






Research Article

The Protective Effects of *Trametes Versicolor* on Arsenic-Induced Male Reproductive Toxicity through Regulation of Oxidative Stress: A Biochemical and Histopathological Survey

Fatemeh Solaimani ¹, Emran Habibi ^{2,3}, Maryam Ghasemi ⁴, Saba Mahboubi ⁵, Ehsan Zamani ⁵, and Fatemeh Shaki ⁶

¹Ramsar Campus, Mazandaran University of Medical Sciences, Ramsar, Iran

²Medicinal Plants Research Center, Mazandaran University of Medical Sciences, Sari, Iran

³Department of Pharmacognosy, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

⁴Department of Pathology, Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

⁵Department of Pharmacology and Toxicology, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran

⁶Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

Correspondence should be addressed to Fatemeh Shaki; fshaki.tox@gmail.com

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Exposure to arsenic is linked to a wide range of diseases, in particular male reproductive toxicities. *Trametes versicolor* is a traditional medicinal fungus with a remarkable potential for antioxidant activity. The purpose of this study was to examine the ameliorating effects of water and methanol extracts of *T. versicolor* on arsenic-induced male reproductive toxicities via the abrogation of oxidative stress. The mice were divided as follows: control: normal saline, As: arsenic (15 mg/kg), WE: water extract (400 mg/kg), ME: methanol extract (400 mg/kg), As + WE: arsenic (15 mg/kg) + water extract (100, 200, 400 mg/kg), As + ME: arsenic (15 mg/kg) + methanol extract (100, 200, 400 mg/kg), and positive control: arsenic (15 mg/kg) + vitamin C (500 mg/kg). Animals were treated via the intraperitoneal route. About 24 hr later, the mice were euthanized, and oxidative stress parameters (reactive oxygen species [ROS], lipid peroxidation, glutathione concentration, protein carbonylation, glutathione peroxidase, and superoxide dismutase activity), histopathological changes and sperm parameters (count, motility, and morphology) were examined in the testicular tissue. Arsenic caused significant pathological changes in the testicular tissue and sperm morphology and significantly reduced sperm count and motility. Moreover, arsenic mediated oxidative stress via significant increases in ROS generation, lipid peroxidation, and protein carbonyl content, as well as significant depletion in glutathione concentration and superoxide dismutase and glutathione peroxidase activities. Although, coadministration of water and methanol extracts of *T. versicolor* at 200 and 400 mg/kg counteracted arsenic-induced oxidative and histopathological damages and improved sperm parameters. Our study indicated that *T. versicolor* ameliorated arsenic-induced testis toxicity and sperm dysfunction via attenuation of oxidative damage.

1. Introduction

Arsenic (As) is a ubiquitously distributed heavy metal in the environment [1]. Human exposure to As is believed to be unavoidable because traces of this element have been detected in the groundwater, soil, food sources, and even as particulates in the air [2]. Drinking water contamination from high As concentrations has recently gained urgency on a global scale [1, 3, 4]. While the World Health Organization

(WHO) guidelines emphasize that the concentration of As in drinking water should be determined to a maximum level of 10 $\mu\text{g/L}$, it is estimated that virtually 220 million individuals throughout the world still access drinking water contaminated with higher concentrations of As [5]. As a case in point, excessive accumulation of As in the groundwater and soil has been observed in different regions of Iran [6, 7]. It has been widely demonstrated that long-term exposure to As can

compromise human health and culminate in various health conditions [8–10]. In addition, the detrimental impacts of As on the male reproductive system have been spotlighted in some studies [11–14]. It is indicated that As has the potency to penetrate the blood-testis barrier (BTB) [15]. The bioaccumulation of As in the testicular tissue can trigger numerous toxicities, including impairment of spermatogenesis and steroidogenesis; induction of histopathological abnormalities; and depletion of sperm counts, motility, and viability. However, the data regarding the mechanisms associated with male reproductive toxicity caused by As is limited [14, 16, 17]. Some evidence suggests that oxidative stress is one of the prominent mechanisms involved in As-induced toxicities. It is assumed that two significant pathways are associated with As-mediated oxidative stress. These pathways include interaction with the antioxidant defense system and changes between oxidation states [18].

Oxidative stress is a pathological condition due to the antioxidant defense system's inability to neutralize the immoderate production of reactive oxygen species (ROS) and the possible damage they may cause [19]. The physiological concentration of ROS is essential for sperm functionality, acrosome reaction, and oocyte fusion. Higher levels might lead to sexual organ dysfunction and infertility due to sperm peroxidation, deoxyribonucleic acid (DNA) damage, and apoptosis [20].

A wide variety of antioxidants have exhibited ameliorative effects on male reproductive toxicities, especially infertility [21–23]. *Trametes versicolor*, formerly *Coriolus versicolor*, is a saprotrophic Basidiomycete commonly found in Asia, Europe, and North America. *T. versicolor* has been used as a medicinal plant in China for over two millennia [24]. The extracts of this fungus have shown considerable antioxidant, antimicrobial, and immunomodulatory activities in various animal models and trials [24, 25]. The health-promoting impacts of *T. versicolor* can be attributed to the presence of components such as polysaccharide-Krestin (PSK) and polysaccharopeptide (PSP) [26]. There is a similarity between PSK and PSP in that they both have demonstrated remarkable free-radical scavenging activity [27]. Furthermore, it has been reported that the biological activity of PSK resembles Superoxide Dismutase (SOD) activity [28]. In addition, a study on PSP derived from *T. versicolor* showed that PSP had the potency to prevent paracetamol-mediated hepatotoxicity by reducing GSSG/Glutathione (GSH) ratio [29].

In light of the previous research, *T. versicolor* has shown promise as an antioxidant that could alleviate oxidative stress in humans. Consequently, we have evaluated the protective effects of the water and methanol extracts of *T. versicolor* (WE and ME) against As-mediated reproductive toxicity in male mice.

2. Materials and Methods

2.1. Chemicals. Mannitol, Sucrose, Ethylenediaminetetraacetic acid (EDTA), Phosphoric acid, Disodium hydrogen phosphate, Methanol, Thiobarbituric acid, HCl, Trichloro acetic acid (TCA), KH_2PO_4 , Dimethyl sulfoxide (DMSO), and *n*-butanol were from Merck; Mops buffer, Paraformaldehyde, Tris-HCl, Potassium Chloride, MgCl_2 , Sodium Succinate,

Dichloro-dihydro-fluorescein diacetate (DCFH-DA), and 2,4-dinitrophenylhydrazine (DNPH) were from Sigma-Aldrich.

2.2. Preparation of *T. Versicolor* Extracts. The fruiting bodies of *T. versicolor* were collected from the forests in northern Iran, Mazandaran. The *T. versicolor* was authenticated by Saeed Ali Mousazadeh (a mycology expert). An herbarium voucher specimen (No-IRAN, MZ.290 F) was deposited at the Agriculture and Resource Research Center Museum, Mazandaran, Iran. After autoclaving for 48 hr, *T. versicolor* was kept in a -20°C refrigerator for 72 hr to eliminate any vermin, xylophagous, and mold. Next, the specimens were dried at 38°C for 7 days. Ground specimens were macerated for 48 hr using absolute methanol to achieve the methanolic extract (ME). Furthermore, ground specimens were dried under a fume hood and then boiled in a flask of hot water for 2 hr to attain water extract. Each of the extraction processes was repeated three times. In the next stage, both the water and MEs were condensed using a rotary evaporator. The condensed extracts were placed in a freeze drier (-50°C) for 72 hr to obtain dry powder extracts. Ultimately, powders were transferred into tight containers and preserved in a cool place [30, 31].

