Ameliorative effect of *Allium sativum* extract on busulfan-induced oxidative stress in mice sperm

Ali Soleimanzadeh1*, Leila Mohammadnejad1, Abbas Ahmadi2

1 Department of Theriogenology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran; 2 Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

**Abstract**

Busulfan is known to cause several adverse effects including reproductive toxicity in humans. Garlic (*Allium sativum*), a widely distributed medicinal plant, is highly regarded for its medicinal activities including antioxidant property. This study was conducted to assess whether garlic extract could serve as protective agents against testicular toxicity during busulfan treatment in a mice model. Seventy-two adult male mice were randomly divided into nine groups. In groups 1, 2 and 3, distilled water, busulfan, and dimethyl sulfoxide and in the treatment groups hydro-alcoholic extract of garlic was administered orally at different doses per day (groups 4, 5 and 6; 200, 400, 800 mg kg⁻¹ respectively). Groups 7, 8 and 9 were treated with the extract (200, 400 and 800 mg kg⁻¹, respectively) plus busulfan. Following euthanasia, blood samples and epididymal sperm were collected. The busulfan-treated group showed significant decreases in sperm quality parameters, and serum levels of testosterone, LH and FSH was observed in the busulfan-treated mice. In addition, the TAC levels and antioxidant enzymes activities were reduced and malondialdehyde (MDA) levels were increased in the busulfan-treated mice. Notably, garlic extract co-administration caused a considerable recovery in sperm quality parameters, TAC levels, antioxidant enzymes activities, hormonal changes and MDA level. Based on our results, garlic has antioxidant effects against busulfan-induced testicular damages in mice.

© 2018 Urmia University. All rights reserved.
Introduction

Busulfan is an effective chemotherapy drug widely used for cancer treatment. Busulfan as a cytostatic agent absorbs from the gastro-intestinal tract and quickly disappears from blood with a half-life of 2 to 3 hr. It is also potentially carcinogenic and teratogenic and has many side effects on gonadal function and different body organs such as skin, bladder, liver and nervous system. Spermatogenesis in mammals is a complex process depending on spermatogonial stem cells. Unlike other drugs, busulfan is a potent agent that preferentially kills spermatogonial stem cells. From the mechanisms by which busulfan can damage the cells of different organs, production of reactive oxygen species (ROS) are important and they have major impact in development of oxidative stress. It seems busulfan inhibits the spermatogenesis process, especially by oxidative damage. Other mechanism suggested that busulfan increases the level of CK and it causes spermatogenesis disorder and infertility.

To protect molecules against toxic free radicals and other ROS, cells have developed antioxidant defenses including the enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), which destroy toxic peroxides and small molecules including glutathione (GSH). External sources of antioxidant nutrients that are essential for antioxidant protection include antioxidant vitamins C and E, vitamin A and the mineral selenium, a component of selenium-dependent GPx.

Medicinal plants have been used for treatment and prevention of certain diseases throughout the world; however, the effects of some plants have been studied scientifically. Phytochemicals from plant-rich diets including garlic, provide important additional protection against oxidant damage. Allium species such as onion and garlic are consumed as seasonings, foods, spices and local drugs. Allium sativum is extended throughout the world. Compounds of garlic are vitamins A, C, B6, B1 and B2, sulfur, ajoine and alicine. Reducing cardiovascular and cancer risk factors, stimulation of immune activities, antioxidant activity and antiviral and antimicrobial effects are biological responses of garlic. The antioxidant effects of garlic extract on reproductive performance have been studied previously.

To the best of our knowledge, there is no scientific study concerning the effect of A. sativum hydro-alcoholic extract on busulfan-induced male reproductive damages. This study was designed to investigate the effect of A. sativum (garlic) hydro-alcoholic extract on changes of serum concentrations of testosterone, LH and FSH hormones, semen parameters, antioxidant enzyme activities and total antioxidant capacity (TAC) and malondialdehyde (MDA) levels in adult male mice treated with busulfan.

