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Effect of moxa smoke on sperm parameters and oxidative stress in rats with asthenozoospermia

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Abstract

Asthenozoospermia is a leading cause of male infertility, characterized by reduced sperm motility. In this study, we determined sperm motility and the activities of antioxidant enzymes and oxidation products in the testis of rats with ornidazole (ORN)-induced asthenozoospermia and further examined and compared the differential effects of moxa smoke (MS) and cigarette smoke (CS) on sperm motility and oxidative stress (OS) of asthenozoospermic rats. The smoke intervention was initiated 11 days after intragastric administration of ORN, followed by the examination of testis index, sperm parameters, OS-related gene levels, and testicular histopathology. Sperm motility and antioxidant enzyme activities, as well as oxidation products significantly decreased in ORN-induced rats compared with MS-treated rats ($p < .05–.001$). MS treatment restored the reduced sperm motility and activities of glutathione peroxidase, superoxide dismutase, and catalase, but increased the malondialdehyde and nitric oxide synthetase levels in ORN-induced rats ($p < .05–.001$). Also, the histopathological changes in the testis of ORN-induced rats were improved by MS treatment. The study highlighted that MS was an effective factor in moxibustion therapy, which notably improved the sperm motility of asthenozoospermic rats by inhibiting OS in the reproductive system.

KEYWORDS
asthenozoospermia, moxa smoke, oxidative stress, sperm motility

【摘要】
弱精子症是以精子活力下降为主要表现的病症，是引起男性不育症的常见病因之一。本实验我们探讨了不同浓度的艾烟对弱精子症大鼠精子参数的影响和对生殖系统氧化应激损伤产生的作用，比较了艾烟与香烟对机体产生的效应差异。实验从奥硝唑溶液灌胃的第11天开始进行烟雾干预。干预结束后，检测每组大鼠的体重增量、睾丸质量、睾丸指数、精子参数、氧化应激指标以及睾丸组织形态病理学变化。结果表明，弱精子症模型大鼠的精子活力和抗氧化酶活性显著下降，氧化产物含量显著升高。不同浓度的艾烟干预后，能够显著改善弱精子症大鼠的精子活力和抗氧化酶活性，降低氧化产物含量，睾丸组织形态结构损伤也得到恢复。因此，艾烟作为艾灸疗法的起效因素之一，可能通过改善生殖系统氧化应激反应的方式，提高弱精子症大鼠的精子活力。

【关键词】
弱精子症, 艾烟, 精子活力, 氧化应激

1 | INTRODUCTION

Defective sperm function is considered to be the predominant cause of human infertility (Shahrokhi et al., 2020; Sun et al., 2020). As one of the most frequent causes of male infertility, asthenozoospermia is featured by impaired sperm motility (Jo et al., 2015). Usually, there exists excessive production of reactive oxygen species (ROS) triggered by oxidative stress (OS) in seminal plasma and exacerbated ROS-mediated damage of sperm membranes in asthenozoospermic patients (Agarwal et al., 2006). OS injury in the reproductive system is mainly manifested by poor semen parameters, low sperm concentration, reduced motility, abnormal sperm morphology, aberrantly upregulated ROS and malondialdehyde (MDA) levels, and downregulated superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities (Marzony et al., 2016). Given that the etiology and pathogenesis of asthenozoospermia remain obscure, there is still a lack of effective clinical treatment at present, which warrants further research.

Moxibustion is known as a Traditional Chinese therapy that uses moxa floss to burn and fumigate at acupoints or specific areas of the body to produce warm heat and deliver medical effects (Wang et al., 2013; Xu et al., 2020). Through the conduction of meridians and collaterals, moxibustion can stimulate the activities of meridians and Qi, thereby warming Qi and blood, strengthening the body's capacity to eliminate pathogenic factors, and preventing and treating diseases with its comprehensive therapeutic effect (Smith, 2013; Sun et al., 2008). Modern research have shown that the combustion products of moxa can demonstrate a variety of effects, such as bactericidal, antiviral (Ho et al., 2006), immune regulation (Zhang & Wu, 2006), free radical scavenging, antioxidant (Meng et al., 2011), lipid metabolism regulation (Yu et al., 2016), and inflammatory response reduction (Ha & Zhao, 2016). As a combustion product of moxa with a special aromatic odor, moxa smoke (MS) exhibits multiple biological activities and can ensure the efficacy of moxibustion treatment.

Our previous research found that electroacupuncture exerted therapeutic effects on asthenozoospermia by enhancing sperm viability and motility in rats (Jin et al., 2017). MS was proved to be effective in improving the reproductive function of male rats, and the low concentration of MS seemed to be superior to the high concentration of MS in terms of improving sperm parameters, testis index, and serum sex hormones (Wang et al., 2016). In this context, we hypothesized that MS constituted an effective factor of moxibustion therapy. The impacts of MS at different concentrations were investigated and compared in the aspects of sperm parameters and OS of the reproductive system in rats with ornidazole (ORN)-induced asthenozoospermia to demonstrate its therapeutic effect on the reproductive function. Moreover, our results highlighted the alleviatory effects of MS on asthenozoospermia in comparison with cigarette smoke (CS) to provide experimental proof for the effectiveness and safety of MS.

2 | MATERIALS AND METHODS

2.1 | Experimental animals and grouping

Male Sprague–Dawley rats with sexual maturity (weighting 200–230 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China; SCXK [Beijing] 2016-0006). The rats were individually housed in the controlled vivariums of cages with free access to food and water, the humidity of 50%–55%, and the illumination of 12 h light/12 h dark at the temperature of 20–22°C. All animal experimental procedures were ratified by the Institute of Animal Care Committee and the Ethics Committee of Beijing University of Chinese Medicine (Permit No. BUCM-4-2019022703-1023).

Following 1 week of acclimatization to the animal laboratory area, 72 rats were randomly assigned into six groups (12 rats in each group): vehicle, ORN, ORN + CS, ORN + MS1 (the low concentration of 0.4%), ORN + MS2 (the moderate concentration of 2%), and ORN + MS3 (the high concentration of 15%) groups. Eight rats were randomly selected from each group to detect testis index, sperm parameters, and OS-related gene levels. Additionally, the remaining four rats were utilized for the testicular histopathological examination.

2.2 | Establishment of a rat model of asthenozoospermia

The ORN-induced rat model has been a frequently used animal model to study asthenozoospermia over the last several decades (Drobnis & Nangia, 2017). In our lab, rats underwent daily intragastric administration of ORN for 10–14 days to establish the disease model (Bone et al., 2000) as per the previously described method with minor modifications (Du et al., 2019). In the groups of ORN and ORN combined with smoke exposure, ORN was dissolved in 0.2% (*w/v*) sodium carboxymethylcellulose (CMC-Na) in double-distilled water and fed orally to the rats once a day at 400 mg/kg body weight for 10 days, following by a dose of 200 mg/kg body weight throughout the intervention. The vehicle rats were fed with 0.2% (*w/v*) CMC-Na in double-distilled water without ORN. The development of asthenozoospermia in rats was determined by the assessment of sperm motility.

