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Benzo-a-pyrene induced genotoxicity and cytotoxicity in germ cells of mice: Intervention of radish and cress

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Abstract Exposure to chemicals like benzo(a)pyrene (BaP) can lead to structural changes in DNA and as a consequence to increased incidence of diseases with a genetic basis, as well as oxidative stress in the testis. However its ability to induce oxidative DNA damage in germ cells is not fully investigated. In the present study, BaP was used to induce 8-hydroxydeoxyguanosine (8-OHdG), a specific DNA adducts for oxidative DNA damage, in testis and epididymal sperm and the possible protection role of radish and/or cress was investigated. The results revealed that BaP induced a significant increase in DNA damage in both tissues, as indicated by increased DNA strand breaks in a fluorimetric analysis of DNA unwinding (FADU). Furthermore, it increased the oxidative damage in epididymal sperm, as indicated by the increase in sperm abnormalities, lipid peroxidation (LPO), accompanied with a decrease in glutathione content (GSH), sperm count and sperm motility as well as induction of filtration in the histology of the testis. Treatment with radish and/or cress oil prior to BaP injection succeeded in reducing the germ cell genotoxicity as indicated by the decrease in DNA damage, 8-OHdG levels, sperm abnormalities, LPO level and increased sperm counts, motility and GSH content. Moreover, cress was found to be effective than radish and the combined treatment was more effective than the single treatment. It could be concluded that, pretreatment with radish and/or cress improved the epididymal sperm quality and reduced the genotoxicity and DNA damage induced by BaP, thereby declaring the protective role of radish and cress.

1. Introduction

Benzo(a)pyrene (BaP) is an important polycyclic aromatic hydrocarbon (PAH) carcinogen that undergoes metabolic activation through CYP1A1, CYP1A2, and CYP1B1. BaP is a potent systemic and local carcinogen which induces skin, lung, and stomach tumors in animal models [42]. During the metabolic process, BaP produces reactive oxygen species (ROS) via cytochrome P4501A1 (CYP1A1) [9]. These ROS and their metabolites (benzo(a)pyrene diol epoxide) can
cause oxidative DNA damage and form adducts with DNA. The reactive intermediates of BaP metabolism also have the ability to alkylate nucleophilic sites of DNA [18,9], which can lead to the formation of basic sites and DNA strand breaks. Exposure to BaP can lead to structural changes in DNA of somatic cells and as a consequence to increase incidence of somatic diseases with a genetic basis [14,45] and [44]. The mutagenic potential of BaP in male germ cells, however, has still not been fully established. BaP-related DNA damage was observed at all stages of spermatogenesis and in testis [44,30], but it is largely unknown how germ cells deal with DNA damage to protect their genetic material, and to prevent the accumulation of mutations in the germ line [30]. However, there are dietary agents which have been found to be beneficial in promoting good health and reducing the carcinogenic risks associated with the exposure to some of these dietary agents [28,25].

Increased vegetable intake, particularly of cruciferous vegetables such as cabbage, cauliflower, radish, watercress and mustard greens, is associated with a decreased risk of several cancers in human population studies [40,52] and [6]. Cruciferous vegetables have been shown to display several anticarcinogenic properties in vivo, as reviewed by Steinkellner et al. [41] with various underlying mechanisms. Some of these mechanisms include alterations in the activities of metabolic enzymes [29] and [21], through reduction of oxidative DNA damage levels in humans after supplementation with Brussels, Cuciferous and Leguminous sprouts [17].