Materials and Methods

Extract preparation. In this experimental study, A. sativum samples were obtained from the Agricultural and Natural Research Center of west Azerbaijan province. Identification of genus and species samples was done by plant taxonomy experts from Faculty of Science, Urmia University, Urmia, Iran. Hydro-alcoholic extract of A. sativum was obtained using the method of Erdemoglu et al.

Drugs preparation. Busulfan (1,4-Butanediol dimethanesulfonate; Sigma, St. Louis, USA) was first dissolved in dimethyl sulfoxide (DMSO; Sigma), then an equal volume of sterile water was added to reach a final concentration of 5.00 mg mL⁻¹.

Animals. Adult male mice with the age range of 8 to 10 weeks and average weight of 26.00 ± 2.00 g were provided from Animal House of Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. Animals were kept under a controlled environmental condition at room temperature (22.00 ± 2.00 °C) with humidity of 5.00 ± 10.00% and a 12/12 hr photoperiod. This project was carried out in accordance with international guidelines for the care and use of laboratory animals and approval of the ethics committee of Faculty of Veterinary Medicine, Urmia University (3/T. DT/1813-2016).

Experimental design. After one week of acclimation period, the mice were divided randomly into nine groups of eight mice each. Animals within different treatment groups were maintained on their respective diets for 35 days as follows: group 1 (control), received distilled water (0.1 mL per day), group 2 (busulfan), received busulfan (20 mg kg⁻¹, IP), group 3 (sham), received a single dose of DMSO (10 mg mL⁻³, IP), group 4, 5 and 6 was treated with 200, 400, and 800 mg kg⁻¹ hydro-alcoholic extract of A. sativum per day, respectively. In groups 7, 8 and 9, hydro-alcoholic of A. sativum (200, 400, and 800 mg kg⁻¹ per day, respectively) plus busulfan (20 mg kg⁻¹, IP) was administered orally.

Semen collection. Epididymal sperms were collected by slicing the cauda region of the epididymis in 5 mL of human tubal fluid and incubated for 30 min at 37 °C in an atmosphere of 5.00% CO₂ to allow sperm to swim out of the epididymal tubules. After collection, the sperm count, motility, viability, morphology and DNA integrity and semen TAC were evaluated using conventional methods.

Sperm count assessment. In order to count sperms, a 1:20 dilution was prepared in a 1 mL microtube through pouring 190 µL of distilled water and addition of 10 µL sperm mixture. Then, 10 µL of the mixture was dropped on a Neubauer slide and the sperms were counted.

Sperm motility evaluation. In order to evaluate sperm motility, 10 µL sperm suspension was placed on a pre-heated slide and covered with a slip and then the motility was observed under a light microscope (Nikon, Tokyo, Japan) with 400× magnification.
Sperm viability. Sperm viability was evaluated as follows: 20 µL of 0.05% Eosin Y-nigrosin was added into an equal volume of sperm suspension. After 2 min incubation at room temperature, slides were observed by a light microscope with magnification of 400x. Dead sperms were stained pink but the live ones took no color. Viable sperms (n = 400) were counted in each sample and the viability percentage was computed.23

Sperm morphology. To evaluate sperm morphology in the present study, aniline blue staining method was implemented and abnormal morphologies percentage was determined. Especially, the cytoplasmic residual of sperms was considered as an abnormal morphology.24

Sperm DNA damage determination. Fragmentation of sperm DNA was applied as a biomarker for male infertility. Acidine orange (AO) staining was used, after challenging at low pH, to distinguish between denatured, native and double-stranded DNA regions in sperm chromatin25 and high level of fluorescent can be observed in denatured DNA. Thick smears were placed in Carnoy’s fixative (methanol/acetic acid 1:3) for 2 h for fixation.26 The slides were removed from the fixative and left on the outside to be dried for 5 min at laboratory temperature. Then, slides were placed in a stock solution of 1 mg of AO in 1000 mL distilled water and stored in a dark place at 4 °C.27 After 5 min staining, sperms were examined using fluorescent microscope (model BX51; Olympus, Tokyo, Japan). Green-colored sperms were observable as normal sperms and yellow-red ones were considered as sperms with abnormal or damaged DNA.20

Hormonal assay. Serum concentration of testosterone was measured by enzyme-linked immunosorbsent assay (ELISA) as described in the instructions provided by manufacturer’s kit (Demeditec Diagnostics GmbH, Kiel, Germany).