2.3. Experimental Design. A total number of 66 male albino mice with an average weight of 20–25 g and an average age of 8–10 weeks were supplied from Mazandaran University of Medical Science's animal research institute, Sari, Iran. The animals were retained in conventional cages under standard controlled temperature ($25 \pm 1^\circ\text{C}$) and a 12 hr light/dark cycle. Food and water were accessible *ad libitum*. All experimental procedures were conducted following the guidelines approved by the Research Ethics Committee of Mazandaran University of Medical Sciences, Mazandaran, Iran (Ethic number: IR.MAZUMS.REC.1396.2783). The animals were randomly divided into 11 groups ($N = 6$) as follows:

- Control group: received Normal saline (0.5 ml)
- As group: received arsenic (15 mg/kg) (LD50 = 16.5 mg/kg) [17, 32–34]
- WE group: received water extract (400 mg/kg)
- ME group: received methanol extract (400 mg/kg)
- As + WE groups: received arsenic (15 mg/kg) with water extract (100, 200, 400 mg/kg)
- As + ME groups: received arsenic (15 mg/kg) with methanol extract (100, 200, 400 mg/kg) [35, 36]
- Positive control group: received arsenic (15 mg/kg) with vitamin C (500 mg/kg)

The treatments were administered via the intraperitoneal route as a single dose. After 24 hr, the mice were euthanized following the intraperitoneal administration of ketamine (80 mg/kg) + xylazine (5 mg/kg) based on standard protocols and the mice's testes were instantly harvested. By this stage, the testes were irrigated with cold normal saline so as to remove any residue of connective tissue [37]. As regards the oxidative stress biomarkers, ROS formation, glutathione, lipid peroxidation, protein carbonyl contents, SOD, and GPx

activities in the testicular tissue were evaluated. In addition, we analyzed the histopathological changes in the testicular tissue and sperm parameters.

2.4. Measurement of Oxidative Stress Parameters

2.4.1. Tissue Preparation. To conduct biochemical assays associated with oxidative stress, the testes pertaining to each group were first minced and then homogenized on an ice bath using a handheld glass homogenizer. The tissue homogenate of each group was then divided to equal aliquots.

2.4.2. Measurement of ROS Content. The tissue ROS concentration was determined using 2,7-dichlorofluorescein diacetate (DCFH-DA) as previously described with minor modifications [38]. In brief, aliquots of tissue homogenate pertaining to each group were treated with DCFH-DA and incubated at 37°C for 15 min. During incubation, the nonfluorescent DCFH-DA would transform into fluorescent dichlorofluorescein (DCF) in the presence of ROS. Finally, the fluorescence intensity of each group was evaluated at 312 nm excitation/420 nm emission using a spectrofluorometer (Shimadzu spectrofluorometer RF-5000U, Kyoto, Japan).

2.4.3. Measurement of Glutathione (GSH) Concentration. Reduced GSH content was analyzed using 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) as the indicator [38]. Samples of all groups were individually vortex mixed with TCA 20% for the protein contents to precipitate, and right after that, the mixtures were centrifuged at 1,000 × g for 20 min. Next, the supernatants were collected and mixed with 0.3 M Disodium hydrogen phosphate and DTNB 0.4%. The interaction between DTNB and the Sulfhydryl groups existing in the structure of glutathione would culminate in the formation of a detectable yellow thiolate anion group. Finally, the absorption of each group was read at 412 nm on a spectrophotometer (Shimadzu UV-1601PC, Kyoto, Japan). GSH concentration was expressed as μM.

2.4.4. Measurement of Lipid Peroxidation. Lipid peroxidation was determined by evaluating the formation of thiobarbituric acid reactive substances (TBARs), as previously stated [38]. The concentration of malondialdehyde (MDA) in μM was reported as the index of lipid peroxidation. Thiobarbituric acid (TBA) reagent (consisting of 15% w/v TCA and 0.3% w/v TBA in 0.5 N HCl) was added to tissue homogenates and vortexed appropriately. Subsequently, for 30 min, the samples were incubated in boiling water. At this step, the samples were transferred to an ice bath, followed by the addition of *n*-butanol to them. Ultimately, the *n*-butanol layer was separated by centrifugation at 1,500 × g for 10 min, and the absorbance of sample groups was assessed at 532 nm using a microplate reader (Tecan Spectra Rainbow Microplate Reader, Zurich, Switzerland).

2.4.5. Measurement of Protein Carbonyl Content. Protein carbonyl contents are produced as a consequence of protein oxidation as well as the interaction between amino acid residue with lipid peroxidation by-products [39]. The highly sensitive 2,4-dinitrophenylhydrazine (DNPH) spectrophotometric assay

was implemented to determine protein carbonyl content [38]. At the first stage of the process, the protein contents of all groups were precipitated by cold TCA 20% and separated through centrifugation at 6,500 × g for 10 min. Then, 10 mM DNPH in 2 M HCl was added to the pellet pertaining to each group, and samples were placed in a dark chamber for 1 hr at room temperature, where they were vortexed in 10 min intervals. Again, at this stage, protein precipitation was conducted using TCA 20% followed by centrifugation at 6,500 × g for 10 min. The pellets containing protein were collected, and to eliminate free DNPH, they were washed with ethanol/ethyl acetate (1 : 1) three times. Later, protein contents were solubilized through the resuspension of samples in 6 M Guanidine hydrochloride. Finally, the optical absorption was evaluated at 366 nm with a molar absorption coefficient of 22,000 M⁻¹cm⁻¹.

2.4.6. Evaluation of Glutathione Peroxidase (GPx) Activity. The activity of the antioxidant enzyme GPx in the testicular tissue was assessed using commercial kits (Zellbio GmbH, Lonsee, Germany; Cat. No: ZB-GPX-A 96). The assay was conducted as instructed. The GPx activity was calculated after measuring the samples' absorption at 412 nm by a microplate reader (Tecan Spectra Rainbow Microplate Reader, Zurich, Switzerland).

2.4.7. Evaluation of Superoxide Dismutase (SOD) Activity. To determine SOD activity, the tissue homogenate, and reagents were prepared according to the protocols stated in the commercial kit (Zellbio GmbH, Lonsee, Germany; CAT No. ZB-SOD-96A). Gradually, the absorption of samples was assessed at 420 nm using a microplate reader (Tecan Spectra Rainbow Microplate Reader, Zurich, Switzerland), and SOD activity was calculated.

2.4.8. Histopathologic Investigation. Initially, the testes were immersed in paraformaldehyde 4% solution at 4°C to be fixed. After 24 hr, graded concentrations of ethanol were used to dehydrate samples. Following the dehydration, the samples were embedded in a paraffin oven and instantly saturated by paraffin. Next, the blocks of each tissue sample were sectioned at 5-μm-thick intervals on a microtome. The tissue biopsies were stained using hematoxylin and eosin (H&E) stain. Eventually, samples were observed and examined under an optical microscope (Nikon Labophot, Tokyo, Japan) at 400× field. Finally, an experienced pathologist, who was blinded to the study groups, randomly examined testicular tissues [40, 41]. The Johnsen criteria scoring was used to quantify spermatogenesis [42].