2.3 | Smoke interventions

MS was generated by burning moxa sticks (length, 20–21 cm; diameter, 1.9–2.1 cm; Hubei Li Shizhen Herbal Pieces Co., Ltd., Hubei, China). CS was produced by flaming tobacco (SHUANGXI; tar, 11 mg; Guangdong Zhongyan Industrial Co., Ltd., Guangdong, China). The concentration of MS was measured using a light-scattering digital dust test (DT, Beijing BINTA Green Technology Co., Ltd., Beijing, China). The MS was controlled using a Dynamic Toxicant Exposure Cabinet (HOPE-MED 8050 series, Tianjin HOPE Co., Ltd., Tianjin, China), which was applied to monitor smoke concentration through optical density (OD). OD refers to the proportion of MS particulates visible in the beam. Moxa was burned in a smoke generating device. The flow of MS into the cabinet was properly controlled for exposure.

The smoke intervention was commenced 11 days after the intragastric administration of ORN. Twelve rats in the vehicle group were placed in the cabinet for 20 minutes with the door closed. Rats in the ORN group were subjected to the same operation. Twelve rats were placed in the cabinet for 20 minutes when the concentrations of smoke were stabilized at 0.4%, 2%, and 15% for the ORN + MS1, ORN + MS2, and ORN + MS3 groups, respectively. The concentration of CS in the ORN + CS group was set to 2%, which was consistent with the ORN + MS2 group. Rats in the smoke intervention groups underwent 20-minute smoke exposure per day, 6 days per week, for 8 weeks. Finally, the rats were euthanized by decollation and the specimens were harvested for the examination of corresponding indexes.

2.4 | Body weight and testis index

All rats were weighed after 8 weeks of intervention and prior to euthanization. The two testicles of each rat were quickly removed and weighed. The testis index of a rat was calculated as the ratio of the weight of two testicles to the body weight of the rat. Epididymides were obtained for further examination.

2.5 | Sperm parameters

To assess sperm parameters, sperms in the caudal epididymides were attained and prepared as previously described (Rizzetti et al., 2017). In brief, each caudal epididymis was placed in 2 ml of 0.9% normal saline prewarmed at 37°C. The caudal epididymis was then incised in several places to allow the semen to ooze out and was incubated for 2 min in a 37°C water bath. Subsequently, 20 μl sperm suspension was placed on the sperm counting chamber (Nanjing Songjing Tianlun Biotechnology Co., Ltd., Nanjing, China). Afterward, the sperm motility was

assessed by performing a computer-assisted sperm assay (CASA) with a sperm motility analyzer (WLJY-9000, Beijing Weili Century Science & Technology Development Co., Ltd., Beijing, China). The following sperm parameters were evaluated, including progressive motility (grade A + B) (%), sperm viability (%), sperm concentration ($\times 10^6$/ml), curve-line velocity (VCL, μm/s), straight-line velocity (VSL, μm/s), average path velocity (VAP, μm/s), amplitude of lateral head displacement (ALH, μm), linearity (LIN, %), wobble (WOB, %), and straightness (STR, %).

2.6 | Determination of GSH-Px, SOD, catalase, MDA, and nitric oxide synthetase activities

GSH-Px activity can be expressed by the rate of its enzymatic reaction, which was assayed by the colorimetry method using a GSH-Px assay kit (A005-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China). SOD activity was measured by the xanthine oxidase method in the light of the protocols of a SOD assay kit (A001-3, Nanjing Jiancheng Bioengineering Institute). Catalase (CAT) activity was estimated by detecting its variance at the wavelength of 405 nm using a CAT assay kit (A007-1, Nanjing Jiancheng Bioengineering Institute). MDA was condensed with thiobarbituric acid to form a red product with a maximum absorption peak at 523 nm so that its activity was evaluated using an MDA assay kit (A003-1, Nanjing Jiancheng Bioengineering Institute). Nitric oxide synthetase (NOS) content was assessed at 530 nm by the colorimetry method according to the manuals of a NOS assay kit (A014-2, Nanjing Jiancheng Bioengineering Institute).

2.7 | Histopathological evaluation

Testis tissues were excised and fixed in 4% paraformaldehyde solution and then embedded in paraffin. Hematoxylin and eosin (H&E) staining was performed following the standard protocols after the embedded tissues were sectioned at 5 μm. Finally, histological changes were observed under an inverted optical microscope (CI-S, Nikon, Tokyo, Japan) at a magnification of ×40.

2.8 | Statistical analysis

SAS 9.4 (SAS Institute Inc., Raleigh, NC) was employed to conduct statistical analyses, and GraphPad Prism 8 for Windows (GraphPad Software Inc., La Jolla, CA) was adopted for plotting. All data were expressed as mean ± standard error of the mean. The two-sample *t*-test was utilized for the comparisons of the mean values between the two groups. One-way analysis of variance (ANOVA) and least significant difference (LSD) *t*-test were applied for the comparisons among multiple groups. Differences with $p < .05$ were considered statistically significant.

3 | RESULTS

3.1 | Effects of MS on the body weight and testis index of rats

No significant difference was observed in the body weight between ORN-treated rats and vehicle rats (255.65 ± 34.94 g vs. 308.81 ± 50.81 g; $p > .05$). In comparison with the ORN group, rats in the ORN + CS and ORN + MS2 groups had decreased body weight (251.66 ± 13.81 g vs. 249.91 ± 18.48 g), whereas rats in the ORN + MS1 and ORN + MS3 groups had increased body weight (265.85 ± 17.10 g vs. 274.61 ± 20.91 g). Our data showed no obvious difference in the gained body weight among the six groups ($p > .05$; Figure 1a). After the intervention of CS and MS, the testis index demonstrated no remarkable difference among the six groups ($p > .05$; Figure 1b).

3.2 | Impacts of MS on the sperm parameters of rats

CASA results revealed that almost all sperm motility parameters were substantially reduced in the ORN-treated rats, as compared with the vehicle rats ($p < .05$–.001; Figure 2). The proportion of progressive motility (grade A + B) was conspicuously diminished in the ORN-treated rats in contrast to the vehicle rats ($p < .001$; Figure 2a). As depicted in Figure 2b–d, VCL, VSL, and VAP were evidently decreased ($p < .01$; $p < .001$; $p < .001$, respectively), resulting in the decline in the proportion by 19%–49%. The relatively lower velocity of the ORN-treated spermatozoa could be attributed to the reduced ALH versus the vehicle-treated spermatozoa ($p < .001$; Figure 2e). As for the sperm swimming patterns, LIN (Figure 2f), WOB (Figure 2g), and STR (Figure 2h) were considerably diminished ($p < .001$; $p < .001$; $p < .05$, respectively) with the proportion decreasing by approximately 10%–30% in the ORN-treated spermatozoa compared with the vehicle-treated spermatozoa. These results suggested that ORN induced prominently impaired sperm motility, indicating the successful establishment of the asthenozoospermic rat model.