Serum levels of LH and FSH were determined by ELISA using specific commercial kits (Amersham, Buckinghamshire, UK) according to a previous study.29

TAC assay. The TAC of the semen was measured by ferric reduction antioxidant power (FRAP) assay.30 Cellular supernatants (100 µL) was added to 1 mL of fresh FRAP reagent [(2,4,6-Tri(2-pyridyl)-s-triazine; Sigma)] and incubated in 37 °C for 10 min at dark condition. Reading of the blue-colored reagent was then taken at 595 nm every 20 sec for 10 min. Aqueous solution of Fe^II (FeSO_4·7H_2O; Merck; Darmstadt, Germany) and appropriate concentration of freshly prepared ascorbic acid were used as blank and standard solutions, respectively.

Assessment of antioxidant activity. For biochemical tests, after thawing of the testicular tissue, one g of the samples was homogenized in ice-cold 20 mM Tris-HCl Buffer (pH = 7.40; Sigma) and then centrifuged for 10 min in 4 °C at 4000 g. For examination of antioxidant enzymes activities, CAT level was determined as described by Sinha.31 GPx level was determined according to Paglia and Valentine32 and SOD level was determined according to Nishikimi et al.33

Measurement of GSH. To measure reduced GSH, testicular tissue was homogenized in phosphate-EDTA buffer (pH=8.00; Merck) and 25% HPO_3 and centrifuged for 10 min at the temperature of 4 °C at 15,000 rpm. Reduced GSH level was estimated according to Rajesh and Latha.34

Measurement of MDA. A volume of 300 µL of 10% trichloro-acetic acid (Sigma) was added to 150 µL of the sample, centrifuged at 1000 rpm for 10 min at 4 °C and then incubated in 300 µL of 67.00% thiobarbituric acid (TBA; Sigma) at 100 °C for 25 min. Five min after cooling the solution, pink color was appeared because of MDA-TBA reaction and evaluated using a spectrometer (Novaspec II; Biochrom Ltd., Cambridge, UK) at a wave length of 535 nm.35

Statistical analysis. The results are expressed as the mean ± standard error of mean. Differences between the groups were assessed by analysis of variance using SPSS (Version 17.0, SPSS Inc., Chicago, USA). Statistical significance between groups was determined by Tukey’s multiple comparison post hoc test and the p-values less than 0.05 were considered to be statistically significant.

Results

Average sperm count. The results revealed that the number of sperms was decreased significantly (p < 0.01) in busulfan group in comparison with the control group. Group 6 revealed a significant enhancement compared to control group (p < 0.01). There was no significant difference in sperm count between group 5 and group 6 as well as group 8 and group 9 (Table 1).

Table 1. Effects of Allium sativum extract on sperm parameters in study groups.

| Groups                      | Count (10^6) | Motility (%) | Viability (%) | Morphology (%) | DNA integrity (%) |
|-----------------------------|--------------|--------------|---------------|----------------|-------------------|
| Control                     | 21.77 ± 0.14a| 75.19 ± 0.23a| 89.23 ± 0.33a | 88.72 ± 0.41a  | 1.23 ± 0.14a      |
| Busulfan                    | 14.32 ± 0.19b| 58.38 ± 0.44b| 67.08 ± 0.45b | 63.41 ± 0.05b  | 19.17 ± 0.34b     |
| Sham                        | 21.75 ± 0.01a| 74.69 ± 0.09c| 89.21 ± 0.22c | 86.34 ± 0.18c  | 1.25 ± 0.51a      |
| A. sativum (200 mg kg⁻¹)    | 22.18 ± 0.07c| 76.34 ± 0.01d| 91.00 ± 0.01d | 88.20 ± 0.08a  | 1.09 ± 0.01c      |
| A. sativum (400 mg kg⁻¹)    | 22.85 ± 0.09d| 78.09 ± 0.19e| 92.60 ± 0.25e | 88.91 ± 0.17d  | 0.89 ± 0.27c      |
| A. sativum (800 mg kg⁻¹)    | 22.90 ± 0.12d| 78.22 ± 0.26f| 93.20 ± 0.16f | 89.42 ± 0.20e  | 0.87 ± 0.17d      |
| A. sativum (200 mg kg⁻¹) + Busulfan | 19.20 ± 0.23e| 71.07 ± 0.09g| 72.05 ± 0.12f | 68.28 ± 0.07f  | 7.62 ± 0.24e      |
| A. sativum (400 mg kg⁻¹) + Busulfan | 20.03 ± 0.31f| 71.96 ± 0.07h| 72.08 ± 0.07f | 72.08 ± 0.36g   | 6.33 ± 0.21f      |
| A. sativum (800 mg kg⁻¹) + Busulfan | 20.08 ± 0.01f| 72.80 ± 0.31a| 75.36 ± 0.24h | 75.36 ± 0.31h  | 4.17 ± 0.07g      |