2.4.9. Assessments of Sperm Parameters.

(1) Sperm Motility. Sperm motility can be categorized into three distinct types according to WHO, namely progressive motility (PR), nonprogressive motility (NP), and immotility (IM) [43]. The cauda epididymis of each sample group was collected and incised slightly with a scalpel. A droplet of epididymal fluid was diluted with a few droplets of tris buffer and transferred on a preheated slide (37°C). The slides were observed under a light microscope at 40× magnification. The number of progressive sperms and nonprogressive sperms was calculated to determine sperm motility. Each group was

analyzed in triplicates, and the percentage of motile sperms was calculated based on the average of three assessed slides [44]. In this study, nonprogressive motility and immotility were accounted for sperm motility abnormalities.

(2) *Determination of Sperm Count.* To determine the epididymal sperm count, the epididymides were minced in a normal saline and incubated for 10 min at 32°C. Following that, the number of sperms was obtained by a hemocytometer. The sperms were counted at 40× magnification by hemocytometer, and the results were reported based on 1 million sperms in 1 ml sample [44].

(3) *Evaluation of Sperm Morphological Features.* A small portion of sperm suspension was dropped on a slide and then smeared out with another slide. Slides were air-dried overnight so that the morphological abnormalities of sperms could be examined. As regards the determination of viable/nonviable ratio, the slides were stained by 1% eosin and 5% nigrosine in 3% sodium citrate dehydrate solution, and the samples were examined under a light microscope at 100× magnification [44]. All the head, midpiece, and tail defects were accounted for as morphological abnormalities [45].

2.5. *Statistical Analysis.* Results were expressed as mean ± SD. All statistical analyses were performed using the SPSS software, version 13. Assays were performed in triplicate and the mean was used for the statistical analysis. Statistical significance was determined using the one-way ANOVA test, followed by the post hoc Tukey test. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Measurement of Oxidative Stress Parameters

3.1.1. *Measurement of ROS Content.* As demonstrated in Figure 1, As-induced significant increases in ROS generation ($P < 0.001$). In comparison with the As group, water extract (WE) of *T. versicolor* significantly reduced As-induced ROS generation in the testicular tissue at 200 mg/kg ($P < 0.05$) and 400 mg/kg ($P < 0.01$). Whereas the concentration of 100 mg/kg of WE did not impose any significant impact compared to the As group (Figure 1(a)). Similarly, the ME significantly diminished the generated ROS at 200 mg/kg ($P < 0.05$) and 400 mg/kg ($P < 0.01$), whereas it did not exhibit significant effect at the dose of 100 mg/kg compared to the arsenic group (Figure 1(b)). There are no significant differences between WE and ME of *T. versicolor* at different doses (Figure 1(c)).

3.1.2. *Measurement of Glutathione (GSH) Concentration.* Figure 2 shows that there was a significant depletion in GSH concentration in As-treated group ($P < 0.05$). By contrast, in the group treated with 400 mg/kg of WE of *T. versicolor*, the concentration of GSH increased significantly in comparison with As group ($P < 0.05$) (Figure 2(a)). Similarly, a significant increase in the concentration of GSH in the group receiving 400 mg/kg of ME was observed ($P < 0.05$) (Figure 2(b)). Interestingly, neither WE nor ME did not indicate significant increases in GSH levels at the dose of 100 and 200 mg/kg compared to As group (Figure 2). There

are no significant differences between the effects of WE and ME of *T. versicolor* at different doses (Figure 2(c)).

3.1.3. *Measurement of Lipid Peroxidation.* MDA levels increased significantly in the group treated with As ($P < 0.01$) (Figure 3). Treating groups with WE of *T. versicolor* at the dose of 400 mg/kg reduced MDA concentration significantly ($P < 0.05$) compared to As group whereas no significant reductions were observed at the dose of 100 and mg/kg of WE (Figure 3(a)). On the other hand, in the groups that received ME at the doses of 200 and 400 mg/kg, significant decreases in the concentration of MDA were observed in comparison with the As group ($P < 0.05$) whereas the ME at the dose of 100 mg/kg did not have significant effect on the levels of MDA (Figure 3(b)). No significant differences were observed between the effects of WE and ME of *T. versicolor* at different doses (Figure 1(c)).

3.1.4. *Measurement of Protein Carbonyl Content.* As demonstrated in Figure 4, the concentration of protein carbonyl in the testicular tissue increased significantly in As-treated group ($P < 0.001$). We observed significant decreases in the testicular protein carbonyl content in the WE treated groups at the doses of 200 and 400 mg/kg compared to As group ($P < 0.01$). By contrast, the dose of 100 mg/kg of WE did not demonstrate significant effects on protein carbonyl content (Figure 4(a)). Similarly, the levels of protein carbonyl significantly depleted in groups treated with ME at 200 mg/kg ($P < 0.01$) and 400 mg/kg ($P < 0.001$) in comparison with As group. Similar to WE, the dose of 100 mg/kg of ME did not impose significant effect on protein carbonyl content (Figure 4(b)). We did not observe any significant differences between the effects of WE and ME of *T. versicolor* at different doses (Figure 4(c)).

3.1.5. *Evaluation of Glutathione Peroxidase (GPx) Activity.* It was observed that the administration of As decreased GPx activity significantly ($P < 0.05$). Figure 5 demonstrates that coadministration of WE at the maximum concentration (400 mg/kg) did not increase GPx activity significantly compared to As group. Similarly, no significant improvement in the activity of GPx was observed at the maximum dose of ME (400 mg/kg) in comparison with the As group.

3.1.6. *Evaluation of Superoxide Dismutase (SOD) Activity.* As presented in Figure 6, As significantly reduced SOD activity in the testicular tissue ($P < 0.01$). Contrary to As group, neither treating with the maximum dose of WE (400 mg/kg) nor the maximum dose of ME (400 mg/kg) improved the activity of SOD.