We further dissected the influences of CS and MS on ORN-induced asthenozoospermia in the rat model. It was observed that except for progressive motility (grade A + B)

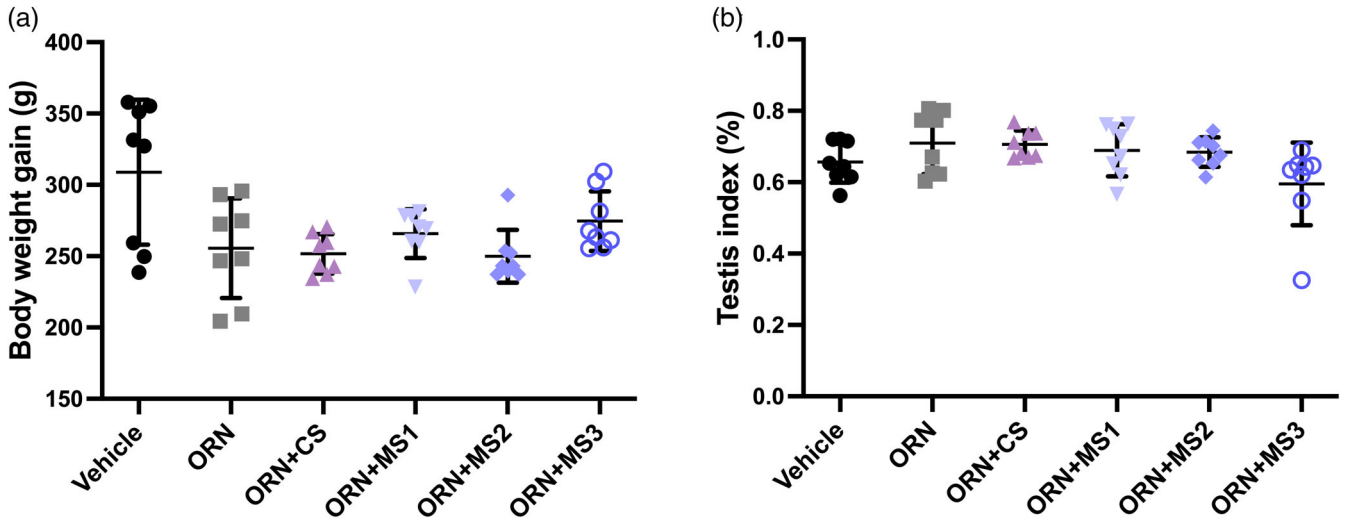


FIGURE 1 Body weight gain and testis index in different rat groups. (a) Body weight gain; (b) testis index. Data were presented as mean ± SEM ($n = 8$). ORN, ornidazole; CS, cigarette smoke; MS, moxa smoke

and LIN, there existed insignificant differences in sperm motility parameters between ORN-treated rats and ORN + CS rats. All of the deteriorated sperm motility parameters induced by ORN were rescued by the different concentrations of MS in the treatment groups in comparison to the vehicle group ($p < .05$–$.001$; Figure 2). Notably, the data of the ORN + MS3 group displayed that it exhibited the most promising effect than that in all other groups. These results indicated that MS exerted an ameliorative effect on ORN-triggered asthenozoospermia in rats.

Moreover, the data demonstrated that ORN contributed to the noticeable decline of sperm viability, which was abrogated by CS and various concentrations of MS ($p < .05$; $p < .001$, respectively; Figure 3a). However, no statistically significant alteration was detected in sperm concentration among all groups ($p > .05$; Figure 3b). These results illustrated that in addition to sperm motility, administration of ORN impaired spermatogenesis and compromised the quality of spermatozoa and that MS exerted a reverse effect on decreased sperm viability caused by ORN.

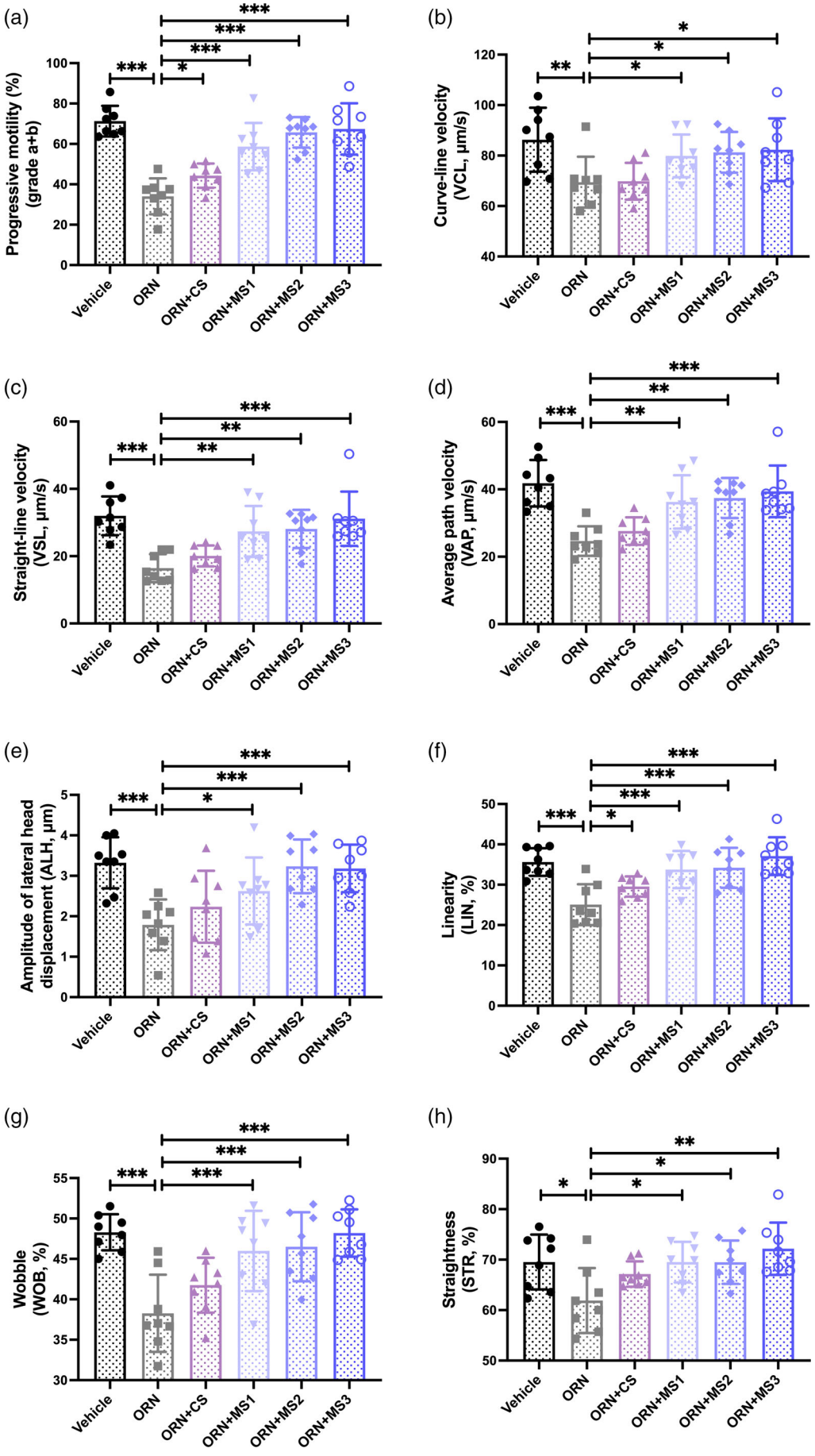
3.3 | Influences of MS on GSH-Px, SOD, CAT, MDA, and NOS activities