Different superscript letters indicate significant differences between groups.
Sperm motility. Comparison of sperm motility in busulfan group with control group showed significant decrease ($p < 0.01$). However, significant variations ($p < 0.01$) were observed among *A. sativum* supplemented groups and control group (Table 1).

Sperm viability. The comparison of sperm viability in busulfan group with control group showed that it was significantly reduced ($p < 0.01$), (Fig. 1). However, with administration of *A. sativum* in group 7, group 8 and group 9, this reduction was improved, but there was significant difference ($p < 0.01$) between control and busulfan groups. Also, no significant variations ($p < 0.01$) were observed between group 7 and group 8 (Table 1).

Sperm morphology. The mean percent of sperm with normal morphology in busulfan group was significantly lower ($p < 0.01$) than control group, but with administration of *A. sativum* in busulfan groups, this reduction was improved. Also, in treatment groups (groups 4-6), in spite of high percentages of normal sperm, it was significantly different ($p < 0.01$) from the control (Table 1).

Sperm DNA damage. The percentage of sperm with damaged DNA in busulfan group was significantly increased ($p < 0.01$) compared to the control group (Fig. 1). However, in groups 4-9, it was reduced in comparison with busulfan group, yet it was significantly higher than control group ($p < 0.01$). The percentage of sperm with damaged DNA in sham group was almost the same as the control group (Table 1).

**Level of TAC.** Comparisons of the TAC levels are shown in Table 2. The TAC levels in groups 4-9 were significantly higher ($p < 0.01$) than busulfan group.

Hormonal levels. Treatment with busulfan caused a significant decrease ($p < 0.01$) in serum levels of testosterone, LH and FSH as compared to the control group. The administration of hydro-alcoholic extract of *A. sativum* along with busulfan significantly restored serum levels of testosterone, LH and FSH towards the control values (Table 2).

Antioxidant enzyme activities. In this study, intraperitoneal (IP) administration of busulfan significantly ($p < 0.01$) decreased tissue GSH contents compared to the control group (Table 2). Also, oral treatment with hydro-alcoholic extract of *A. sativum* significantly ($p < 0.01$) increased tissue GSH contents in testes compared to busulfan group (Table 2). In this study, IP administration of busulfan in mice significantly ($p < 0.01$) decreased the activity of antioxidant enzymes (GPx, CAT and SOD) in testes compared to the control group. Oral administration of hydro-alcoholic extract of *A. sativum* significantly ($p < 0.01$) increased the activity of GPx, CAT and SOD in testes compared to the busulfan group (Table 3).

**Lipid peroxidation.** The level of MDA, as a major product of lipid peroxidation was significantly increased ($p < 0.01$) in busulfan group compared to the control group. There was a significant restoration in treatment groups (groups 7-9). Also, in some treatment groups (groups 4-6), there was a significant decrease ($p < 0.01$) in MDA levels compared to control groups (Table 2).

**Discussion**

This study was planned to investigate the protective role of *A. sativum* hydro-alcoholic extract against busulfan-induced testicular toxicity in male mice. The obtained results showed that IP administration of busulfan in male mice induced reproductive toxicity, increased MDA levels and decreased serum levels of testosterone.

