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Investigating the pathological changes and activity of antioxidant enzymes in testicular tissue of male Wistar rats exposed to welding fumes

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Abstract
Background: Welding is an extensively utilized industrial technique, which involves the fusion of fumes containing various heavy metals. To protect exposed workers, it is crucial to investigate the subsequent adverse effects. This study aimed to carry out the welding process within a regulated welding chamber and examine the effects of glutathione peroxidase (GPX) and superoxide dismutase (SOD) as antioxidant enzymes and pathological damage on rat testicular tissue.
Methods: Male adult Wistar rats (n = 7/experiment group) were exposed to an average concentration of (44.48 mg/m^3) welding fumes (WFs) for 30 min/d × 8 days through shielded metal arc welding (SMAW). The control group (n = 7) was in similar conditions without direct exposure to WFs.
Results: The metal composition of WFs was found to contain iron (Fe), manganese (Mn), copper (Cu), aluminum (Al), lead (Pb), and chromium (Cr). Among these metals, Fe had the highest average concentration (12.06 mg/m^3), while Cu displayed the lowest one (0.019 mg/m^3). The majority of the produced particles were found within the micron size range (0.25 to 0.5 μm). The mean activity of GPX and SOD was determined 15.16777 (mU/mg protein) and 81.955 (U/mg protein) in the exposed group, respectively. In the microscopic examination of testis tissue, some complications were observed.
Conclusion: Oxidative damage occurred in the testicular tissue of experimental rats by decreasing the mean activity of antioxidant enzymes. Besides, the exposed group showed reduced sperm quality and quantity indexes.
Keywords: Welding, Antioxidant enzymes, Glutathione peroxidase, Superoxide dismutase
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Introduction
Welding is a widely used industrial process that involves joining different metals together using high pressure and temperature (1,2). Many workers around the world perform welding-related activities but are not considered as full-time welders, while approximately 800 000 workers are regarded as full-time welders (3). Welding-related employees and welders suffer from a variety of harmful factors such as ultraviolet radiation, noise, extreme heat, burns, improper body postures, electrical hazards, gas, and fumes (1,4,5). It seems that among all welding harmful factors, metal fumes have the most adverse effects on workers' health status (5). The industrial process of welding exists in more than 80 different ways, but the most prevalent one is arc welding. The most commonly used feeding system in arc welding is stainless and mild steel, and the resulting welding fumes (WFs) can cause various reactions depending on the metal composition, soluble ion content, and ability to generate free radicals (5,6). The shielded metal arc welding (SMAW) procedure involves passing an electric current through both the flux-covered electrode and workpiece, creating an electric arc that generates heat and melts metal droplets (7,8). The produced WFs can consist of 14 various metals including manganese (Mn), chromium (Cr), beryllium, cadmium, copper (Cu), cobalt, lead (Pb), iron (Fe), aluminum (Al), and nickel (5,9). Metal fumes are particles with an aerodynamic diameter of less than one micron and are