In terms of active chemical species, cruciferous vegetables are rich sources of glucosinolates, a class of sulfur- and nitrogen-containing glycosides that are hydrolyzed (by plant myrosinase or intestinal microflora) to form isothiocyanates. Isothiocyanates protect laboratory animals against chemically-induced cancer through inhibition of phase I enzymes and/or induction of glutathione-S-transferase [50,23]. 4-(Methylthio)-3-butenyl isothiocyanate (MTBITC) is one of the most extensively studied compounds in this class, and recent epidemiological and experimental studies showed that MTBITC and other isothiocyanates may possess promising cancer chemo-preventive agent towards different classes of environmental carcinogens [19]; Ben [2]. Cruciferous vegetables such as cress (Lepidium sativum) and radish (Raphanus sativus) contain glucotropaeolin, the precursor compound from which butenyl isothiocyanate is formed. Therefore, we used garden cress and radish as a model cruciferous plant in the present study. Although the chemo-preventive effects of isothiocyanates were found to be due to protection against DNA damage caused by the different carcinogens, only few reports are available on the antimutagenic effects of cruciferous vegetables [21] and none of the available reports indicate potential protective effects of radish and cress in germ cells. The aim of the current study was to investigate the chemo-preventive role of radish and cress oil-extracted from Egyptian R. sativus and L. sativum-against the genotoxic and cytotoxic effect of benzo-a-pyrene in sperm and testicular tissue of mice.

2. Materials and methods

2.1. Chemicals

Benzo(a)pyrene (BaP) (CAS No. 50-32-8. Sigma, USA) was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1 mg/ml. Radish and Cress seed oils were purchased from El-Captain Company (CAP PHARMA), 6th October City, Egypt. The major fatty acid in Cress seed oil was ω-linolenic acid (34%) followed by oleic (22%), linoleic (11.8%), eicosanoic (12%), palmitic (10.1%) erucic (4.4%), arachidic (3.4%) and stearic acids (2.9%). The total tocopherol and carotenoid content of Cress seed oil was 327.42 and 1.0 µmol/100 g oil, respectively. The oil was stable up to 4 months at 4 °C. While Radishes seed oil are rich in ascorbic acid, folic acid, and potassium. They are a good source of vitamin B6, riboflavin, magnesium, copper, and calcium. All other chemicals were of analytical grade.

2.2. Animals

Adult Swiss male mice (10–12 week-old, 25 gm) were randomly drawn from the stock colony of “National Research Center”. Mice were housed in polypropylene cages in a controlled atmosphere with a temperature range of 25 ± 5 °C and mean relative humidity of 50 ± 5%. The animals were maintained on commercial mouse pellets ad libitum and had free access to water during a week of acclimatization and throughout the experiment.

2.3. Experimental protocol

The mice were randomly divided into nine groups (10 mouse/group). Group I, served as control and received normal diet throughout the experimental period. Group II, intraperitoneally injected with BaP (175 mg/kg body weight) dissolved in DMSO at a concentration of 1.0 mg/ml for 2 consecutive days. Group III, served as negative control and intraperitoneally injected with DMSO (4 ml/kg body weight) for 2 consecutive days. Groups IV, V, VI, VII, VIII and IX orally administered radish and/or cress (0.1 ml/25 gm b.w/day) alone or 2 weeks prior to BaP injection for 2 days. At the end of the experimental period, all animals were killed by decapitation. The epididymis and testes were excised immediately and processed for the following analysis.

2.4. Cytogenetic techniques

2.4.1. Quantification of DNA damage in testis and epididymal sperm

The fluorimetric analysis of sperm and testis DNA unwinding (FADU) was performed according to the procedure described by Birnboim [5] with minor modifications. In brief, final cell pellet was suspended in known volume of Krebs Ringer bicarbonate solution. An aliquot of testicular cell suspension (1 × 10⁶ cells) or epididymal sperm (2 × 10⁶) were transferred into test tubes and cells were lysed for 10 min. The assay was performed in triplicates. The pH was increased by adding, successively and carefully, the alkaline solutions in order to allow DNA unwinding. Following neutralization, the percentage of double-stranded DNA (ds DNA) formed was detected by measuring the fluorescence of samples after addition of ethidium bromide. Measurements were performed in a Shimadzu F-2000 fluorescence spectrophotometer with 520 and 575 nm as excitation and emission wavelengths, respectively. The percentage of ds DNA remaining after the unwinding process was calculated according to the method of Birnboim [5] by the ratio (unwound DNA fluorescence-denatured DNA...
fluorescence))/native DNA fluorescence–denatured DNA fluorescence).