Table 2. Effects of *Allium sativum* extract on TAC, GSH, MDA and testosterone, LH and FSH levels in study groups.

| Parameters          | Control   | Busulfan | Sham     | Group 4 | Group 5 | Group 6 | Group 7 | Group 8 | Group 9 |
|---------------------|-----------|----------|----------|---------|---------|---------|---------|---------|---------|
| TAC (μmol L⁻¹)      | 1.20 ± 0.25 | 0.54 ± 0.34 | 1.17 ± 0.12 | 1.40 ± 0.21 | 1.82 ± 0.37 | 2.10 ± 0.55 | 0.69 ± 0.17 | 0.83 ± 0.20 | 0.99 ± 0.28 |
| Testosterone (μmol L⁻¹) | 6.11 ± 0.14 | 3.24 ± 0.18 | 6.00 ± 0.09 | 6.12 ± 0.09 | 6.10 ± 0.07 | 6.21 ± 0.19 | 4.17 ± 0.26 | 4.77 ± 0.05 | 5.27 ± 0.38 |
| LH (mIU mL⁻¹)       | 2.43 ± 0.46 | 0.93 ± 0.69 | 2.42 ± 0.85 | 2.45 ± 0.61 | 2.79 ± 0.97 | 3.16 ± 0.51 | 1.14 ± 0.43 | 1.39 ± 0.70 | 1.76 ± 0.89 |
| FSH (mIU mL⁻¹)      | 3.75 ± 0.84 | 1.48 ± 0.70 | 3.75 ± 0.33 | 3.83 ± 0.61 | 3.89 ± 0.97 | 4.19 ± 0.50 | 1.64 ± 0.41 | 1.95 ± 0.68 | 2.20 ± 0.29 |
| MDA (μmol mg⁻¹)     | 3.26 ± 0.01 | 8.13 ± 0.20 | 3.40 ± 0.17 | 3.21 ± 0.16 | 3.18 ± 0.39 | 3.12 ± 0.04 | 5.94 ± 0.23 | 5.19 ± 0.06 | 4.47 ± 0.24 |
| GSH (μg mg⁻¹ Proteins) | 64.10 ± 1.28 | 45.12 ± 0.79 | 64.08 ± 0.95 | 66.23 ± 1.34 | 69.38 ± 1.19 | 72.11 ± 0.62 | 49.27 ± 0.47 | 53.61 ± 1.05 | 53.94 ± 1.23 |

TAC: Total antioxidant capacity; LH: Luteinizing hormone; FSH: Follicle-stimulating hormone; MDA: Malondialdehyde; GSH: Glutathione.

Different superscript letters indicate significant differences between groups.

![Fig. 1. A) Dead sperm (red arrow) with red color head and viable sperm (green arrow) with colorless head are observable (Eosin/nigrosin staining, 400×); B) Green-colored normal sperm (red arrow) and yellow-colored damaged sperm (white arrow) can be seen (Acridine orange staining, 1000×).](image-url)
In this study, observed lowered sperm quantity and quality in busulfan group compared to control and sham groups were in agreement with the results of others.36–38 The inhibition of spermatogenesis in busulfan group in this study can be the result of decreased testosterone level. In addition, free radical products in the testicular tissue may exert detrimental effects on spermatogenesis.39

In this study, lowered serum levels of testosterone, LH and FSH in busulfan-treated mice were in agreement with previous reports indicating a dropped level of aforementioned factors following IP administration of busulfan in male mice.36,40 However, Dehghani et al. have reported no significant changes in serum testosterone level of male mice following IP administration of busulfan.38 In another study, Gerl et al. have reported that busulfan can affect exocrine and endocrine compartments of testis and a persistent impairment of Leydig cell function can occur following administration of high doses of busulfan.41 In addition, findings of this study showed that IP injection of busulfan can lead to antioxidant enzymes activities reduction and testicular lipid peroxidation increase. The mechanism of the detrimental effect of IP injection of busulfan can be attributed to its specific direct toxic effects on the testis and it is not the result of its general toxicity and/or non-specific indirect effects through lowering serum testosterone level.42 The oxidative stress in testes increased MDA level and concomitantly decreased the activities of protective antioxidant enzymes (SOD, CAT and GPx) in testicular tissue. The busulfan-induced oxidative stress observed in this study was similar to previous reports in male reproductive system.37 Oxidative stress in testicular tissue can lead to serum testosterone level disturbance and testicular damages as well.36,38