typically generated in large quantities during common industrial processes such as welding (10,11). Exposure to welding metal fumes has been shown to cause various diseases in humans and animals by inhalation and direct contact (6,12). The International Agency for Research on Cancer (IARC) classified WFs as probable carcinogens (Group 2B) in 1990 and even as "carcinogenic to humans" (Group 1) in 2017 (13–16). Oxidative stress plays a significant role in the development of many disorders caused by WFs (17). This damage is caused by an imbalance between the formation of reactive oxygen species (ROS) and the antioxidant defense of the biological system (18). ROS compounds are among the free radicals that are produced by exogenous agents such as chemicals, radiation, or some endogenous mechanisms. When ROS reach certain levels, they can cause oxidative stress by overwhelming the capacity of the endogenous cellular antioxidant defense system (19). Due to the high reactivity of ROS and the difficulty in assessing related levels, several biomarkers associated with oxidative stress such as protein carbonyls, antioxidant enzymes, manganese superoxide dismutase (MnSOD), and total antioxidant capacity are evaluated to indicate changes caused by WFs (15). The existence of ROS and a huge mass of highly unsaturated fatty acids makes testes susceptible to oxidative stress (20). ROS can cause DNA fragmentation and lipid peroxidation, which can disrupt cell motility and impair the ability of spermatozoa to support normal embryonic development (17). Pb, mercury, and cadmium have the potential to affect the endocrine system and even disrupt gonadal evolution (20,21). According to some studies, male welding workers have been shown decreased reproductive operation related to reduced fertility, various degrees of reproductive hormones, and deficient sperm quality (13). Exposure to cadmium and Cu can cause a reduction in testis weight and histopathological damage, leading to defective sperm motility, decreased sperm counts, reduced male fecundity, and induced testicular apoptosis in male experimental animals (22,23). Scientific research have demonstrated that cadmium and Pb are among the heavy metals that have been correlated with lower sperm concentrations, abnormal spermatic morphology, and lower sperm viability (24). The present study aimed to conduct the welding process in a controlled welding chamber and investigate the reduction of the mean activity of glutathione peroxidase (GPX) and superoxide dismutase (SOD) as antioxidant enzymes and pathological damage to testicular tissue in adult Wistar rats. Since previous studies have considered the number of days and duration of exposure to be longer, the researchers of this study tried to find out whether changes in the mean activity of GPX and SOD and pathological damages occur in shorter periods or not. In addition, none of the similar studies had measured the relationship between exposure to WFs and the reduction of the mean activity of GPX and SOD, the reduction of quantitative and qualitative measurement parameters of sperms, and the pathological changes of testicular tissue at the same time. This study aimed to contribute to the development of strategies to control the exposures of welders and to promote their health status.

Materials and Methods

Animals and treatments

Fourteen healthy adult male Wistar rats, aged 8 weeks and weighing 180-220 g, were obtained from the animal house of the faculty of pharmacy at Isfahan University of Medical Sciences in Iran. During the study, the animals were fed a regular diet and provided with ad libitum access to food and water. They were housed in standard physical conditions, including a temperature range of 22-28°C, relative humidity of 55%, a 12-hour light/dark cycle with 325 lux illumination at midday, and a noise level of 85 dBA (18,25).

Experimental design

The animals were acclimated to the laboratory environment for one week, and then, randomly divided into two groups, each composed of six male Wistar rats. However, one animal from each group was subsequently excluded from the study, leaving six rats per group. Following the laboratory protocol, WFs containing various heavy metals were produced in a specially designed chamber. In the experimental group (Group 1), the rats were exposed to an average concentration of 44.48 mg/m^3 of WFs for 30 minutes per day, for a total of 8 days. The animals in the control group (Group 2) were in similar physical conditions but without direct exposure to WFs (25,26).

Welding fume generator system and exposure chamber

The WF generator consisted of an electrical welding device (Mini Arc 200, IRAN TRANS), a standard torch, and a wire feeder that supplied wire to the torches as the welding power source. The SMAW was performed manually by a professional welder using a stainless-steel electrode (SUPER MICA E6013) on a steel piece. The process was carried out for 30 minutes per day, over a total of 8 days, using 12 electrodes each day. The WF generation chamber was cone-shaped and constructed from galvanized steel sheet. The generated fumes were transferred from the upper orifice of the generator chamber to the exposure chamber via a flexible trunk. The welding process was carried out under standard ventilation conditions, using a canopy fan. The welder wore appropriate personal protective equipment, including special clothing, a shield, a mask, and gloves. The exposure chamber was made of six plexiglass sheets, each 8 mm thick, with dimensions of 40 × 50 × 40 cm. The chamber had an elliptical hole on the upper side for animal entry. A 40% dilution was applied to the WFs in the exposure chamber. Three outlets with adjustable valves were applied on the other side of the chamber. A peripheral pump (Brook Crompton Parkinson Motors, 1206, England) was used as an air pressure supply with a flow rate of 9 liters/min. A personal sampling pump (Leland Legacy 100-3002 sample pump, SKC, Inc.) was applied to sample the internal air of the exposure chamber with a flow rate of 2.5 L/min. Additionally, a cascade impactor was used with the Leland Legacy Sample Pump (SKC Cat. No. 100-3002) with a flow rate of 9 L/min for particle size distribution. Figure 1 depicts a general view of the pilot, which was designed and tested in this study (13,26).