2.4.2. Determination of 8-hydroxy-2′-deoxyguanosine (8-OHdG), in testis/sperm-extracted DNA
Sperm and testis DNA were extracted by phenol/chloroform/isoamyl alcohol according to Racevskis [31]. Extracted DNA was digested by DNase-1 (1 U/1 μg DNA) and the digested DNA was subjected to the determination of 8-OHdG by ELISA according to the Kit instructions (BIOXYTECH, 8-HDG-EIA Kit, OXIS, Health Product. Portland Inc., OR, USA).

2.5. Sperm characteristics
The cauda epididymis was removed after sacrificing the animals, and placed in a Petri dish containing 2–3 ml of HBSS at room temperature. The epididymis was minced into small pieces to allow the sperm to swim out. One drop of sperm suspension was placed on a microscope slide, and a cover slip was placed over the droplet. At least 10 microscopic fields were observed at 400× magnification using a phase contrast microscope, and the percentage of motile sperm was recorded according to WHO [48] recommendations. Sperm motility was expressed as a percentage of motile sperm of the total sperm counted. The obtained sperm suspension was centrifuged at 1000 rpm for 5 min. After centrifugation, 1 ml of the supernatant was taken and the epididymal sperm count was determined using Neubauer’s hemocytometer. For sperm head morphology, the sperm suspension in HBSS was stained with 1% nigrosin and kept undisturbed for 1 h. Smears were prepared using the above solution, air dried and fixed with absolute methanol for 5 min. Five hundred sperms per animal were examined to determine the morphological abnormalities at 1000× magnification [7,22]. Sperm head morphology was scored under the category of normal, sperm without hook, amorphous head and banana head essentially as described by [49]. Data were shown in terms of% of abnormal sperms.

2.6. Biochemical analysis
The epididymal sperm suspension was centrifuged at 800g for 10 min at 4°C and the pellet was resuspended in 0.01 M Tris–HCl buffer (pH 7.4). The sperm suspension was homogenized with the help of a glass-Teflon homogenizer and the aliquots of the homogenate were suitably processed for the assessment of following biochemical parameters.

2.7. Reduced glutathione (GSH)
GSH analysis was performed fluorimetrically. Briefly, 0.5 ml of previously prepared homogenate was added to 0.5 ml of 5% trichloro acetic acid then centrifuged, the supernatant (200 µl) was added to 1750 µl of potassium phosphate buffer and 50 µl of DTNB reagent. The product was measured spectrophotometrically using the extinction coefficient of 13.7 mM−1 cm−1. Protein thiols were expressed as µmol/g tissue.

2.8. Lipid peroxidation
Lipid peroxidation (LPO) was determined by the procedure of Muralidhara and Narasimhamurthy [27]. Malondialdehyde (MDA), formed as an end product of the peroxidation of lipids, served as an index of the intensity of oxidative stress. MDA reacts with thiobarbituric acid to generate a colored product that can be measured optically at 532 nm.

2.9. Histopathological studies
Specimens from testes were collected from all experimental groups and fixed in 10% neutral buffered formalin, dehydrated in ascending concentrations of ethyl alcohol (70–100%) and then prepared using standard procedures for Hematoxylin and Eosin staining [1].

2.10. Statistical analysis
All data were statistically analyzed using analysis of variance (ANOVA). The significance of the differences among treatment groups was determined by Waller–Duncan k-ratio [46]. All statements of significance were based on probability of \( P \leq 0.05 \).