In our study, oral administration of A. sativum hydro-alcoholic extract in busulfan treated animals significantly increased serum levels of FSH, LH and testosterone and improved sperm quantity and quality. Also, the findings of this study demonstrated that administration of A. sativum hydro-alcoholic extract caused a significant improvement in reproductive parameters of male mice compared to busulfan treated group. In addition, there was a significant increase in activities of antioxidant enzymes (SOD, GPx and CAT) and a decrease in testicular lipid peroxidation following administration of A. sativum hydro-alcoholic extract in this study. Several studies have reported that A. sativum contains a large amount of beneficial nutritional components and detectable active antioxidants including alliin, allicin, gamma-glutamyl cysteine, dially sulfide and diallyl disulfide.43,44

The increased level of serum testosterone, LH and FSH due to administration of A. sativum in this study could be responsible for sperm quantity and quality improvement as it has been established that androgen hormone is essential for spermatogenesis.44 In addition, the improvement of sperm quantity and quality via administration of A. sativum hydro-alcoholic extract may be also attributed to its antioxidant activity.15,46,47

The improvement of semen characteristics in this study following administration of A. sativum hydro-alcoholic extract was in agreement with previous findings reporting that an improvement in male reproductive function following A. sativum administration can be attributed to its powerful antioxidant properties.15,46,48,49 In another study, Oi et al. have showed that administration of garlic powder in rats increases the testicular testosterone concentration.50 Also, Mirfarid et al. demonstrated that administration of hydro-alcoholic extract of garlic can increase serum concentrations of LH, FSH and testosterone hormones following cyclophosphamide administration.49

In conclusion, it has been clearly determined that antineoplastic or cytotoxic chemotherapy drugs like busulfan, causes a decrease in FSH, LH and testosterone level, antioxidant status and sperm motility, normal morphology, count and viability and an increase in levels of MDA in male mice. Also, our findings showed that oral administration of A. sativum hydro-alcoholic extract after IP injection of busulfan can reduce the reproductive toxicity of busulfan and help the improvement of reproductive parameters of normal male mice. Our results also revealed that the above mentioned changes will be dose dependent with A. sativum hydro-alcoholic extract.

Acknowledgements

Authors would like to sincerely thank the members of the Faculty of Veterinary Medicine and Urmia University Research Council for the approval and support of this research.

Conflicts of interest

The authors declare that they have no particular conflicts of interest.
References