Collection of welding fumes and particle size distribution

The WFs were collected on a 25-mm Mixed Cellulose Ester (MCE) membrane filter using the personal sampling pump. The particle size distribution of the collected samples was assessed by the cascade impactor, which was connected to a specialized pump. Particles over each cut-point were collected on a 25-mm MCE after-filter and particles under the 0.25 µm cut-point were collected on a 37-mm MCE one. The weight of particles in each stage was measured by gravimetric method (weighing the cascade impactor filters before and after sampling with high accuracy). It should be noted that all sampling pumps were calibrated with a reagent sampler connected to the related pump (26-28).

Welding particle composition

According to NIOSH method 7302, which was qualified for microwave digestion, welding particles were collected on MCE filters with 0.8-µm pore size in 25-mm and 37-mm cassettes during 30 minutes of welding. The particle samples were digested, and the composited metals were examined by inductively coupled plasma atomic emission spectroscopy (ICP-OES). To analyze the sample, 10 mL of nitric acid and distilled water were used as a reagent solution, and the final solution was measured at a wavelength of 220.4 nm. After this assessment, the metals that comprised mild steel and stainless electrodes were quantified: Pb, Cr, Al, Cu, Mn, and Fe. Table 1 demonstrates the limits of detection (LODs) and limits of quantification (LOQs) (26,27).

Analysis of animal samples

After the 8-day exposure period, the rats were transferred to a glassy chamber. They were anesthetized and sacrificed by diffusion of carbon dioxide after an overnight fast. Testis tissues were collected and processed for further investigations. The sperms removed from the epididymis were placed on a slide in Ham's Fl0 culture medium, and their motility, viability, and number were immediately examined under a 10-objective microscope. The right testicle was immediately placed at -20 °C for biochemical investigation, and the left testicle was also stored in 10% formalin for histopathological analysis. To burn the carcasses in a special oven, they were collected in plastic bags and frozen (14).

Homogenize testicular tissue

The testis homogenate was prepared in a lysate buffer supplemented with cocktail protease inhibitor and centrifuged at 3000 rpm for 10 minutes at 4 °C. The supernatant was frozen at -80 °C and used for various biochemical analyses.

Determining oxidative damage by measuring GPX and SOD activity

The tissue activities of GPX and SOD were measured using a local commercial kit (Kiazist, Iran) and the data were normalized based on the protein content of the supernatant according to the Bradford method.

Table 1. The limits of detection (LODs) and limits of quantification (LOQs)

Metal	LOD (µg)	LOQ (µg)
Fe	0.2	0.6
Mn	0.2	0.6
Pb	0.1	0.3
Al	0.4	1.2
Cr	0.2	0.6
Cu	0.1	0.3

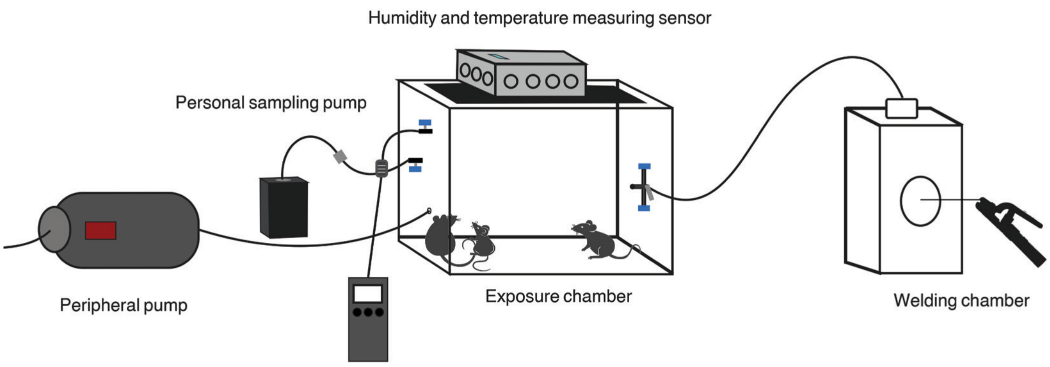

Figure 1. A designated model of the studied pilot

Histopathological evaluation of testis

For histopathological evaluation, testis tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm thickness, and stained with hematoxylin-eosin staining for light microscopic examination.

Statistical analysis

SPSS software version 2021 was used for statistical analysis. The inferential data were analyzed using either one-way or two-way ANOVA models with Tukey's post-hoc comparisons on GraphPad Prism version 8.02.