Marked differences were determined in regard to testicular GSH-Px, CAT, MDA, and NOS activities between the vehicle and ORN groups ($p < .05$–$.001$). However, unlike it in the vehicle group, SOD activity was not prominently downregulated in the ORN group (Figure 4).

As illustrated in Figure 4a–c, the activities of GSH-Px, SOD, and CAT were enhanced to varying degrees after the intervention of MS at different concentrations. In contrast to the ORN group, GSH-Px activity was appreciably augmented in the ORN + MS2 and ORN + MS3 groups ($p < .05$; $p < .01$, respectively), SOD activity was improved in the ORN + MS1 and ORN + MS2 groups (both $p < .05$), and CAT activity was enhanced in the ORN + MS1, ORN + MS2, and ORN + MS3 groups ($p < .001$; $p < .05$; $p < .01$, respectively). MDA activity was strikingly lower in the ORN + MS1 group than in the ORN group ($p < .01$; Figure 4d). The ORN + MS1 and ORN + MS2 groups had markedly reduced NOS activity compared with the ORN group (both $p < .05$; Figure 4e). MS at manifold concentrations did not improve OS in a definite concentration-dependent manner. In comparison to the ORN group, no conspicuous variation was noted in the ORN + CS group in terms of GSH-Px, SOD, MDA, CAT, and NOS activities ($p > .05$; Figure 4).

3.4 | Histopathological observation of testis

In the vehicle group, the seminiferous tubules were round or oval with intact and full structure, and the spermatogenic cells were arranged orderly in distinct layers. The sperm cells were clearly visible, with a large number of sperms stored in the lumen. In contrast to the vehicle group, the spermatogenesis of rats in the ORN group was notably diminished, as evidenced by seminiferous tubules manifesting slight atrophy, mild disorder and loose connection of spermatogenic cells with decreased layers and numbers, slightly widening of the lumen space, and a small decrease in spermatid cell size. The incidence of spermatogenesis hypofunction reduced with the



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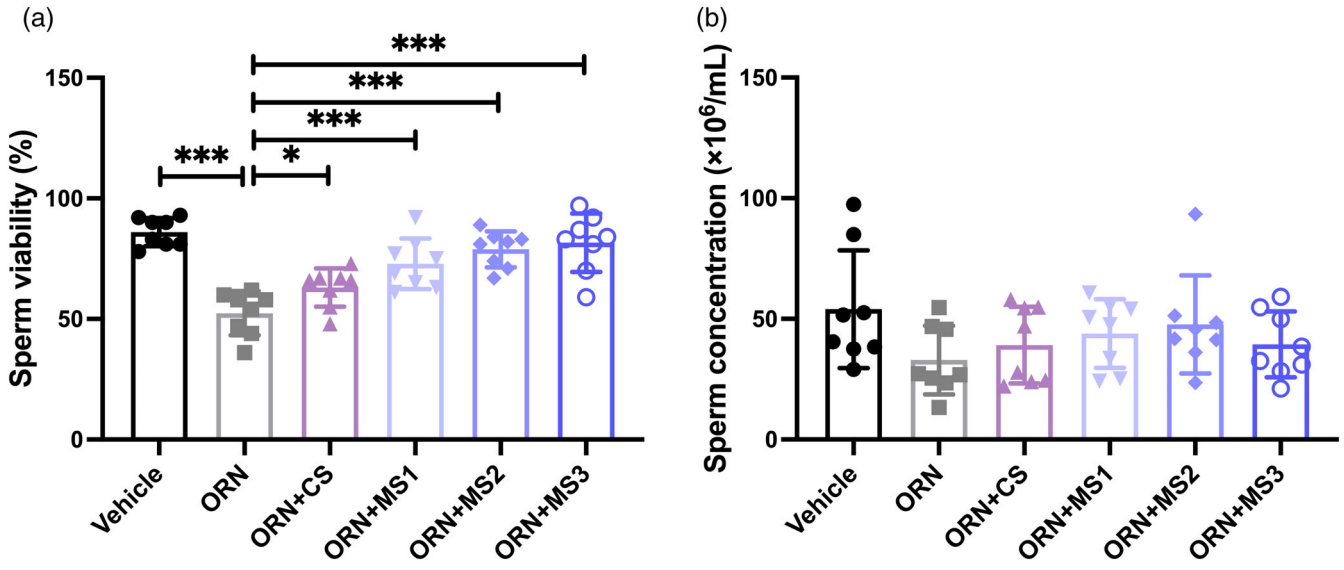

FIGURE 3 Computer-assisted analysis of sperm viability and concentration in different rat groups. (a) Sperm viability; (b) sperm concentration. Data were presented as mean ± SEM ($n = 8$). *$p < .05$; **$p < .01$; ***$p < .001$. ORN, ornidazole; CS, cigarette smoke; MS, moxa smoke

intervention of CS and high concentration of MS, yet improved after the treatment of the low and moderate concentrations of MS. This revealed that the low and moderate concentrations of MS exhibited certain therapeutic effects on spermatogenesis hypofunction (Figure 5).

4 | DISCUSSION

Although asthenozoospermia has been considered a common cause of male infertility, the underlying mechanism remains unknown in most cases. The previous studies have reported that reduced sperm motility in patients with asthenozoospermia may be related to multiple risk factors, for instance, obesity, diabetes, smoking, and exposure to bisphenol-A (Bisconti et al., 2021). Besides, proteomic profiles unveiled that oxidative stress and inflammatory response play crucial roles in sperm abnormal (Martins et al., 2020). In the present study, the downregulated antioxidant enzymes and upregulated oxidation products were identified in rats with ORN-reduced asthenozoospermia. Moreover, MS abrogated ORN-induced reduction of GSH-Px, SOD, and CAT activities, enhanced MDA and NOS levels, and revised the histopathological changes in the testis, thereby exerting an observably mitigating effect on asthenozoospermia. Our study provides the basic experimental evidence for the use of MS in the treatment of asthenozoospermia, which can improve sperm motility by facilitating antioxidant enzymes and repressing oxidation products.