1. Suriapraba E, Mani PR, Kumar RA, et al. Protective effect of NAC on busulfan induced clastogenesis in human peripheral blood lymphocytes. Int J Inst Pharm LifeSci 2012; 2(1): 172-177.
2. Sweetman SC. Martindale. London, UK: Pharmaceutical Press 2002; 33.
3. McClive PJ, Sinclair AH. Type II and type IX collagen transcript isoforms are expressed during mouse testis development. Biol Reprod 2003; 68(5): 1742-1747.
4. Kanatsu-Shinohara M, Toyokuni S, Morimoto T, et al. Functional assessment of self-renewal activity of male germine stem cells following cytotoxic damage and serial transplantation. Biol Reprod 2003; 68: 1801-1807.
5. Probin V, Wang Y, Zhou D. Busulfan-induced senescence is dependent on ROS production upstream of the MAPK pathway. Free Radic Biol Med 2007; 42(12):1858-1865.
6. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition 2002; 18(10): 872-879.
7. Maymon BB, Yogev L, Marks A, et al. Sertoli cell inactivation by cytotoxic damage to the human testis after cancer chemotherapy. Fertil steril 2004; 81(5): 1391-1394.
8. Valko M, Leibfritz D, Moncol J, et al. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39(1): 44-84.
9. Saxena R, Garg P, Jain DK. In vitro anti-oxidant effect of vitamin E on oxidative stress induced due to pesticides in rat erythrocytes. Toxicol Int 2011; 18(1): 73-76.
10. Bozin B, Mimica-Dukic N, Samojlik I, et al. Phenolics as antioxidants in garlic (Allium sativum L., Alliaceae). Food Chem 2008; 111(4): 925-929.
11. Hammami I, Nahdi A, Mauduit C, et al. The inhibitory effects on adult male reproductive functions of crude garlic (Allium sativum) feeding. Asian J Androl 2008; 10(4): 593–601.
12. Abid-Essefi S, Zaied C, Bouazziz C, et al. Protective effect of aqueous extract of Allium sativum against zearalenone toxicity mediated by oxidative stress. Exp Toxicol Pathol 2012; 64(7): 689-695.
13. Song K, Milner JA. The influence of heating on the anticancer properties of garlic. J Nutr 2001; 131(3S): 1054S-1057S.
14. Santos J, Almagano MP, Carbo R. Antimicrobial, and antioxidants activity of crude onion (Allium cepa L.) extracts. Int J Food Sci Technol 2010; 45(2): 403-409.
15. Hosseini N, Khaki A. Effect of aqueous extract of garlic (Allium sativum) on sperms morphology, motility, concentration and its antioxidant activity in rats. Afrinad 2014; 80(566): 201-204.
16. Ghalehkandi JG. Garlic (Allium sativum) juice protects from semen oxidative stress in male rats exposed to chromium chloride. Anim Reprod 2014; 11(4):526-532.
17. Erdemoglu N, Kupeli E, Yesilada E. Anti-inflammatory and anti-nociceptive activity assessment of plants used as remedy in Turkish folk medicine. J Ethnopharmacol 2003; 89(1): 123-129.
18. Homafar MA, Soleimanirad J, Ghanbari AA. A morphologic and morphometric study of adult mouse testis following different doses of busulfan administration. J Reprod Infertil 2006; 7(1):25-36.
19. Ahmadi A, Bamohabat Chafjiri S, Sadrkhanlou R. Effect of Satureja khuzestanica essential oil against fertility disorders induced by busulfan in female mice. Vet Res Forum 2017; 8(4): 281-286.
20. Mirfard M, Johari H. The effect of hydro-alcoholic Allium sativum extracts on sexual hormones in mature male rats under chemotherapy with cyclophosphamide. Zahedan J Res Med Sci 2015; 15: 29-33.
21. Zobeiri F, Sadrkhanlou RA, Salami S, et al. The effect of ciprofloxacin on sperm DNA damage, fertility potential and early embryonic development in NMRI mice. Vet Res Forum 2012; 3: 131-135.
22. World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 3rd ed. Cambridge, UK: Cambridge University Press 1992; 45-100.
23. Wyrobek AJ, Gordon LA, Burkhart JG, et al. An evaluation of the mouse sperm morphology test and other sperm tests in nonhuman mammals. Mutat Res 1983; 115: 1-72.
24. Narayana K, D’Souza UJ, Seetharama Rao KP. Ribvirin induced sperm shape abnormalities in Wistar rat. Matal Res 2002; 513(1): 193-196.
25. Bungum M, Humaidan P, Spano M, et al. Predictive value of sperm chromat structure assay (SCSA) Parameters for the outcome of intrauterine insemination, IVF and ICSI. Hum Reprod 2004; 19(6): 1401-1408.