Results

Physical condition, fume sampling, and particle size distribution

In this study, the physical conditions of the environment were determined during all sampling days. Humidity and temperature were measured by a digital sensor placed on the top of the exposure chamber, and the mean value ± standard deviation was recorded as 58.41 ± 10.7% and 23.51 ± 2.55°C, respectively. The illumination level was measured by DIGITAL LIGHT METER, YF-170, and the mean value of it was calculated as 446.56 ± 35.57 lux. The sound level was also determined by SOUND LEVEL METER, HT169, and the mean level was reported as 66.337 ± 0.3 dBC (Table 2).

The metal composition of the fume particles produced during the welding process was determined. The MCE filters were analyzed by ICP-OES. Fe, Mn, Cu, Al, Pb, and Cr were identified as metal compositions. The highest mean concentration belonged to Fe (12.06 mg/m^3) and the lowest one to Cu (0.019 mg/m^3) (Figure 2).

According to the collected data by the cascade impactor, the particles in stages D, A, C, and B were the most abundant size ranges in the sample, respectively. Also, the mean weight of particles in various diameters was calculated, and the results are as follows:

- Stage A (up to 2.5 µm): 1.775 mg
- Stage B (1-2.5 µm): 1.075 mg
- Stage C (0.5-1 µm): 1.537 mg
- Stage D (0.25-0.5 µm): 3.887 mg (Table 3).

Oxidative changes of testicular tissue by evaluation of the tissue activity of GPX and SOD

Oxidative changes of the testicular tissue were observed in the form of a significant decrease in the activity of GPX and SOD as antioxidant enzymes compared to the control group. The mean activity of SOD in the experiment group was 81.955 U/mg protein vs.179.952 U/mg protein in the control group. The mean activity of GPX in the experiment group was also 15.16777 mU/mg protein vs. 20.32871 mU/mg protein in the control group (Figure 3).

Assessment of sperm number, motility, and viability

Relative WF exposure causes significant changes in

Table 2. Physical conditions of the environment

Sample	Physical Parameter			
	Mean humidity (%) ± SD	Mean temperature (C°) ± SD	Mean illumination level (lux) ± SD	Mean sound level (dBC) ± SD
1	54.05 ± 12.94	24.3 ± 0.07	416 ± 1.41	65.3 ± 0.28
2	46.35 ± 0.6	22.7 ± 0.28	442.5 ± 24.74	65.05 ± 0.35
3	66.75 ± 17.18	20.1 ± 0.56	505 ± 7.07	66.85 ± 0.07
4	74.15 ± 8.69	19.1 ± 0.42	437.5 ± 3.53	69.3 ± 0.14
5	71.3 ± 21.21	24.2 ± 0.49	496 ± 11.31	69.25 ± 0.91
6	48.55 ± 2.05	26.2 ± 0.070	408 ± 4.24	64.9 ± 0.14
7	52.6 ± 10.46	25.6 ± 0.84	442.5 ± 10.60	65.85 ± 0.21
8	53.55 ± 9.82	25.3 ± 0.26	425 ± 7.07	64.2 ± 0.28

Table 3. The weight of different particle sizes ranges on four stages of cascade impactor filters

Sample	Particle size up to 2.5 µm (mg)	Particle size (1-2.5 µm) (mg)	Particle size (0.5-1 µm) (mg)	Particle size (0.25-0.5 µm) (mg)
1	5.1	1.7	0.7	1.1
2	0.8	0.9	1	6.8
3	1.4	1.5	3.7	7
4	4.2	0.9	2	9.5
5	0.6	1	2.1	3.6
6	0.5	0.9	1	1.8
7	0.5	0.5	0.2	0.3
8	1.1	1.2	1.6	1
Total	14.2	8.6	12.3	31.1

sperm parameters. They include a significant decrease in sperm count in the experiment group (3.18 ± 0.31) vs. the control group (4.06 ± 0.35), a decrease in sperm viability in the experiment group (69.28 ± 1.97) vs. the control group (79.33 ± 5.39), a decrease in sperm motility in the experiment group (59.71 ± 4.07) vs. the control group (67.66 ± 3.93), and a decrease in progressive sperm in the experiment group (52.42 ± 5.88) vs. the control group (62.5 ± 3.08) (Table 4).

