol/mg protein)</td>
<td>423.6 ± 27.3</td>
<td>212.2 ± 12.7</td>
<td>436.7 ± 53.8</td>
<td>263.3 ± 27.4</td>
</tr>
<tr>
<td>Protein (mg/g)</td>
<td>41.9 ± 2.99</td>
<td>26.36 ± 0.66</td>
<td>27.14 ± 1.3</td>
<td>54.7 ± 5.8</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM for six rats.
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Values are statistically significant at p < 0.05.
development, the decreased activity of this enzyme in cadmium-administered animals represents a defect in spermatogenesis and testicular maturation. Lactate and pyruvate are found to play an important role in the metabolism of testis [54]. In addition, FSH has been reported to stimulate pyruvate production in the rat Sertoli cells and this is a rate-limiting factor for germ cell activities [58]. Therefore, the lowered FSH level in this study may also account for the reduction in LDH activity due to its possible inhibitory effect on lactate and pyruvate, which generally reflects the reduced metabolic activities of the testis. Moreover, a unique isoenzyme of LDH has been found to be located in the inner mitochondrial membrane of the spermatogenic cells of the mature and developing testis. This isoenzyme plays an important role in transferring hydrogen from cytoplasm to mitochondria by redox coupling α-hydroxy acid/α-keto acid related to spermatozoal metabolism [59]. Cadmium was found to increase lipid peroxidation in the mitochondria of rat testis [9]. The enhanced lipid peroxidation in mitochondria may result in the disintegration of the mitochondrial membrane ultrastructure, which in turn can affect the membrane-bound LDH function resulting in its inhibition. γ-GT is the marker of Sertoli cell function, and its activity parallels with the pattern of Sertoli cell maturation and replication [60]. The marked increase in the activity of gesticular-GT in cadmium-treated rats indicates the impaired functions of Sertoli cells and interference of cadmium with the process of spermatogenesis. Similar patterns of testicular SDH, LDH, and γ-GT activities have been reported with a number of chemicals causing testicular toxicity [61].

The decreased activities of testicular ACP and ALP in cadmium-administered animals may reflect the release of these nonspecific phosphatases from lysosomes of the degenerating cells and rapid catabolism of the injured germ cells. The alteration in enzymes activity may lead to the destruction of seminiferous epithelium and loss of germinal elements, resulting in the reduction of the number of spermatids associated with the decrease in the daily sperm production in the testes [62]. Activities of free lysosomal enzymes have been shown to rise when testicular steroidogenesis is increased [63]. A decrease in the acid phosphatase would thus reflect the decreased testicular steroidogenesis in the cadmium-treated rats and may be correlated with the reduced secretion of gonadotrophins. Alkaline phosphatase is involved in the synthesis of nuclear proteins, nucleic acids, and phospholipids as well as in the cleavage of phosphate esters and in mobilizing carbohydrates and lipid metabolites to be utilized by the spermatozoa. A decrease in the alkaline phosphatase activity in cadmium-treated rats indicated that cadmium decreased steroidogenesis, in which the inter- and intracellular transport was reduced as the metabolic reactions to channalize the necessary inputs for steroidogenesis slowed down [64]. G6PD is another key enzyme of the testicular tissue that provides reducing equivalents for the hydroxylation of steroids. G6PD is also directly associated with glutathione metabolism, and the lowered activity of this enzyme provokes increased oxidative stress to the tissues and may lead to cell death [65]. In general, the decrease in studied testicular enzyme activities in the cadmium-treated group is consistent with the previous suggestion that cadmium can affect DNA and RNA synthesis [66].