26. Hodjat M, Akhondi MA, Amirjanati N, et al. The comparison of four different sperm chromatin assays and their correlation with semen parameters. Tehran Uni Med J 2008; 65(Supl3): 33-40.
27. Meistrich ML, Brock WA, Grimes SR, et al. Nuclear protein transitions during spermatogenesis. Fed Porc 1978; 37(11): 2522-2525.
28. Talebi AR, Sarcheshmeh AA, Khalili MA, et al. Effect of ethanol consumption on chromatin condensation and DNA integrity of epididymal spermatzoa in rat. Alcohol 2011; 45(4): 403-409.
29. Deiss WP. Hormone assays and their clinical application. J Am Med Assoc 1967; 200(2):184-185.
30. Benzie IF, Strain JJ. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol 1999; 299:15-27.
31. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972; 47(2): 389-394.
32. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70(1): 158-169.
33. Nishikimi M, Rao NA, Yogi K. Colorimetric determination of superoxide dismutase. Biochem Biophys Comm 1972; 46: 849-854.
34. Rajesh MG, Latha MS. Protective activity of Glycyrrhiza glabra Linn. on carbon tetrachloride-induced peroxidative damage. Indian J Pharmacol 2004; 36(5): 284-287.
35. Hosseinzadeh H, Sadeghnia HR. Safranal, a constituent of Crocus sativus (saffron), attenuated cerebral ischemia induced oxidative damage in rat hippocampus. J Pharm Pharm Sci 2005; 8(3): 394-399.
36. Mohammad-Ghasemi F, Soleimanirad J, Ghanbari AA. An ultrastructural study on the apoptotic features of spermatogenic cells following busulfan treatment in adult mice. Reprod Infertil 2008; 8(4): 319-329.
37. Nasimi P, Tabandeh MR, Vahdati A, et al. Busulfan induces oxidative stress-and Bcl-2 family gene-related apoptosis in epididymal sperm and testis of adult male mice. Physiol Pharmacol 2015; 19(3): 208-215.
38. Dehghani F, Hassanpour A, Pooest-Pasand A, et al. Protective effects of L-carnitine and homogenized testis tissue on the testis and sperm parameters of busulfan-induced infertile male rats. Iran J Reprod Med 2013; 11(9): 693-704.
39. Ghosh D, Das UB, Ghosh S, et al. Testicular gametogenic and steroidogenic activities in cyclophosphamide treated rat: A correlative study with testicular oxidative stress. Drug Chem Toxicol 2002; 25: 281-292.
40. Bordbar H, Esmaeilpour T, Dehghani F, et al. Stereological study of the effect of ginger's alcoholic extract on the testis in busulfan-induced infertility in rats. Iran J Reprod Med 2013; 11(6): 467-472.
41. Gerl A, Mühlbayer D, Hansmann G, et al. The impact of chemotherapy on Leydig cell function in long term survivors of germ cell tumors. Cancer 2001; 91(7): 1297-1303.
42. Ilbey YO, Ozbek E, Simsek A, et al. Potential chemoprotective effect of melatonin in cyclophosphamide-and cisplatin-induced testicular damage in rats. Fertil Steril 2009; 92(3): 1124-1132.
43. Banerjee SK, Mulherjee PK, Maulik SK. Garlic as an antioxidant: The good, the bad and the ugly. Phytother Res 2003; 17(2): 97-106.
44. Charles DJ. Antioxidant properties of spices, herbs and other sources. New York, USA: Springer Science & Business Media 2012; 612-613.
45. Ruwanpura SM, Mclachlan RI, Meachem SJ. Hormonal regulation of male germ cell development. J Endocrinol 2010; 205(2): 117-131.
46. Hajiouon B, Elahizadeh H. Effects of garlic (Allium sativum L.) hydro alcoholic extract on estrogen, progesterone and testosterone levels in rats exposed to cell phone radiation. Armaghane Danesh 2014; 19(5): 390-400.
47. Zarei L, Shahrrooz R, Sadrkhanlou R, et al. Protective effects of corum mas extract on in vitro fertilization potential in methotrexate treated male mice. Vet Res Forum 2015; 6(1): 55-61.
48. Asadpour R, Azari M, Hejazi M, et al. Protective effects of garlic aqueous extract (Allium sativum), vitamin E, and N-acetylcysteine on reproductive quality of male rats exposed to lead. Vet Res Forum 2013; 4(4): 251-257.
49. Mirfard M, Johari H, Mokhtari M, et al. The effect of hydro-alcoholic garlic extract on testis weight and spermatogenesis in mature male rats under chemotherapy with cyclophosphamide. J Fasa Uni Med Sci 2011; 1(3):123-130.
50. Oi Y, Imafuku M, Shishido C, et al. Garlic supplementation increases testicular testosterone and decreases plasma corticosterone in rats fed a high protein diet. J Nutr 2001; 131(8): 2150-2156.