3.1.7. *Histopathologic Manifestation.* Seminiferous tubules are recognized as male reproductive system's functional unit where spermatogenesis takes place. Spermatogenesis is a remarkably complicated process which involves a wide range of molecular, biochemical, and cellular events. It has been demonstrated that the active interaction between germ cells and Sertoli cells, which play an elaborate role in different stages of spermatogenesis [46]. To assess the detrimental effects of As and ameliorative impacts of *Trametes versicolor* on the testicular tissue, we observed the histopathological

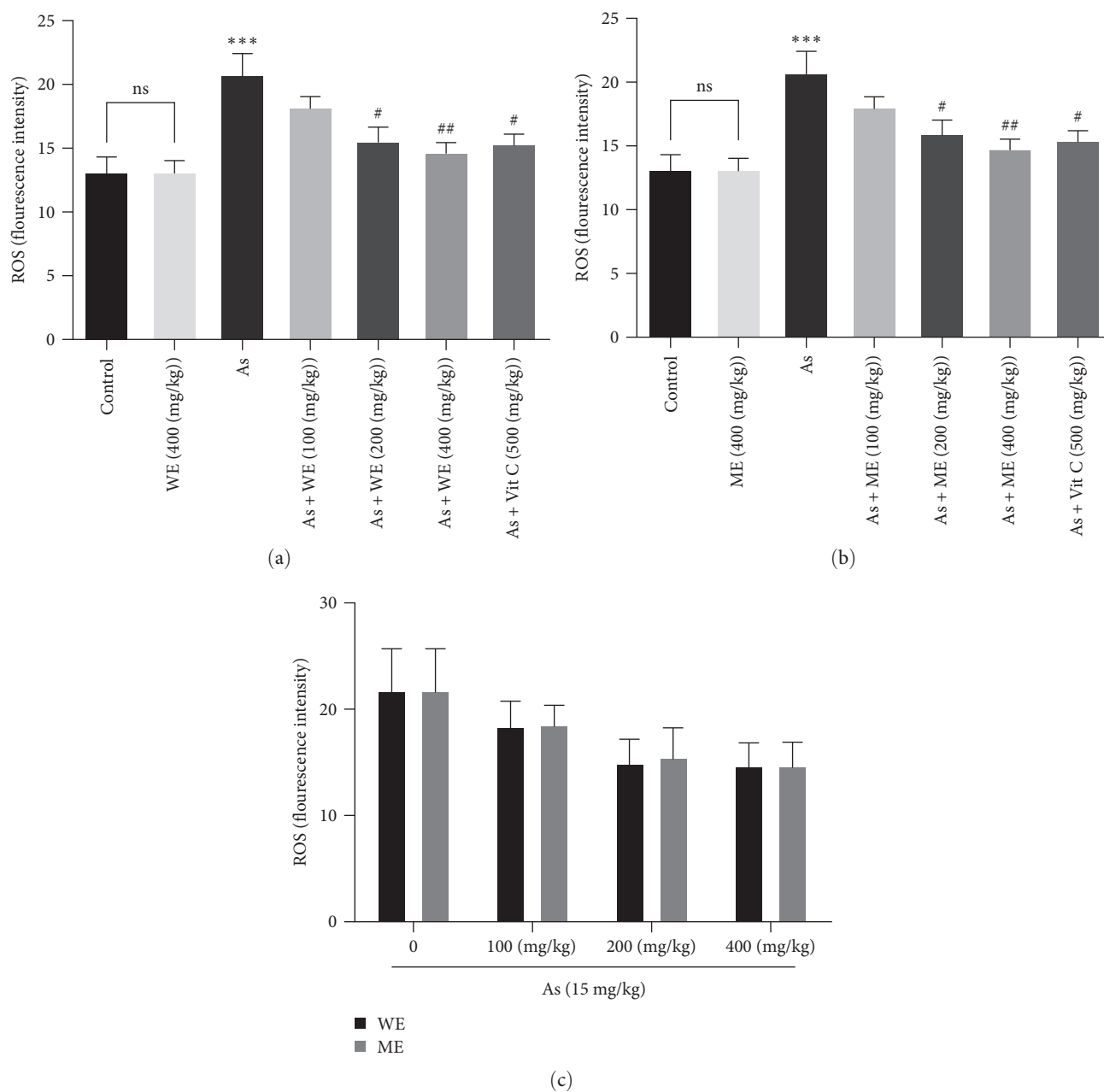


FIGURE 1: The protective effects of water and methanol extracts of *T. versicolor* on arsenic-induced ROS formation in mice's testicular tissue. (a) Effect of water extract, (b) effect of methanol extract, and (c) comparison of water and methanol extract. Values are expressed as mean \pm SD; ***significantly different compared to the control group ($P < 0.001$); #significantly different from arsenic (As) group ($P < 0.05$); ##significantly different in comparison with As group ($P < 0.01$); ns, not significant; As, arsenic; ME, methanol extract; WE, water extract; Vit C, vitamin C.

changes that occurred in the epithelium of seminiferous tubules (where both germ cells and Sertoli cells are located) through microscopy methods. Moreover, Johnsen scoring system was used to evaluate the changes statistically (Figure 7) [42]. Figure 8 represents the histological changes that occurred in the testicular tissue in study groups. As can be seen, the appearance of testicular tissue and spermatogenesis were normal in control group. Whereas, administration of arsenic induced severe histopathological changes in the tissue including degeneration of seminiferous tubules and increased

vacuolization. As-induced histological damages mitigated in groups treated with WE at the dose of 200 mg/kg. Interestingly, the administration of WE at the dose of 400 mg/kg and ME at doses of 200 mg/kg and 400 mg/kg reversed the deleterious effects of arsenic on the tissue and the appearance of the testicular tissue in these groups were quite close to that of the control group. As regards the Johnsen scoring system, Figure 7 demonstrates that As significantly induced damages to spermatogenesis ($P < 0.001$). On the other hand, cotreatment of the mice with WE significantly reduced the damages at