In contrast, intake of DAS and zinc reverted the deleterious effects of cadmium on steroidogenesis as manifested by restoring the activities of the two key enzymes. The observed significant increase in serum testosterone level in the cadmium and DAS-treated group is consistent with a previous suggestion that allyl-containing sulfides in garlic increase testicular testosterone content due to the increase in the plasma noradrenaline concentration [67] (Table 2). The way DAS reversed cadmium-induced reduction of steroidogenesis might be through scavenging of cadmium-induced ROS, which can affect steroidogenic enzyme activities at protein and transcription levels [9]. In addition, concomitant administration of DAS was capable of reducing oxidant stress as evidenced by reversion of testicular TAC level (Table 3). This restoration of TCA may be accompanied by an increase in the ascorbic acid content that has a protective role in the cadmium-induced impaired steroidogenesis as reported previously in [5]. Unsal et al. [68] have reported that pretreatment of rats with oral garlic extract seems to attenuate testicular tissue damage by preventing the generation of toxic-free radicals, as evidenced by the reduction of the elevated testicular malondialdehyde level and xanthine oxidase activity.

Interestingly, the normalization of two key enzymes of steroidogenesis and the nonsignificant change in serum LH and FSH levels in DAS- and zinc-treated groups allow to postulate that factors other than LH and FSH are involved in the modulation of testicular function. Thus, the increased serum testosterone production in the cadmium and DAS-treated group may be attributed to paracrine modulation of testosterone synthesis in rat Leydig cells by ROS, mainly nitric oxide. DAS may result in reversal of inhibition imposed on resting Leydig cells by testicular macrophages-derived nitric oxide but not LH-stimulated Leydig cells [69]. This finding is also in harmony with the normalization of the testicular TAC level achieved in the DAS-treated group (Table 3).

Administration of DAS or zinc along with cadmium has been shown to prevent the testicular injury as revealed by increasing testicular weights and restoring
SDH, LDH, and G6PD activities in testicular tissue (Table 3). In addition, zinc restored body weight and testicular protein level whereas DAS increased relative testicular weight significantly (Tables 1 and 3). Recently, El-Demerdash et al. [70] have shown that garlic juices exerted antioxidant effects and consequently alleviated testicular damage caused by alloxan-induced diabetes. Garlic administered to rat simultaneously with cadmium was demonstrated to possess protective effect against heavy metal poisoning, detected by reduced accumulation of heavy metals in the liver, kidneys, bone, and testes, and enhanced excretion of cadmium through feces [71].

Despite the fact that zinc deficiency leads to several clinical symptoms such as decreased spermatogenesis and impaired male fertility, the exact pathophysiology has not been clarified. Previously, Reeves [72] has confirmed that part of the effect of zinc deficiency in male rat may be due to the reduction in testicular activity of dipeptidyl carboxypeptidase. This enzyme is required for maturation and development of sperm cells. Cadmium has been shown to displace zinc in many vital enzymatic reactions, causing disruption or cessation of enzyme activities because the metabolism of cadmium is closely related to zinc metabolism, metallothionein binding, and transport [73]. This is clearly demonstrated hereby the normalization of reduced SDH in the zinc-treated group, as shown in Table 3. This is in harmony with the fact that a zinc ion is a cofactor for SDH [74]. Protective effects of zinc may be related to MT induction. It is believed that zinc administration can induce MT expression levels in different tissues (liver, kidney, etc.) [75]. A role of MT is to detoxify heavy metals, such as cadmium and mercury, and MT induction can prevent apoptosis and oxidative stress induced by them [76]. Zinc has been shown to inhibit cadmium-induced apoptosis and production of ROS in cell cultures [7]. Zinc-induced protection against the cytotoxicity of cadmium may also be related to the maintenance of normal redox balance inside the cell [77].

Taken together, the present study indicates that cadmium causes impairment of testicular functions and affects the androgenicity in male rats. Concurrent treatment with DAS or zinc can ameliorate cadmium-induced testicular oxidative damage and ensure normal steroidogenic activities. Therefore, garlic and zinc supplementation is highly recommended to guard against male infertility especially in environmentally polluted areas. In addition, more research is needed as to clearly elucidate the molecular mechanisms by which natural supplements including DAS and zinc exert their testicular protective effect on both human and rat.

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