The prior sampling survey elucidated that the average mass concentration of MS inhalable substance was 3.54 mg/m^3 in moxibustion clinic (Huang et al., 2012). With the knowledge of this concentration and the concentration set in the earlier experiments (Xu et al., 2014; Xu et al., 2021), the clinical MS concentrations of 1, 3, and 9 times were set as the experimental concentrations. To confirm the unification in different environments, the mass concentration and optical concentration (shading rate) of MS were converted (Yang et al., 2014). Specifically, the dynamic toxicant exposure cabinet was set to a certain shading rate value, and the light-scattering PM10 digital dust test was adopted to detect the mass concentration corresponding to the certain shading rate value of MS in our research, followed by the calculation of the average value after multiple measurements. Finally, the shading rate value of MS was fitted to its mass

FIGURE 2 Computer-assisted analysis of sperm motility in different rat groups. (a) Progressive motility (grade a + b); (b) curve-line velocity (VCL); (c) straight-line velocity (VSL); (d) average path velocity (VAP); (e) amplitude of lateral head displacement (ALH); (f) linearity (LIN); (g) wobble (WOB); (h) straightness (STR). Data were presented as mean ± SEM ($n = 8$). *$p < .05$; **$p < .01$; ***$p < .001$. ORN, ornidazole; CS, cigarette smoke; MS, moxa smoke

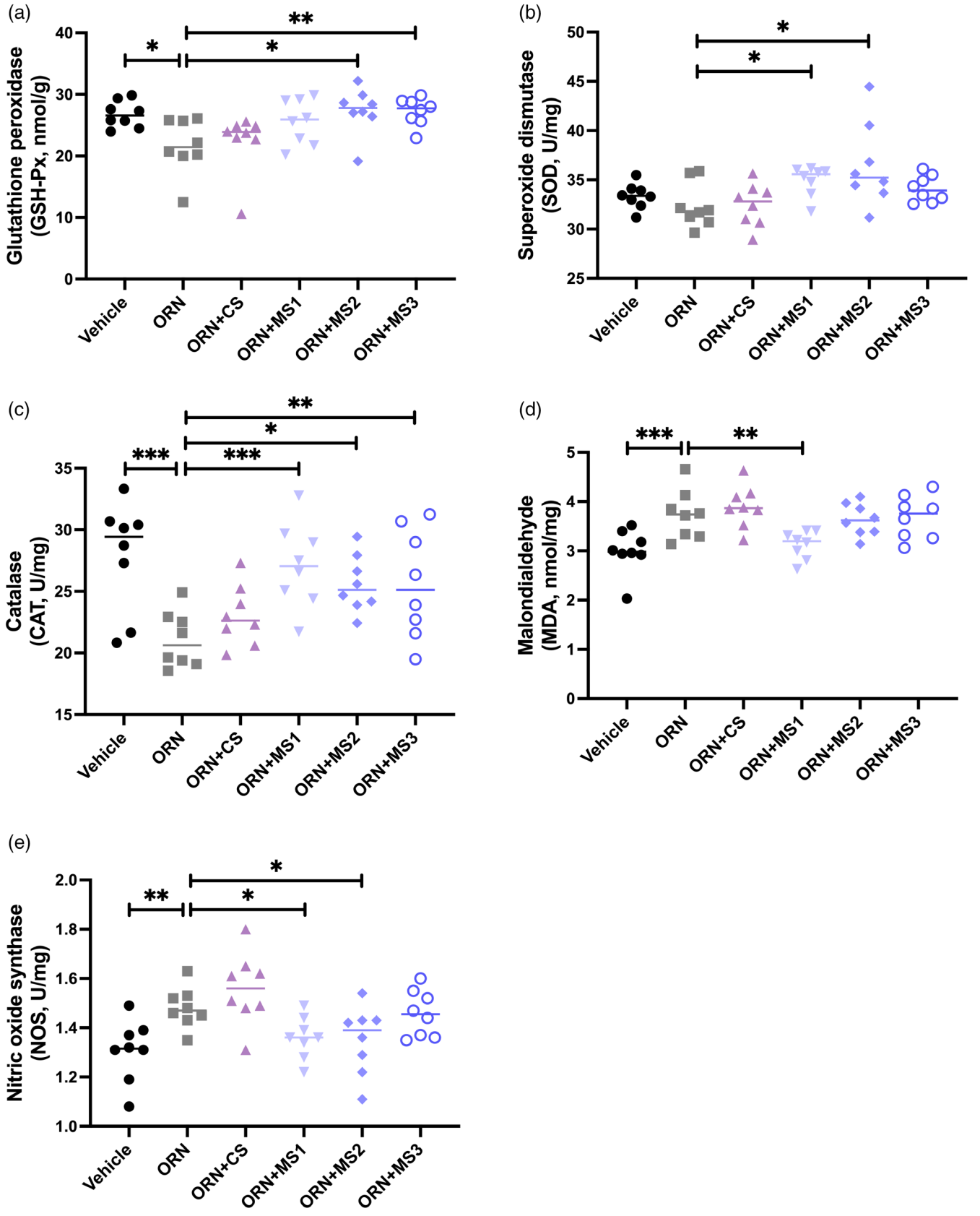

FIGURE 4 Comparative results of GSH-Px, SOD, CAT, MDA, and NOS levels in different rat groups. (a) GSH-Px. (b) SOD. (c) CAT. (d) MDA. (e) NOS. Data were presented as mean ± SEM ($n = 8$). *$p < .05$; **$p < .01$; ***$p < .001$. ORN, ornidazole; CS, cigarette smoke; MS, moxa smoke