Histological changes of testicular tissue

In the microscopic examination of testis tissues, no abnormal structures were observed in the control group. The arrangement of cells from spermatogonia to mature sperm inside the seminiferous tubules and their number as well as the thickness of the basement membrane and the diameter of the seminiferous tubules were normal. The testes of animals in the experiment group showed some structural changes compared to the control group. Although there was no evidence of a decrease in the diameter of seminiferous tubules or a decrease in their number, the thickening of basement membrane in the tubules, disorganized architecture of germinal epithelium, decrease in height of germinal epithelium, loss of intercellular junction between Sertoli cells and spermatogenic cell line and dilatation of blood vessels were observed (Figure 4).

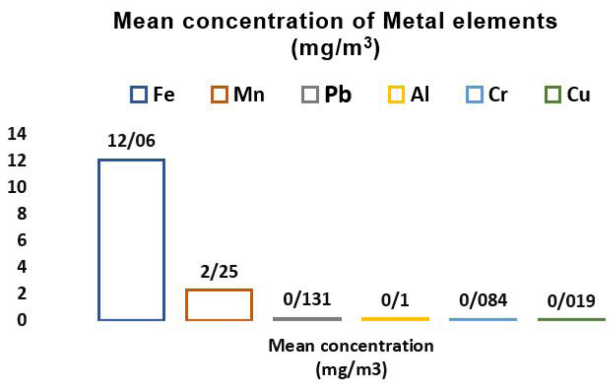

Figure 2. The mean concentration of metal elements in sampled fumes

Discussion

The main aim of this research was to perform welding procedures within a specifically designated welding chamber to investigate how WFs affect the testicular tissue of male Wistar rats. The study particularly focused on exploring the mean activity of GPX and SOD as antioxidant enzymes, sperm quality and quantity parameters, and pathological damage. The present study showed that the mean activity of SOD and GPX enzymes, along with sperm parameters, decreased significantly in the experimental group, and also, pathological changes diagnosed in this group.

Despite the assessment of health hazards among welders, exposure to WFs within the workplace remains a significant health issue, especially in developing countries (29). The toxicity of WFs is related to particle morphology, chemical composition, and particle size distribution, besides exposure duration and concentration (6). Among all the detrimental effects on almost every organ of the body, environmental and occupational exposures to heavy metals can lead to adverse effects on the human reproduction system by inducing oxidative stress (18). According to different studies, male welders suffer from lower sperm quality and fecundity, poor fertility, and changed levels of reproductive hormones compared to non-exposed cases (30).

Figure 2 demonstrates an algorithm for the mean concentration of WFs, which is arranged in the sequence of Fe > Mn > Pb > Al > Cr > Cu. Fe, Mn, and Pb exceeded the occupational exposure limit (OEL) level, which

Table 4. Sperm parameters analysis in the experiment and control groups

Parameters	Control	Experiment	*P* value
Sperm number × 10^6/ml	4.06 ± 0.35	3.18 ± 0.31	< 0.001
Viable sperm (%)	79.33 ± 5.39	69.28 ± 1.97	< 0.001
Motile sperm (%)	67.66 ± 3.93	59.71 ± 4.07	0.004
Progressive sperm (%)	62.5 ± 3.08	52.42 ± 5.88	0.003

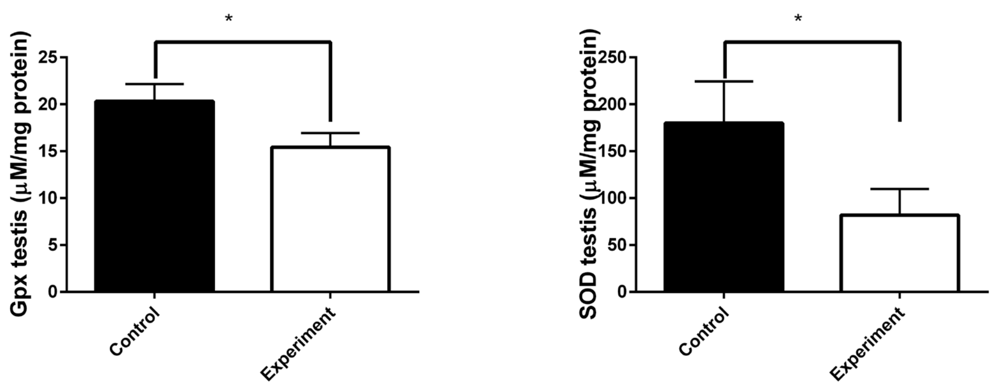

Figure 3. These diagrams demonstrate a definite decline in the activity of GPX and SOD in the experiment group compared to the control group. The diagnosed data, display a steady correlation between exposure to WFs and decreased antioxidant enzyme activity which leads to an increasing The risk of oxidative stress. Each value represents mean ± SD, n = 7, ***P* < 0.01