3. Results
3.1. Cytogenetic analysis
3.1.1. DNA damage in testis and epididymal sperm
The FADU method was based on the rate of alkaline unwinding of DNA being dependent on the length of the DNA molecule. A decrease in the percentage of double stranded DNA following treatment relates to the formation of strand breaks. The effect on DNA unwinding at 15°C for different treatments is shown in Table 1. The percent double-stranded DNA in control animals varied from 84.4 to 89.8 in testicular DNA and from 86 to 91.6 in sperm DNA. Samples from BaP treated-animals showed a significant decrease (\( P < 0.05 \)) in% double-stranded DNA in the testis (61.4 ± 0.99) and epididymal sperm (65.6 ± 1.31) compared to the control. However, animals treated with radish and/or cress prior to BaP injection showed a significant inhibition in the percentage of DNA damage in both tissues. The maximum efficacy being found in the animals received the combined treatment (radish plus cress) as the inhibition percentage reached 92.1% and 93.6% in testicular and sperm DNA, respectively (Fig. 1).

As illustrated in Table 1, BaP induced a testicular and sperm oxidative DNA damage as detected by a significant increase in the amounts of 8-OHdG in the testicular and sperm-extracted DNA by 4.9- and 3.9-folds, respectively, compared to those of control group. Moreover, in groups of animals that received radish or cress alone or in combination, the amounts of 8-OHdG were similar to that of the untreated animals in both testicular and sperm DNA. On the other hand, pretreatment with radish or cress prior to BaP injection resulted in a significant decrease in the level of 8-OHdG in both tissues. However, the combined treatment (radish plus cress) resulted in 82% prevention of the increase in the level of 8-OHdG in the sperm-extracted DNA.

3.1.2. Sperm characteristics
The sperm count in BaP-treated group decreased significantly (21.5 ± 1.76) compared to those of the control groups. Whereas, animals treated with radish cress and radish plus
cress showed a significant increase in sperm count (39 ± 1.48, 43.6 ± 1.8, and 43.8 ± 1.36, respectively), compared to the control group (34.06 ± 1.73). Animals treated with radish and/or cress prior to BaP injection showed a significant improvement in sperm count compared to BaP-treated group. This improvement was pronounced in the animals treated with cress alone or radish plus cress (Table 2). The motility of sperm in animals treated with BaP showed a significant decrease (P < 0.05) compared to the control animals. Pretreatment with radish and/or cress resulted in a significant increase in the motility percentage compared to BaP-treated animals.

The current results clearly indicated that treatment with radish and/or cress had no influence on sperm abnormality (Table 3). However, animals treated with BaP alone showed a significant increase (16%) in the frequency of abnormal sperm compared to the control group (2.32%). Treatment with radish and/or cress prior to BaP injection resulted in a considerable reduction of sperm abnormalities. The maximum efficacy being found in the animals received the combined treatment (radish plus cress) prior to BaP (43.6%). In spite of this reduction, the percentage of sperm abnormalities was still significantly higher than those in the control group. Various morphological sperm abnormalities in head and tail were recorded in Table 3.

### 3.1.3. Glutathione (GSH) level

The present results revealed that BaP induced a significant depletion in sperm GSH content compared to that of the control value (Fig. 2). Administration of radish before BaP injection significantly improved the sperm GSH content compared to the BaP-treated animals. However pre-administration of cress plus radish restored the sperm GSH content.

### 3.1.4. Lipid peroxidation (LPO) level

Treatment with BaP resulted in a significant (P < 0.05) increase in the LPO in epididymal sperm compared to the control group. Pretreatment with radish or cress significantly decreased the alterations induced by BaP. However, treatment with radish plus cress succeeded in preventing the increase in sperm LPO content and the values were similar to the control (Fig. 3).

### 3.1.5. Histopathological findings

The histological examination of the testis sections of the control, DMSO-treated mice, radish, cress or radish plus cress revealed no histopathological changes (Fig. 4A). Meanwhile, testis of BaP-treated mice revealed vacuolations and necrosis of germ cells lining seminiferous tubules as well as vacuolizations of leydig cells (Fig. 4B) as well as complete absence of germ cells, atrophy of seminiferous tubules associated with interstitial oedema (Fig. 4C). Moreover, some sections showed multinucleated spermatid giant cells (symplasts) in the lumen of seminiferous tubules (Fig. 4D). A moderate improvement in the histological structures was observed in the testis of mice pre-treated with radish. This group showed slight degeneration of germ cells lining seminiferous tubules associated with incomplete spermatogenesis (Fig. 4E). However, marked improvement was observed in the group treated with cress alone or in combination with cress prior to BaP injection (Fig. 4F).