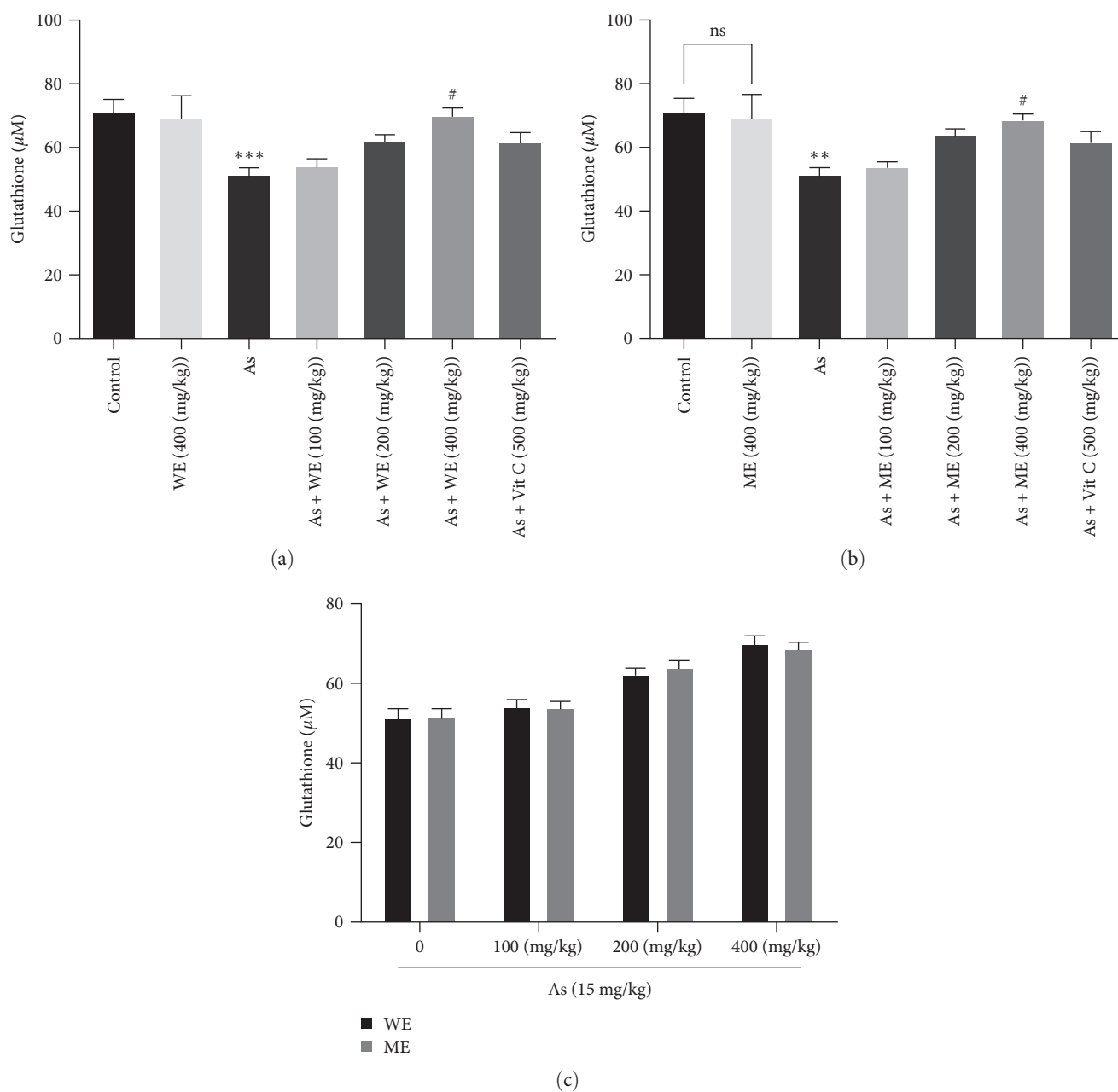


FIGURE 2: The protective effects of water and methanol extracts of *T. versicolor* on As-induced glutathione depletion in mice's testicular tissue. (a) Effect of water extract, (b) effect of methanol extract, and (c) comparison of water and methanol extract. Values are expressed as mean \pm SD; ** significantly different compared to the control group ($P < 0.01$); *** significantly different compared to the control group ($P < 0.001$); # significantly different in comparison with As group ($P < 0.05$); ns, not significant; As, arsenic; ME, methanol extract; WE, water extract; Vit C, vitamin C.

200 mg/kg ($P < 0.01$) and 400 mg/kg ($P < 0.001$). Similarly, the administration of ME at the doses of 200 and 400 mg/kg had similar impacts on the testicular tissue and significantly ameliorated the induced damages ($P < 0.001$).

3.1.8. Evaluation of Sperm Parameters. Measured sperm parameters are presented in Table 1. The administration of As led to significant decreases in sperm count ($P < 0.001$) and motility ($P < 0.01$) and increased sperm morphological abnormalities ($P < 0.001$). Although no significant changes in the sperm count and motility were observed in the group

treated with WE at 400 mg/kg, morphological abnormalities decreased significantly compared to As group ($P < 0.001$). It was observed that the administration of ME at 400 mg/kg significantly ameliorated morphological abnormality and increased sperm count ($P < 0.05$).

4. Discussion

As is a natural metalloids that is notorious for being one of the most hazardous contaminants in the environment, in particular the groundwater [47, 48]. As it was aforementioned,

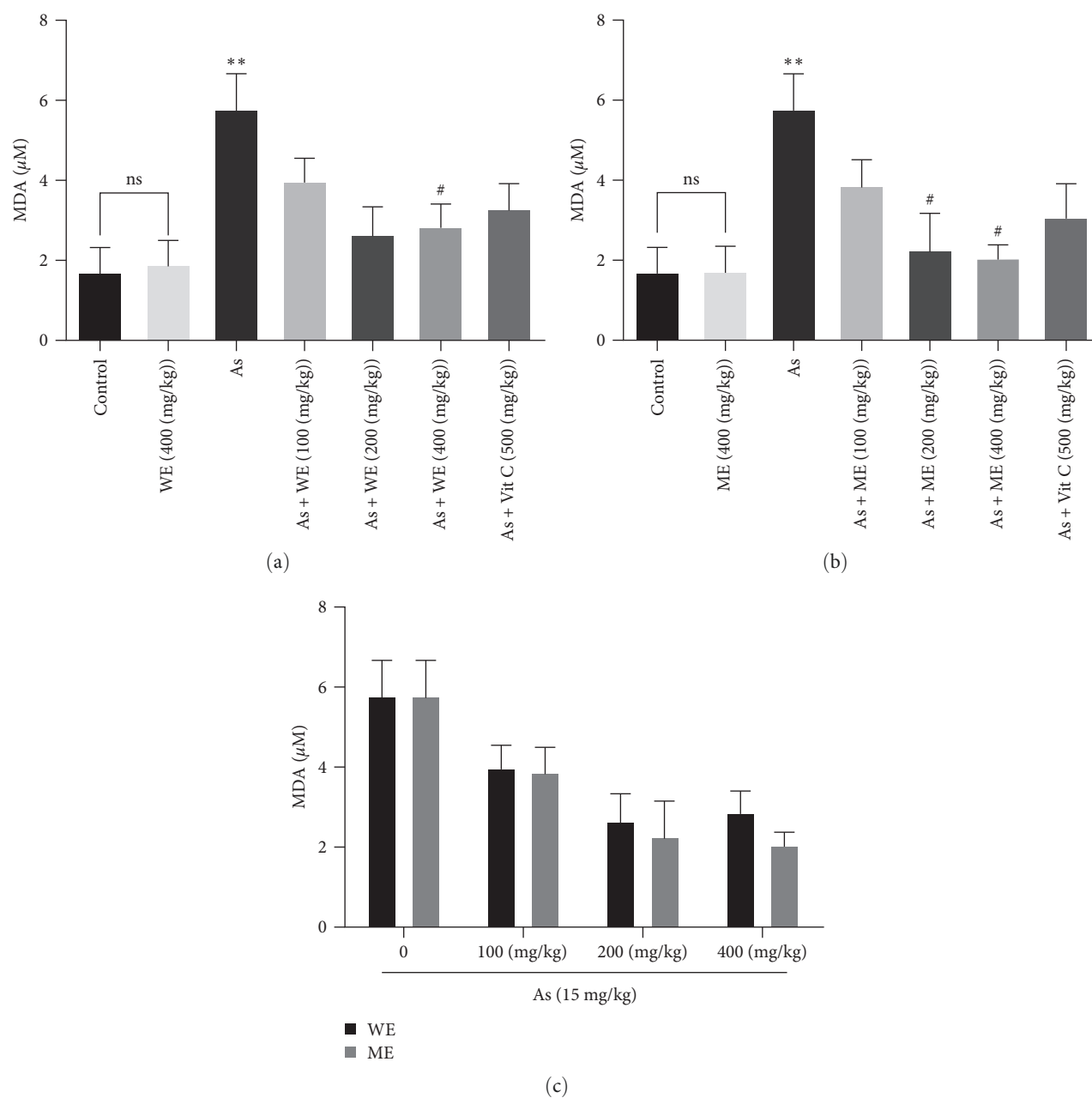


FIGURE 3: The protective effects of water and methanol extracts of *T. versicolor* on As-induced lipid peroxidation in mice's testicular tissue. (a) Effect of water extract, (b) effect of methanol extract, and (c) comparison of water and methanol extract. Values are expressed as mean \pm SD; **significantly different compared to the control group ($P < 0.01$); #significantly different in comparison with As group ($P < 0.05$); ns, not significant; As, arsenic; ME, methanol extract; WE, water extract; Vit C, vitamin C.

millions of individuals are at risk of As toxicity throughout the world [49]. Chronic As poisoning is linked to a number of cancers, dermatological lesions, and cardiac, respiratory, renal, and hepatic dysfunctions [48]. Apart from that, several studies have focused on the possible potential of As in inducing male reproductive system toxicities [11, 13, 14, 23, 48, 50]. However, the precise pathways underlying such toxicities are still ambiguous. Our chief purpose in our study was to highlight the potential of water and methanol extracts of the medicinal fungus *Trametes versicolor* in meliorating As

adverse effects on the male reproductive system by reducing As-induced oxidative stress.