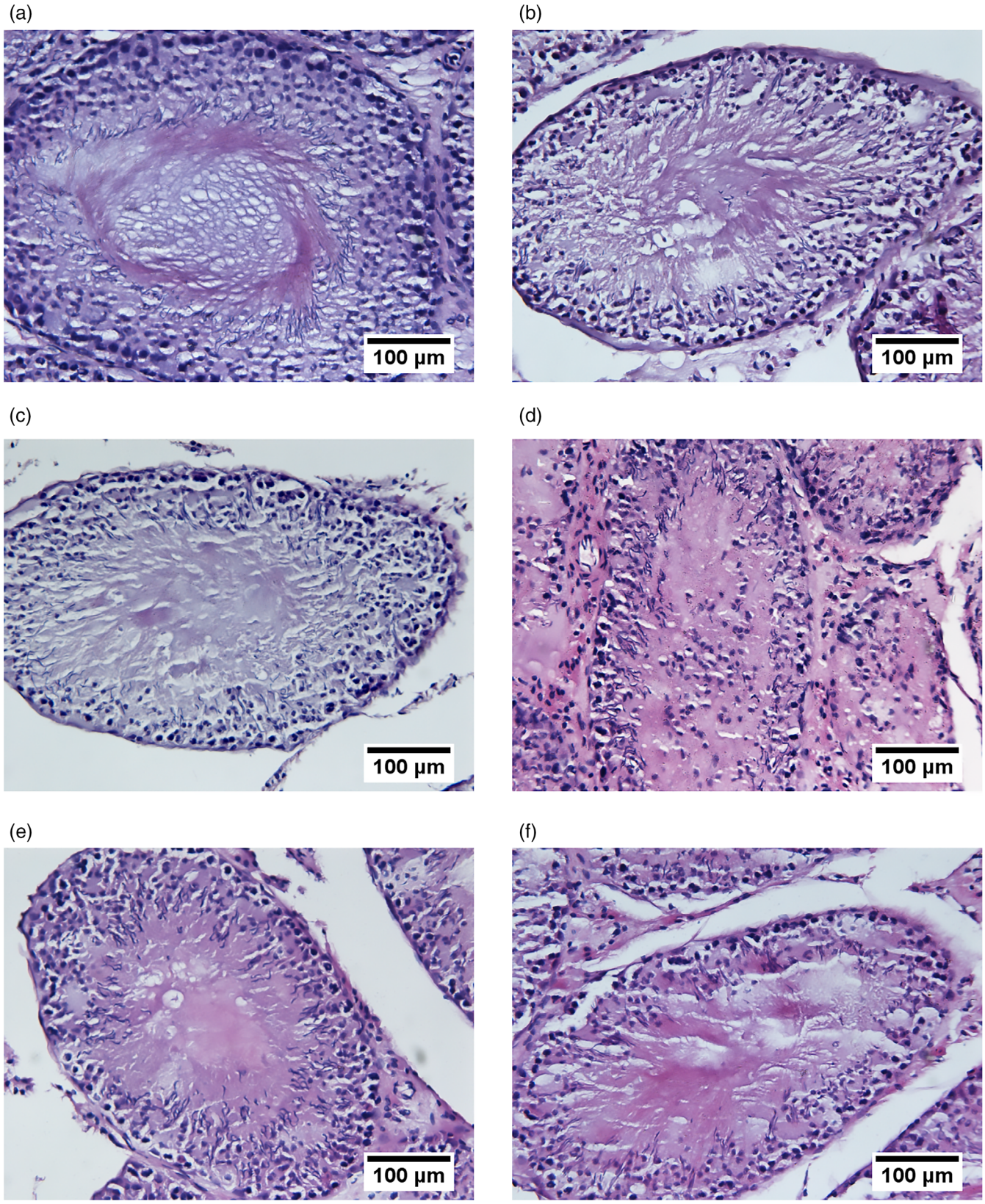

FIGURE 5 HE-stained histological sections of testis (magnification ×40). (a) Vehicle group. (b) ORN-treated group. (c) ORN + CS group. (d) ORN + MS1 group. (e) ORN + MS2 group. (f) ORN + MS3 group

concentration. After conversion, the optical concentration of the low concentration (1 × clinical MS concentration) corresponded to 0.4%, that of the moderate concentration (3 ×) corresponded to 2%, and that of the high concentration (9 ×) corresponded to 15%. These concentrations determined by previous clinical trials and experiments were reasonably and feasibly selected.

In the event of OS, a variety of noxious stimuli in the body result in the aberrantly excess production of highly active molecules, such as ROS and reactive nitrogen species (Sies & Jones, 2020). Due to the high oxidation of ROS, antioxidant enzymes like GSH-Px, SOD, and CAT are applied to eliminate ROS, thereby maintaining the normal level of ROS and protecting cells (Diyabalanage et al., 2020; Dong et al., 2013; Garcia-Rodriguez et al., 2018; Polhsak et al., 2013). As the key product of the peroxidation reaction between ROS and cellular polyunsaturated fatty acids, MDA is a telling indicator for the severity of lipid peroxidation damage (Michno et al., 2005), which impedes cell function, damages the sperm, and suppresses motility (Sun et al., 2011). NOS catalyzes the decomposition of L-arginine into guanidine to generate NO (Srivastava et al., 2006). Abundant NO can react with superoxide to form peroxides, leading to detrimental sperm quality (Shiraishi & Naito, 2007).

The findings of the current research unveiled that the different concentrations of MS relieved sperm parameters in asthenozoospermia rats. Within the range of concentrations utilized in this research, the increase in the concentration of MS also induced an improvement in sperm quality. The activities of GSH-Px, SOD, and CAT elevated to different degrees in all MS groups, whereas MDA level was lowered in the low-concentration MS group and NOS level was decreased in the low- and moderate-concentration MS groups. These data indicated that MS has a speculated antioxidative role and might also improve the sperm motility of asthenozoospermic rats through certain manners. More experiments are warranted to verify whether different concentrations of MS exert various antioxidative effects in a concentration-dependent manner.

Interestingly, our findings further elaborated that progressive motility, LIN, and sperm viability in the CS group increased dramatically versus the ORN group. A previous study (Chen et al., 2014) uncovered that this increase could be attributable to the high NO level in cigarettes. Here, compared with the ORN group, the activities of GSH-Px, SOD, and CAT were slightly enhanced in the CS group, yet no significantly promoted oxidative damages and differences were found. It was speculated that the defensive response mechanism in rats was freed from CS-produced radicals. However, the contents of MDA and NOS augmented evidently, and testicular tissues also exhibited severer pathological damage, suggesting that the stimulated antioxidant enzyme activities were insufficient to protect the body against OS. In addition, the concentration of CS set in this study was concordant with that of the moderate concentration of MS, lower than that in other CS experiments. With the prolongation of the intervention time or the augmentation of the smoke concentration, CS might diminish sperm motility in rats. Further studies are required to ascertain the reasons in the future.

The safety of MS is becoming controversial as research on haze determines its biotoxicity in the environment. In the UK, the smoke toxicity of moxa sticks was tested under the standard conditions of cigarette testing, which unraveled that under normal conditions, only two kinds of volatile substances, including carbon monoxide and aromatic substances were slightly increased, which did not reach the limit value of carcinogenesis (Wheeler et al., 2009). A recent study evaluated pathological changes in rat lung tissue and analyzed differentially expressed genes using RNA-seq and transcriptomic analyses (Xu et al., 2021), the results showed that the maximum tolerable dose of moxa smoke was 290.036 g/m^3 and LC_{50} was 537.65 g/m^3. The concentration of moxa smoke used in our study is far less than the reported study, which provides a basis for evaluations of moxibustion safety and the development of moxibustion-based technology. Accumulating studies have reported that the etiology of asthenozoospermia is closely related to CS (Asare-Anane et al., 2016; Rehman et al., 2019). Although MS and CS can enter the body in the same way with similar physical properties, they are essentially different substances that exert variable effects on the body. As a product of moxa combustion, MS contains a large amount of small molecular aromatic hydrocarbons that are distinctively dissimilar to those produced by CS (Jin et al., 2011), indicating that moxa is an irreplaceable moxibustion material.