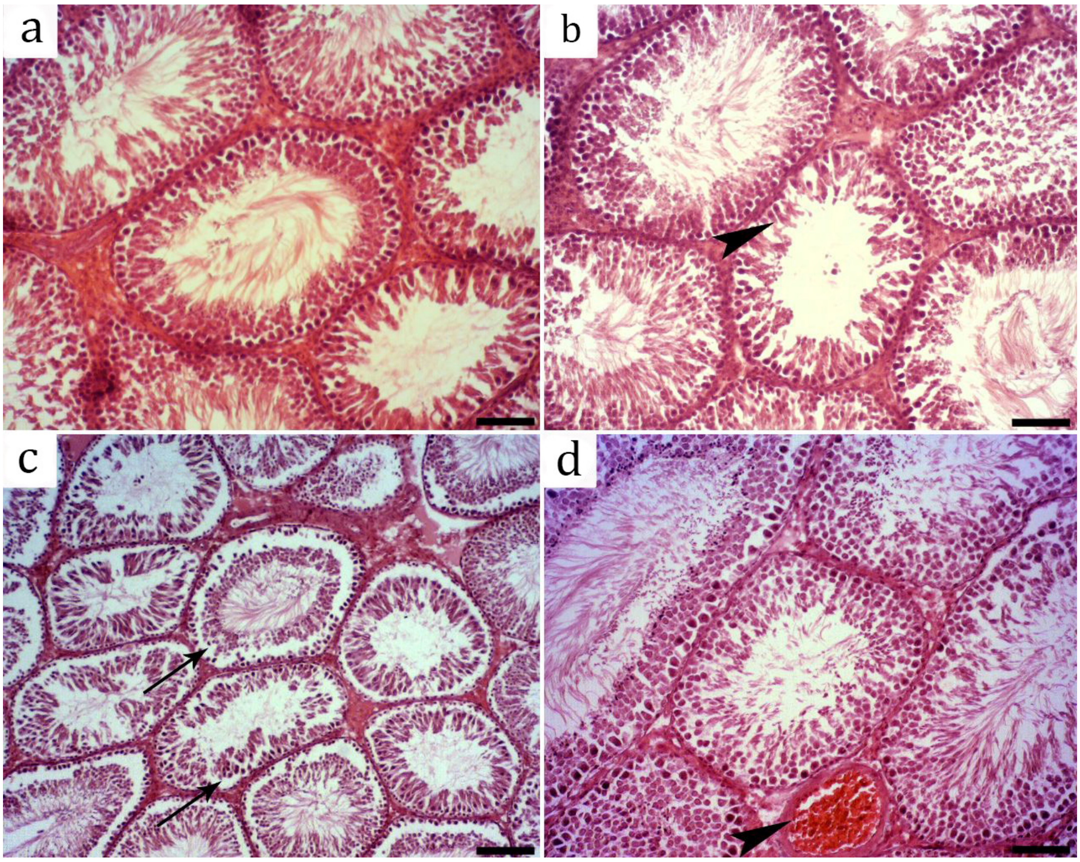

Figure 4. Microscopic features of rat testis tissue in different groups (HE staining). a) normal structure of the testis tissue in the control group (Scale bar = 100 μm); b) WF group, a decrease in height of germinal epithelium (arrowhead) (Scale bar = 100 μm); c) WF group, disorganization of germinal epithelium architecture and loss of cellular junction (arrows) (Scale bar = 250 μm); d) dilatation of blood vessel (arrowhead) (Scale bar = 100 μm)