In our study, As disrupted spermatogenesis by instigating significant decreases in epididymal sperm counts and motility in addition to a significant increase in the number of morphologically abnormal sperms. In consonance with our study, several studies demonstrated that As targets spermatogenesis by exerting detrimental impacts on sperm quality and quantity [13, 14, 50, 51]. Moreover, various studies reported decreases in the mean diameter of seminiferous

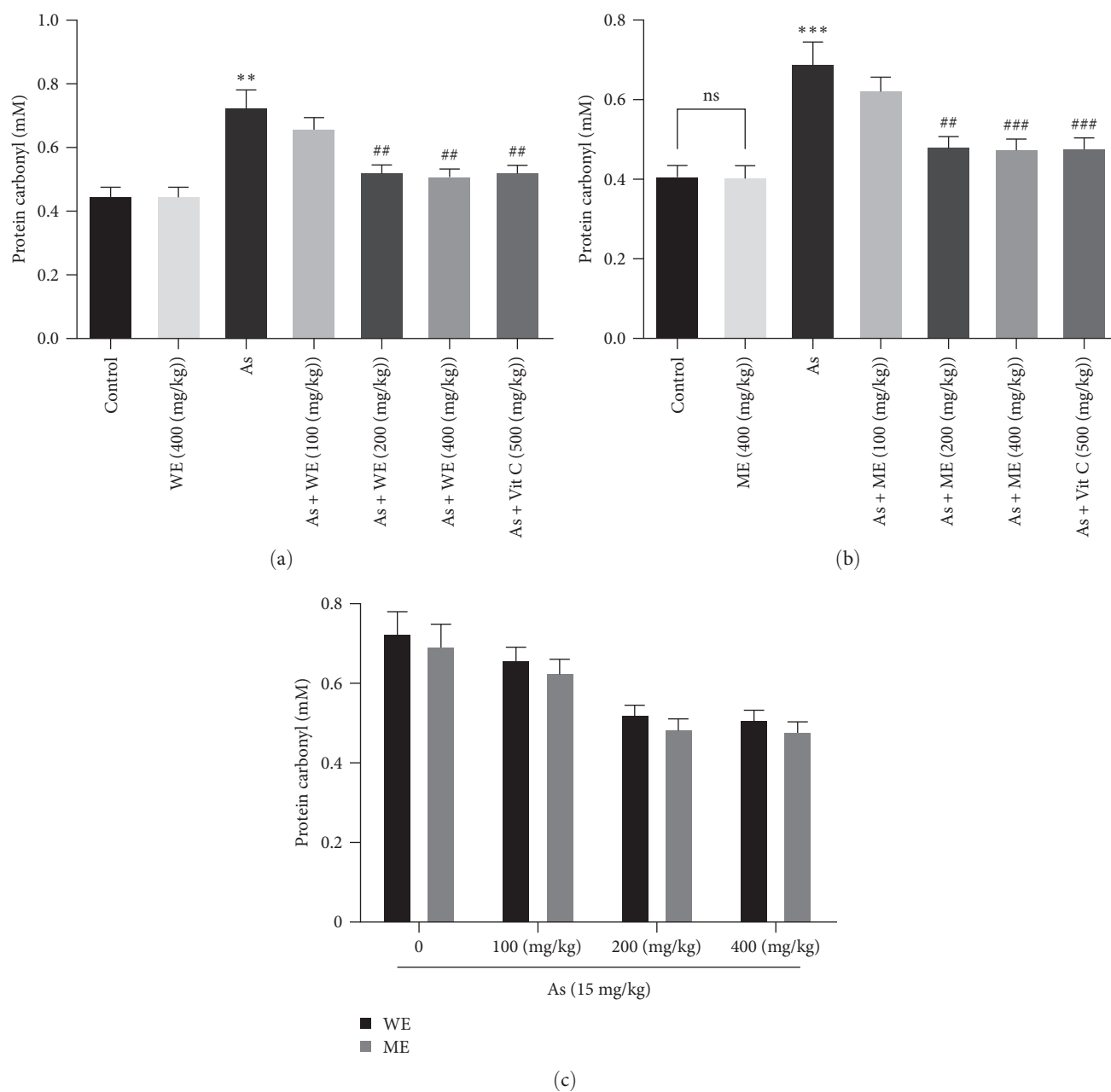


FIGURE 4: The protective effects of water and methanol extracts of *T. versicolor* on As-induced protein carbonylation in mice's testicular tissue. (a) Effect of water extract, (b) effect of methanol extract, and (c) comparison of water and methanol extract. Values are expressed as mean \pm SD; ***significantly different compared to the control group ($P < 0.001$); ###significantly different in comparison with As group ($P < 0.001$); ##significantly different in comparison with As group ($P < 0.01$); ns, not significant; As, arsenic; ME, methanol extract; WE, water extract; Vit C, vitamin C.

tubules, sex organs' weight loss, adverse interference with germ cells and Sertoli cells connections, and increases in seminiferous tubules' lumen as the consequences of As accumulation in the testicular tissue [11, 23, 52, 53].

The substantial role of oxidative stress in As-induced toxicities has been evaluated in different studies [10, 54]. According to the results reported in several studies, it is assumed that As has the potential to initiate a cascade of events culminating in oxidative stress in the testicular

microenvironment [51, 55, 56]. In our study, we indicated that As significantly induced the overgeneration of ROS in the testicular tissue. A likely explanation for this result could be the ability of As to interrupt the mitochondria electron chain resulting in the excessive production of ROS [57–59]. Similar to our results, a study on GC-1 spermatogonial cell line provided evidence that As is able to stimulate the overproduction of ROS [56]. Extensive accumulation of ROS in the cells can consequently injure them through the induction