Limitations are noticed in the present study. First, the rats with ORN-induced asthenozoospermia were exposed to MS at the low, moderate, and high concentrations for 8 weeks, corresponding to 1 × , 3 × , and 9 × clinical MS concentrations in clinics. It remains enigmatic whether they induce the same impacts of MS on sperm motility for a shorter or longer exposure period. Moreover, the golden indicator to evaluate the degree of damage or the recovery of fertility in male rats should be the number of embryos produced by mating with females after modeling or intervention. In this research, the female rats were not caged in the mating test to accurately evaluate the sperm fertilization ability and actual fertility level of each group. Finally, this experiment is an observational research, some signal pathways associated with OS, like Keap1/

Nrf2, PKC/ERK pathway, will be the focus of our further studies.

5 | CONCLUSION

In summary, this study elucidated that MS improved the sperm motility of asthenozoospermic rats by restoring the decreased activities of antioxidant enzymes and the enhanced levels of oxidation products in vivo. In the concentration range of this study, MS ameliorated sperm parameters in asthenozoospermic rats in a concentration-dependent manner. This study identified that MS was an effective factor of moxibustion therapy, which contributed to satisfactory results in the treatment of sperm motility of asthenozoospermic rats by restraining OS.

AUTHOR CONTRIBUTIONS

Yajie Liu: Data curation (lead); investigation (equal); methodology (lead); writing – original draft (lead). **Yu An:** Data curation (supporting); formal analysis (lead); investigation (equal). **Guogang Xing:** Conceptualization (equal); project administration (lead); resources (supporting); writing – review and editing (supporting). **Zirun Jin:** Conceptualization (supporting); investigation (supporting); methodology (supporting). **Ke Xi:** Formal analysis (supporting); investigation (supporting). **Yongwei Huo:** Investigation (supporting). **Rui He:** Visualization (supporting); writing – review and editing (supporting). **Hao Wang:** Writing – review and editing (supporting). **Xiali Ouyang:** Writing – review and editing (supporting). **Yueping Huang:** Writing – review and editing (supporting). **Chang Huang:** Writing – review and editing (supporting). **Li Han:** Visualization (supporting); writing – review and editing (supporting). **Baixiao Zhao:** Conceptualization (equal); funding acquisition (lead); project administration (lead); resources (lead); writing – review and editing (supporting).

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

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REFERENCES

Agarwal, A., Sharma, R. K., Nallella, K. P., Thomas, A. J., Alvarez, J. G., & Sikka, S. C. (2006). Reactive oxygen species as an independent marker of male factor infertility. *Fertility and Sterility*, *86*, 878–885.

Asare-Anane, H., Bannison, S. B., Ofori, E. K., Ateko, R. O., Bawah, A. T., Amanquah, S. D., Oppong, S. Y., Gandau, B. B. N., & Ziem, J. B. (2016). Tobacco smoking is associated with decreased semen quality. *Reproductive Health*, *13*, 90.

Bisconti, M., Simon, J. F., Grassi, S., Leroy, B., Martinet, B., Arcolia, V., Isachenko, V., & Hennebert, E. (2021). Influence of risk factors for male infertility on sperm protein composition. *International Journal of Molecular Sciences*, *22*, 13164.

Bone, W., Jones, N. G., Kamp, G., Yeung, C. H., & Cooper, T. G. (2000). Effect of ornidazole on fertility of male rats: Inhibition of a glycolysis-related motility pattern and zona binding required for fertilization in vitro. *Journal of Reproduction and Fertility*, *118*, 127–135.

Chen, X. H., Dai, J. B., Xu, W. J., Wang, Z. X., & Qiao, Z. D. (2014). Effects of passive smoking on reproductive capacity of male mice. *Reproduction & Contraception*, *34*, 805–808.

Diyabalanage, S., Dangolla, A., Mallawa, C., Rajapakse, S., & Chandrajith, R. (2020). Bioavailability of selenium (se) in cattle population in Sri Lanka based on qualitative determination of glutathione peroxidase (GSH-Px) activities. *Environmental Geochemistry and Health*, *42*, 617–624.

Dong, L., He, Y. Z., Wang, Y. L., & Dong, Z. Y. (2013). Research progress on application of superoxide dismutase (SOD). *Journal of Agricultural Science and Technology*, *15*, 53–58.

Drobnis, E. Z., & Nangia, A. K. (2017). Antimicrobials and male reproduction. *Advances in Experimental Medicine and Biology*, *1034*, 131–161.

Du, Y. T., Liu, H., Zhang, M., Zhang, S., Hu, J. M., Wu, G. F., & Yang, J. C. (2019). Taurine increases spermatozoa quality and function in asthenospermia rats impaired by ornidazole. *Advances in Experimental Medicine and Biology*, *11*(1155), 507–520.

Garcia-Rodriguez, A., de la Casa, M., Gosálvez, J., & Roy, R. (2018). CAT-262CT genotype shows higher catalase activity in seminal plasma and lower risk of male infertility. *Meta Gene*, *18*, 16–22.

Ha, L., & Zhao, B. X. (2016). Experimental study of moxibustion's effect on inflammatory factors and MMP-9 of arteriosclerosis mice. *World Chinese Medicine*, *11*, 1389–1394.

Ho, T., Vrabec, J. T., Yoo, D., & Coker, N. J. (2006). Otomycosis: Clinical features and treatment implications. *Otolaryngology-Head and Neck Surgery*, *135*, 787–791.

Huang, C. X., Zhao, B. X., Liu, P., & Shao, L. Y. (2012). Mass concentration and morphological characteristics of PM10 in moxibustion rooms in Beijing and Tianjin during summer. *China Journal of Traditional Chinese Medicine and Pharmacy*, *27*, 3104–3108.

Jin, R., Zhao, B. X., Yu, M. M., Fu, X. T., Chen, Y. G., & Guo, H. Z. (2011). Qualitative analysis on components of moxa combustion products by solid-phase microextraction-gas chromatography-mass spectrography. *Journal of Beijing University of Traditional Chinese Medicine*, *34*, 632–636.

Jin, Z. R., Liu, B. H., Cai, J., Jing, X. H., Zhu, B., & Xing, G. G. (2017). Experimental study for the treatment of asthenozoospermia by electroacupuncture in rats. *Acupuncture Research*, *42*, 114–118 (In Chinese).