is considered 5, 0.02, and 0.05 mg/m^3 for each metal, respectively. Therefore, it seems that due to the higher concentration of these elements, they probably played a key role in decreasing antioxidant enzymes. These findings were consistent with the results of a similar study, in which Fe had the highest mean concentration among metal fumes (5.43 mg/m^3), followed by Al (4.29 mg/m^3), Mn (2.37 mg/m^3), Cr (1.11 mg/m^3), and Cu (0.23 mg/m^3) (5). The results of another research demonstrated that the metals including Mn, Cr, Cu, and Fe had higher concentrations than other elements in the SMAW technique (10). In another experiment, which exactly used a similar metal piece, electrode, and welding technique, the results revealed that Fe particles had the highest concentration (3.045 mg/m^3) among all the sampled heavy metals (Al, Pb, Zn, and Ti) (31). Conversely, other investigations reported that among determined elements Cr had the highest concentration level (91.9 mg/m^3) and Fe had the lowest one (0.145 mg/m^3) according to the different material of workpiece, electrode, and type of welding technique (32).

The metal size distribution in Table 3 indicates that stage D had the highest weight of sampled particles (3.887 mg), suggesting that the most prevalent fume particles were those measuring between 0.25 to 0.5 micrometers. In a similar study, researchers used a cascade impactor to determine the size distribution of metal particles. They reported the highest concentration for plate D with a cut point of 0.25 µm, which is consistent with the results of the present study (31).

The results of extended research demonstrated that welders had higher levels of reproductive hormones, FSH and LH, and lower levels of testosterone and SOD antioxidant activity compared to the control group. Oxidative stress is associated with increased production of free radicals in the body and a decrease in antioxidant capacity, which can cause damage to cells and tissues (30). Oxidative stress has been linked to decreased sperm quality, quantity, and fertility in humans. The documents have shown that oxidative stress induced by Cr compounds in somatic and germ cells can lead to apoptosis and impair fertility potential (33). Histopathological changes of testicular tissue in Figure 4 are consistent with the results of another study that investigated the testes toxicity in rats exposed to lead. In this research, histological analysis revealed abnormalities in the structure of the testicular tissue in the exposed animals, causing significant harm to the seminiferous tubules. In the lead-treated animals, there was a decrease in the seminiferous epithelium, along with an empty center containing a reduced number of luminal spermatozoa (18). Previous studies reported that inhalation of WFs and subsequent reduction in

Testosterone levels could be a potential cause of infertility (28,34). Overall, these studies highlight the detrimental impact of heavy metal exposure on male reproductive health and emphasize the need for further investigations.

Limitations

Further research is needed to address the limitations of this study, including increasing the sample size, involving female volunteers, and exploring the effects of antioxidant supplements. This study was a laboratory pilot project and the conditions of conducting this category of research are different from the real conditions of the workplace, so it is suggested to establish similar studies in the real environment in the future. Additionally, determining the periodic decrease in antioxidant enzymes leads to assessing a regression model for understanding the relationship between exposure to WFs and oxidative stress.

Conclusion

In conclusion, this study found that exposure to WFs led to a decrease in GPX and SOD as antioxidant enzymes and an increase in oxidative damage in testicular tissue, which could result in male reproductive disorders. To protect the health of employees, medical professionals in the workplace need to implement safety measures such as installing proper ventilation systems, minimizing exposure to harmful substances, conducting regular medical checkups, monitoring levels of heavy metals in the body, providing respiratory protection to welders, and administration of antioxidants.

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Authors' contributions

Conceptualization: Sara Karimi Zeverdegani.
Data curation: Mina Janghorban.
Methodology: Masoud Rismanchian, Sara Karimi Zeverdegani.
Biochemical analysis: Adel Mohammadalipour.
Pathological analysis: Mohammad Hashemnia.
Writing – review & editing: Mina Janghorban, Masoud Rismanchian, Adel Mohammadalipour, Mohammad Hashemnia, Sara Karimi Zeverdegani.

Competing interests

The authors declare that they had no conflict of interests.

Ethical issues

The entire process of maintaining and studying animals was conducted following accepted protocols for laboratory animal testing recommended by the Committee on Ethics in Animal Experimentation at Isfahan University of Medical Sciences (Ethical code: IR.MUI.RESEARCH.REC.1400.455). Safety procedures and ethical guidelines were contemplated throughout the experimental period.

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