### 4. Discussion

Data from epidemiological studies as well as animal experiments show that cruciferous vegetables have cancer protective effects [43,47]. Generally, isothiocyanates (ITCs) and indoles are considered to be the main protective constituents in these vegetables [43]. These agents interact with enzymes that are

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Double-stranded DNA (%)</th>
<th>DNA adducts (ng 8-hydroxy-2'-deoxyguanosine/µg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Testis</td>
<td>Epididymal sperm</td>
</tr>
<tr>
<td>Control</td>
<td>89.8 ± 0.62a</td>
<td>91.6 ± 0.56a</td>
</tr>
<tr>
<td>DMSO</td>
<td>84.4 ± 0.49b</td>
<td>86.0 ± 0.38b</td>
</tr>
<tr>
<td>BaP</td>
<td>61.4 ± 0.99c</td>
<td>65.6 ± 1.31c</td>
</tr>
<tr>
<td>Cress</td>
<td>90.8 ± 0.38a</td>
<td>90.76 ± 0.43a</td>
</tr>
<tr>
<td>Radish</td>
<td>92.3 ± 1.31a</td>
<td>92.4 ± 1.27a</td>
</tr>
<tr>
<td>Cress + Radish</td>
<td>92.2 ± 1.44a</td>
<td>91.6 ± 0.72a</td>
</tr>
<tr>
<td>Radish + BaP</td>
<td>77.0 ± 2.24a</td>
<td>80.8 ± 0.51b</td>
</tr>
<tr>
<td>Cress + BaP</td>
<td>80.5 ± 1.10b</td>
<td>83.4 ± 1.38b</td>
</tr>
<tr>
<td>Cress + Radish + BaP</td>
<td>82.6 ± 0.81b</td>
<td>84.7 ± 1.58b</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE, means with different superscripts (a, b, c, and d) between groups in the same column are significantly different at P < 0.05. Five mice were used in each group.
involved in the activation and detoxification of genotoxic carcinogens thereby reducing chemically-induced DNA damage. However, few data on the antigenotoxic activities of the extracts of cruciferous plants that contain ITCs such as cress and radish are available and were obtained from in vitro experiments [21,13]. In the present study, we evaluate the in vivo protective effect of radish and cress each alone or in combination against BaP-induced DNA damage (strand breaks and DNA adducts), sperm genotoxicity and histological alteration in the testis of the mice.

The carcinogenic and mutagenic effects of BaP have been extensively investigated in mammalian and other animal cell systems [18]. Our results showed that BaP (175 mg/kg b.w.) induced a significant increase in DNA strand breaks in testis and sperm accompanied with an increase in 8-OHdG level in DNA in testis and epididymal sperm by 4.9- and 3.9-folds, respectively. This can be a further evidence for oxidative DNA damage caused by BaP. Strand breaks and increased 8-OHdG level in the testis and sperm may have occurred as results of the action of reactive oxygen species that arise during the metabolism of BaP in the cell. This is consistent with previous studies on BaP reported by Delgado et al. [13] who suggested that BaP metabolites can cause oxidative DNA damage from adducts with DNA, and induce a significant increase of DNA strand breaks in a dose dependent manner. It has also been reported that the increase in the level of 8-OHdG is associated with the oxidative DNA damage [35]. During the metabolic process, BaP produces reactive oxygen species (ROS) via cytochrome P450 (CYP1A1) [9]. These ROS and their metabolites can cause oxidative DNA damage and form adducts with DNA as well as a decline in the production of ATP and protein synthesis [35]. It appears that the most prevalent product of DNA oxidation that is detected in genomic DNA of mammalian cells is 8-OHdG [10]. 8-OHdG is one of the most common adducts formed by oxidative DNA damage [15] and is a reliable marker of oxidative DNA damage [37,38].