Jo, J., Lee, S. H., Lee, J. M., & Jerng, U. M. (2015). Semen quality improvement in a man with idiopathic infertility treated with traditional Korean medicine: a case report. *The Explorer*, *11*, 320–323.

Martins, A. D., Panner Selvam, M. K., Agarwal, A., Alves, M. G., & Baskaran, S. (2020). Alterations in seminal plasma proteomic profile in men with primary and secondary infertility. *Scientific Reports*, *10*, 7539.

Marzony, E. T., Ghanei, M., & Panahi, Y. (2016). Relationship of oxidative stress with male infertility in sulfur mustard-exposed injuries. *Asian Pacific Journal of Reproduction*, *5*, 1–9.

Meng, X. N., Xu, H. F., Cui, Y. X., Liu, P., Li, J., Jin, R., & Zhao, B. X. (2011). Effect of moxa products after burning on SOD, MDA and GSH-Px in the brain of senescence accelerated mice. *Global Traditional Chinese Medicine*, *4*, 413–415 (In Chinese).

Michno, A., Raszeja-Specht, A., Jankowska-Kulawy, A., Pawelczyk, T., & Szutowicz, A. (2005). Effect of L-carnitine on acetyl-CoA content and activity of blood platelets in healthy and diabetic persons. *Clinical Chemistry*, *51*, 1673–1682.

Polhsak, B., Šuput, D., & Milisav, I. (2013). Achieving the balance between ROS and antioxidants: When to use the synthetic antioxidants. *Oxidative Medicine and Cellular Longevity*, *2013*, 956792.

Rehman, R., Zahid, N., Amjad, S., Baig, M., & Gazzaz, Z. J. (2019). Relationship between smoking habit and sperm parameters among patients attending an infertility clinic. *Frontiers in Physiology*, *10*, 1356.

Rizzetti, D. A., Martinez, C. S., Escobar, A. G., da Silva, T. M., Uranga-Ocio, J. A., Pecanha, F. M., Vassallo, D. V., Castro, M. M., & Wiggers, G. A. (2017). Egg white-derived peptides prevent male reproductive dysfunction induced by mercury in rats. *Food and Chemical Toxicology*, *100*, 253–264.

Shahrokhi, S. Z., Salehi, P., Alyasin, A., Taghiyar, S., & Deemeh, M. R. (2020). Asthenozoospermia: Cellular and molecular contributing factors and treatment strategies. *Andrologia*, *52*, e13463.

Shiraishi, K., & Naito, K. (2007). Nitric oxide produced in the testis is involved in dilatation of the internal spermatic vein that compromises spermatogenesis in infertile men with varicocele. *BJU International*, *99*, 1086–1090.

Sies, H., & Jones, D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nature Reviews Molecular Cell Biology*, *21*, 363–383.

Smith, C. A. (2013). Moxibustion for breech presentation: Significant new evidence. *Acupuncture in Medicine*, *31*, 5–6.

Srivastava, S., Desai, P., Coutinho, E., & Govil, G. (2006). Mechanism of action of L-arginine on the vitality of spermatozoa is primarily through increased biosynthesis of nitric oxide. *Biology of Reproduction*, *74*, 954–958.

Sun, H., Qiu, M. Y., Li, B. Q., Wang, S. H., Chen, Z. Y., Jiang, Y., & Luan, J. (2008). Effect of moxibustion on quality of life in hemodialysis patients. *Chinese Acupuncture & Moxibustion*, *28*, 321–324.

Sun, Q. Y., Li, J., Han, Y. C., Xue, C. Y., Guan, H. Z., Xie, S. J., … Zhang, X. Q. (2011). Influence of cigarette smoking on semen quality, seminal reactive oxygen species and sperm apoptosis rate. *Chinese Journal of Laboratory Diagnosis*, *15*, 1046–1048.

Sun, Y., Sun, X. P., Zhao, L. M., Zhang, Z., Wang, Y. P., Dai, Z. F., Zhao, X., & Pu, X. P. (2020). DJ-1 deficiency causes metabolic abnormality in ornidazole-induced asthenozoospermia. *Reproduction*, *160*, 931–941.

Wang, L., Han, L., Liu, P., Yang, J., Cui, Y. X., Bai, H., Liu, J., Huang, Y., Huang, J., Huang, C., & Zhao, B. X. (2016). Moxa smoke: Is it a beneficial or hazardous factor for infertility? A preclinical study on sperm parameters and sex hormones in male rats. *European Journal of Integrative Medicine*, *8*, 28–35.

Wang, Y. S., Zhang, J. B., Jiang, J. F., & Wang, L. L. (2013). Research on effects of the thermal stimulation by moxibustion at different temperatures on cardiac function in rats and on mast cells in the local site of moxibustion. *Evidence-based Complementary and Alternative Medicine*, *2013*, 545707.

Wheeler, J., Coppock, B., & Chen, C. (2009). Does the burning of moxa (artemisia vulgaris) in traditional Chinese medicine constitute a health hazard. *Acupuncture in Medicine*, *27*, 16–20.

Xu, J., Deng, H. Y., & Shen, X. Y. (2014). Safety of moxibustion: A systematic review of case reports. *Evidence-based Complementary and Alternative Medicine*, *2014*, 783704.

Xu, X. Y., Deng, W., Zhang, W. Q., Zhang, J. H., Wang, M. C., Shan, S., & Liu, H. N. (2021). Transcriptome analysis of rat lungs exposed to moxa smoke after acute toxicity testing. *Evidence-based Complementary and Alternative Medicine*, *2021*, 5107441.

Xu, X. Y., Shan, S., Wang, W. L., & Liu, H. N. (2020). Analysis of the components in moxa smoke by GC-MS and preliminary discussion on its toxicity and side effects. *Evidence-based Complementary and Alternative Medicine*, *2020*, 2648759.

Yang, J., Zhao, B. X., Han, L., Liu, P., Wang, L., Bai, H., Huang, J., Liu, J. T., Huang, C., Zhu, M. X., & Yang, Z. H. (2014). Effects of controllable dynamic inhaled exposure of moxa smoke on LDL-r, ICAM-1 and morphology of heart tissue in rats. *Zhongguo Zhen Jiu*, *34*, 573–577.

Yu, M. Y., Ha, L., Huang, C., Huang, X. Y., & Zhao, B. X. (2016). Effects of moxibustion on liver pathology and lipid metabolism in apolipoprotein E-deficient mice. *World Chinese Medicine*, *11*, 1419–1423 (In Chinese).

Zhang, J. F., & Wu, Y. C. (2006). Modern progress of mechanism of moxibustion therapy. *Journal of Acupuncture and Tuina Science*, *4*, 257–260 (In Chinese).

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