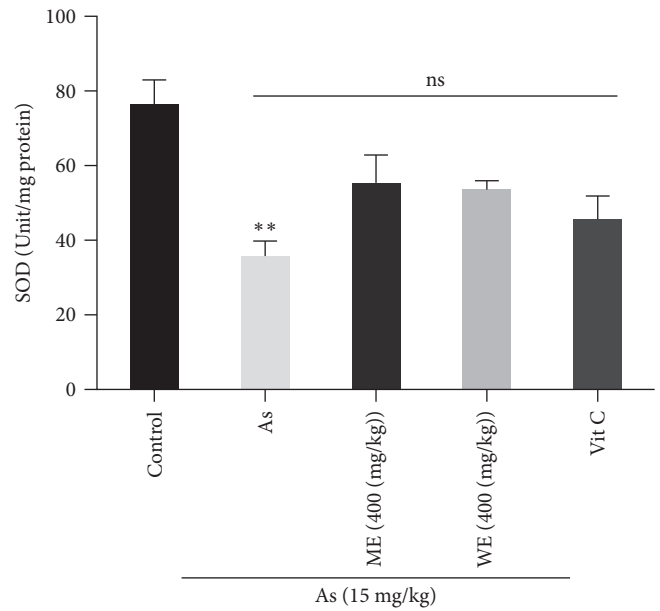
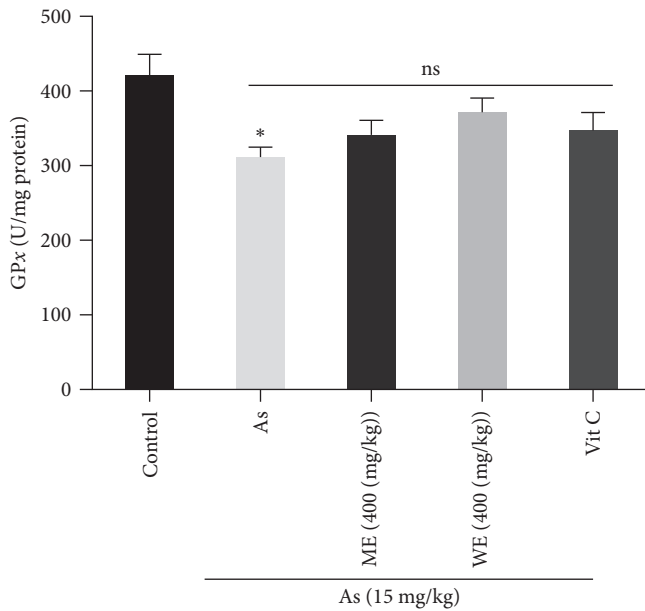


FIGURE 5: The effects of water and methanol extracts of *T. versicolor* on glutathione peroxidase (GPx) activity in mice's testicular tissue after As exposure. Values are expressed as mean \pm SD; *significantly different compared to the control group ($P < 0.05$); ns, not significant; As, arsenic; ME, methanol extract; WE, water extract; Vit C, vitamin C.

FIGURE 6: The effects of water and methanol extracts of *T. versicolor* on superoxide dismutase (SOD) activity in mice's testicular tissue after As exposure. Values are expressed as mean \pm SD; **significantly different compared to the control group ($P < 0.01$); ns, not significant; As, arsenic; ME, methanol extract; WE, water extract; Vit C, vitamin C.

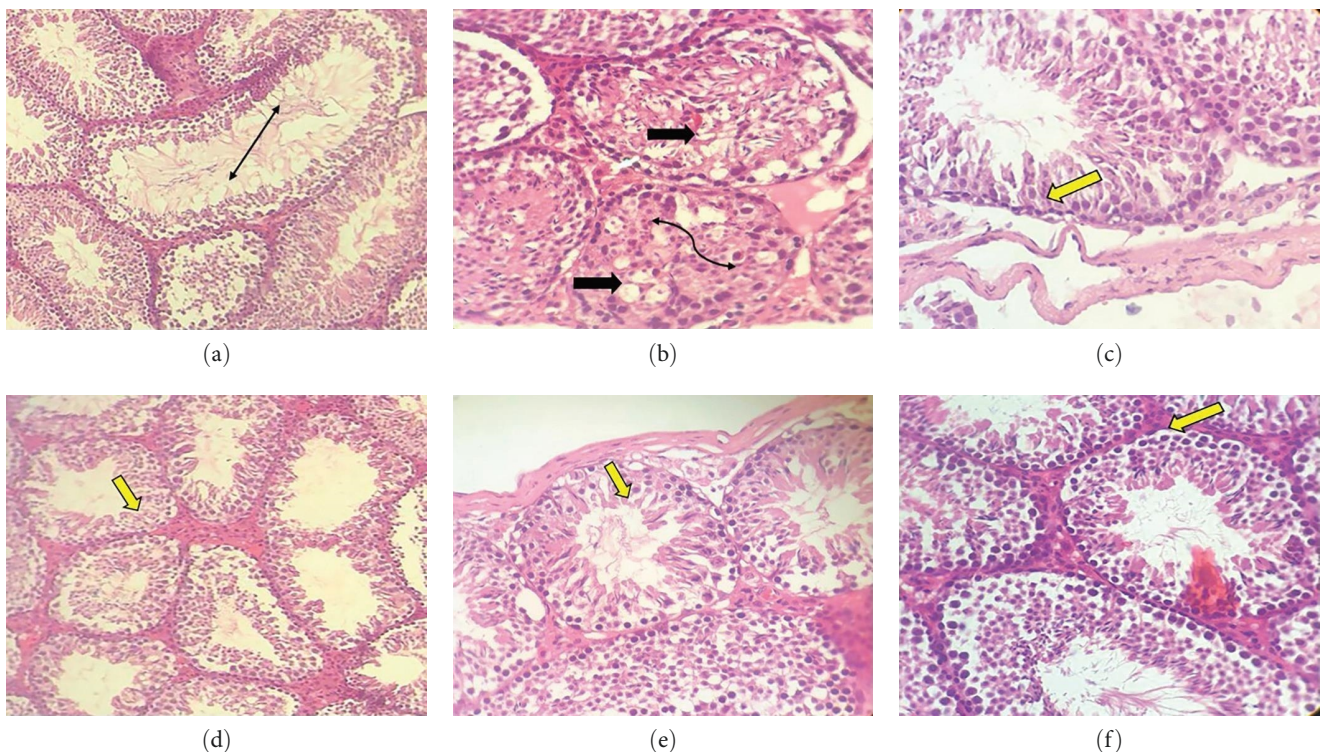


FIGURE 7: Hematoxylin- and eosin-stained section of mice testis: the ameliorative effects of different doses of water and methanol extracts of *T. versicolor* against arsenic-induced histopathological damages in the testicular tissue (400 \times); (a) control group with normal tissue appearance and presence of numerous spermatozoa at luminal border (showed by double arrowhead); (b) the testis from animals treated with As demonstrating degeneration of the seminiferous tubules, absence of spermatozoa, sloughing and disorganization of the germ cell layer, as showed by black curve double arrowhead, lumen of the tubule is obscured and increased vacuolization (black arrowheads); (c) the testis from animals treated with As + water extract (200 mg/kg) showing an increase in germ cell wall thickness (yellow arrowhead); (d) the testis from animals treated with As + water extract (400 mg/kg); (e) the testis from animals treated with As + methanol extract (200 mg/kg); (f) the testis from animals treated with As + methanol extract (400 mg/kg); the appearance of (c), (d), (e), and (f) were highly similar to the control group. The seminiferous tubules exhibited intact and healthy seminiferous epithelium, indicative of optimal cellular composition and organization (yellow arrowheads). Moreover, the restoration of germ cell wall thickness, attributed to the administration of *T. versicolor* extracts, suggested a positive influence on maturation and the preservation of structural integrity. These observations affirm the absence of any notable abnormalities highlighting the beneficial effects of methanol and water extracts of *T. versicolor* on histopathological normalcy.