The present results clearly demonstrated that BaP decreases the sperm count, motility and increases the incidence of sperm head and tail abnormalities. These results confirmed well with the previous report of Ramesh et al. [32] who indicated that BaP induces an epididymis specific effect on sperm count and motility. According to [36], the decrease in the sperm count often results due to the interference in the spermatogenesis process and the elimination of sperm cells at different stages of development and increase apoptosis at specific stages of germinal cycle [33,34]. Hence, the decrease in epididymal sperm count observed in BaP-treated mice might reflect the spermatogenic cell death. BaP decrease ATP which may serve as an energy source for sperm motility, and decrease energy metabolism that may be one of the limiting factors responsible for loss of sperm motility [35]. Furthermore, sperm abnormalities can be attributed to the changes in the physiological, cytotoxic and genetic alterations in the testicular DNA [20]. On the other hand, the abnormalities in sperm head are possibly due to the interference with DNA integrity and/or the expression of the genetic material [49]. The gonadal toxicity of BaP was clearly evident from the histological evaluation of the testis. The increase in testicular vacuolization, necrosis, complete absence of germ cells and atrophy of seminiferous tubules indicated that BaP interferes with the process of spermatogenesis. Mohamed et al. [26] reported that BaP induced testicular malformation and decreased numbers of seminiferous tubules with.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Effects of different treatments on epididymal sperm count, motility and total abnormality percentage.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tested parameters</td>
<td>Control</td>
</tr>
<tr>
<td>Sperm count $\times 10^6$</td>
<td>34.06 ± 1.73$^b$</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>84.4 ± 1.69$^b$</td>
</tr>
<tr>
<td>Abnormal sperms (%)</td>
<td>2.32 ± 0.67$^c$</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE, means with different superscripts (a, b, c, and d) between groups in the same raw are significantly different at $P < 0.05$. Five mice were used in each group.
elongated spermatids and decreased sperm motility and sperm count. In this concern, Ramesh et al. [32] suggested that BaP exposure reduced testis weight in rat and caused significant reductions in the components of the steroidogenic and spermatogenic compartments accompanied with progressive motility and mean density of stored spermatozoa.

There is considerable scientific consensus from epidemiologic studies that cruciferous vegetables may reduce risk of cancer, including cancers of the lung [52,6], prostate [12], colon [40], and of the lymphatic system [51,8]. Cruciferous vegetables contain glucosinolates (GS) from which isothiocyanates (ITCs) are liberated by the enzyme myrosinase. Moreover, ITCs protect laboratory animals against chemically-induced cancer through inhibition of phase I enzymes and/or induction of glutathione-S-transferase [23,11]. The current results showed that cress and radish oils attenuate the genotoxic effects of BaP in terms of decreasing of the DNA strand breaks and the level of 8-OHdG in germ cells (testis and sperm). The protective role of radish and cress may be due to the higher content of isothiocyanate, kaempherol glycosides and l-tryptophan compounds which have the ability to scavenge free radical and enhance the DNA repair system or DNA synthesis. The consumption of watercress, a cruciferous vegetable, can reduce cancer risk in humans via a decrease in DNA damage [16]. Ben Salah-Abbes et al. [4] reported that radish extract has a higher content of ITCs which acts as antigenotoxic complex and enhances DNA repair system or DNA synthesis. Moreover, Kassie et al. [21] reported that water and garden cress juices protect against B(a)P-induced DNA damage in HepG2 cells. In previous studies, we have shown that supplementation of isothiocyanates for 3 consecutive days prior BaP injection displayed a considerably suppressed chromosomal aberrations in testis and bone marrow of mice incidence by BaP [24]. The protective role of radish and cress oil against BaP-induced GSH depletion and increased MDA supported the antioxidant properties which may be due to the higher content of isothiocyanate, and the ability to scavenge free radical intermediates of lipid peroxidation [16]. In this concern, Sipos et al. [39] demonstrated that granule radish root extract protected cell membrane against lipid peroxidation in rats fed on fat-rich diet. Meanwhile, Ben Salah-Abbes et al. [3] reported that isothiocyanates in radish can increase the antioxidant status and lower oxidative damage and free radical generation. Moreover, the increase in GSH activity induced by radish extract may be another way to prevent BaP carcinogenicity through the increase of BaP-glutathione conjugates. Consequently, decrease in the ability of microsomes to metabolize BaP, prevents the

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Sperm tail abnormalities mean ± SE</th>
<th>Sperm head abnormalities mean ± SE</th>
<th>Total sperm abnormalities/500 sperm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amorphous Without-hock Banana</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.4 ± 0.24c</td>
<td>2.4 ± 0.24c</td>
<td>11.6 ± 0.68d 10–14</td>
</tr>
<tr>
<td>DMSO</td>
<td>4.2 ± 0.58c</td>
<td>3.4 ± 0.25c</td>
<td>15.2 ± 1.15 12–18</td>
</tr>
<tr>
<td>BaP</td>
<td>14.6 ± 0.98a</td>
<td>25.0 ± 1.87a</td>
<td>80.2 ± 3.22 73–91</td>
</tr>
<tr>
<td>Radish</td>
<td>2.8 ± 0.37c</td>
<td>3.6 ± 0.51c</td>
<td>12.6 ± 0.80 10–14</td>
</tr>
<tr>
<td>Cress</td>
<td>2.8 ± 0.20d</td>
<td>3.6 ± 0.50f</td>
<td>12.4 ± 0.97 11–16</td>
</tr>
<tr>
<td>Radish + Cress</td>
<td>3.2 ± 0.49f</td>
<td>2.6 ± 0.40e</td>
<td>12.5 ± 1.16 10–16</td>
</tr>
<tr>
<td>Radish + BaP</td>
<td>7.6 ± 0.93b</td>
<td>6.8 ± 0.80b</td>
<td>29.0 ± 2.1b 23–34</td>
</tr>
<tr>
<td>Cress + BaP</td>
<td>6.0 ± 0.71b</td>
<td>6.8 ± 0.66b</td>
<td>26.8 ± 2.26b 19–32</td>
</tr>
<tr>
<td>Radish + Cress + BaP</td>
<td>5.2 ± 0.37bc</td>
<td>5.6 ± 0.38b</td>
<td>21.2 ± 1.0b 19–24</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE, means with different superscripts (a, b, c, and d) between groups in the same column are significantly different at P < 0.05. Five mice were used in each group.
formation of DNA adduct and its toxic effects. Restoration of depleted GSH and prevention of the increase in MDA contents are key mechanisms in protection of radish and cress against oxidative stress induced by BaP. This could be useful in explaining or understanding its ability to prevent oxidative DNA damage induced by BaP.

Pre-treatment of radish and/or cress substantially attenuated the testicular spermatogenic cell damage, restored the sperm counts and decreased the incidence of sperms with head abnormalities in BaP-treated mice. Similar to the current observation, Mahrous et al. [24] reported that isothiocyanates improved sperm count, motility and viability as well as decreased sperm abnormalities in BaP treated mice. The results of the intervention of radish and/or cress and the reduction in the gonadal toxicity induced by BaP were further confirmed by the histological examination of the testes.

In summary, the findings of the present study indicated that administration of BaP induces a significant oxidative stress and genotoxicity in epididymal sperm and testis which are associated with DNA damage and spermatogenetic dysfunction. The intervention of radish and/or cress prior to BaP injection reduces the germ cell genotoxicity as evident from the decrease in DNA damage, 8-OHdG, MDA level, sperm head abnormalities, and increased sperm counts motility and GSH contents.

References