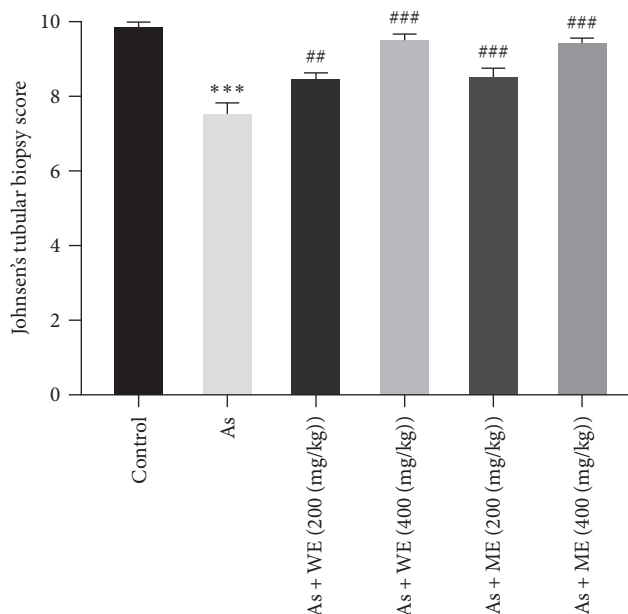


FIGURE 8: Statistical evaluation of the protective effects of water and methanol extracts of *T. versicolor* on As-induced histopathological changes in the testicular tissue; values are expressed as mean \pm SD; ***significantly different compared to the control group ($P < 0.001$); ##significantly different from As group ($P < 0.01$); ###significantly different in comparison with As group ($P < 0.001$); ns: not significant; As, arsenic; ME, methanol extract; WE, water extract.

TABLE 1: The alleviating effects of water and methanol extracts of *T. versicolor* against arsenic-induced changes in mice's sperm parameters in terms of sperm count, sperm motility, and sperm morphological abnormality.

	Sperm count ($\times 10^5$)	Sperm motility (%)	Morphological abnormality (%)
Control	69.6 \pm 4.1	80.6 \pm 7	21.6 \pm 3
As (15 mg/kg)	41.6 \pm 4.04***	60.6 \pm 3.5**	56.6 \pm 4.27***
As + ME (400 mg/kg)	59 \pm 2 [#]	73.3 \pm 4.5	42.2 \pm 4.72 [#]
As + WE (400 mg/kg)	50.6 \pm 2.5	70 \pm 4.5	39.7 \pm 4.98 ^{##}
As + Vit C	51.3 \pm 9.2	68.6 \pm 3	39.6 \pm 5.6 ^{##}

Values are expressed as mean \pm SD; **significantly different in comparison with control group ($P < 0.01$); ***significantly different in comparison with control group ($P < 0.001$); [#]significantly different compared to arsenic (As) group ($P < 0.05$); ^{##}significantly different compared to As) group ($P < 0.01$); As, arsenic; ME, methanol extract; WE, water extract; Vit C, vitamin C.

of oxidative stress. For instance, increased levels of ROS might enable specific signaling pathways which would ultimately induce lipid peroxidation and produce protein carbonyl contents [60, 61]. In the present study, we attempted to measure MDA as one of the most critical products of lipid peroxidation as well as protein carbonyl content. As previously stated, it was observed that treating the mice with As significantly elevated the levels of MDA and protein carbonyl content in the testicular tissue. In accordance with these results, various studies reported that exposure to As commenced lipid peroxidation and induced damage to the protein structures within the mice's testicular tissue [51, 53, 55, 62, 63]. Another significant aspect of As male reproductive toxicity is that glutathione level and the activities of antioxidant defense enzymes might be detrimentally affected following the administration of As [51, 55, 62, 64]. Correspondingly, the results of our study demonstrated significant decreases in the level of glutathione and a significant reduction in antioxidant enzymes GPx and SOD activities in the group that

received arsenic. Apart from inducing oxidative stress, it is assumed that As's binding affinity to sulfhydryl groups might explain the decline in glutathione concentrations and GPx and SOD activities [47, 65].

Taken together, it is presumed that oxidative stress might be the common ground among male reproductive toxicities, particularly infertility [66]. A number of studies have used natural compounds to reduce As toxicity in different tissues [67, 68]. *Trametes versicolor* is a medicinal fungus found predominantly in various parts of the world, including Iran [69]. It has been reported that the presence of bioactive compounds such as PSK and PSP in *T. versicolor* extracts is linked to the mushroom's antioxidant ability [70]. As explained in the introduction section, PSK's antioxidant ability is supposed to be due to its highly similar activity to SOD. Besides, both PSP and PSK have the ability to counteract oxidative stress by scavenging free radicals. For instance, the addition of PSK to the chemotherapy regimen of the patients has reportedly relieved them from oxidative damage

[27]. Moreover, a study on the rabbit model of atherosclerosis indicated that derived PSK from *T. versicolor* significantly declined the stimulated oxidative damage [71]. In another study, the enzymatic hydrolysate of PSPs extracted from *T. versicolor* exhibited vigorous antioxidant activities in keratinocytes [72]. Furthermore, the results of a study on human lymphocytes suggested that *T. versicolor* might protect DNA against oxidizing damage [73]. In agreement with these studies, we demonstrated that both WE and ME of *T. versicolor* at relatively high doses (200 and 400 mg/kg) alleviated the markers associated with oxidative stress. We witnessed significant decreases in the levels of As-induced ROS generation, MDA, and protein carbonyl content in the groups that received WE at 200 and 400 mg/kg as well as the groups that received ME at the respective doses of 200 and 400 mg/kg. In addition, the levels of GSH were significantly improved in the groups treated with WE and ME at 200 and 400 mg/kg. In addition, the treatment with WE at 200 and 400 mg/kg and ME at 200 and 400 mg/kg reversed the deleterious histological damages that As-induced in the testicular tissue.

On the other hand, simultaneous administration of *T. versicolor* did not significantly improve GPx and SOD activities. These results may appear relatively contrary to the previous works, which stated that PSK could enhance the activity of SOD and GPx [74, 75]. It is highly significant to explain that both WE and ME of *T. versicolor* improved GPx and SOD activity (Figures 5 and 6, respectively). However, the results were not statistically significant compared to the control and As group.

5. Conclusions

The results of the present study provided further evidence that oxidative stress is one of the main mechanisms underlying As-mediated male reproductive toxicities. In addition, it was outlined that the WE and ME of *T. versicolor* have the potential to alleviate arsenic-mediated oxidative stress in the testicular tissue.

Data Availability

Data of our study are available from the corresponding author upon reasonable request.

Ethical Approval

All experimental procedures were approved by the Animal Research Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran. All the experiments were carried out the following approved guidelines. All efforts were made to minimize the number of animals and its suffering (Ethic number: IR.MAZUMS.REC.1396.2783).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Fatemeh Shaki designed and supervised the experiment, Fatemeh Solaimani executed and conducted the experiments with help of Saba Mahboubi, and Emran Habibi supervised the extraction processes. Maryam Ghasemi ran the pathological analyses. Ehsan Zamani and Saba Mahboubi prepared the manuscript. Fatemeh Shaki and Ehsan Zamani processed the statistical analyses.

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