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Investigating the Impact of Carbon Nanotube Nanoparticle Exposure on Testicular Oxidative Stress and Histopathological Changes in *Swiss albino* Mice

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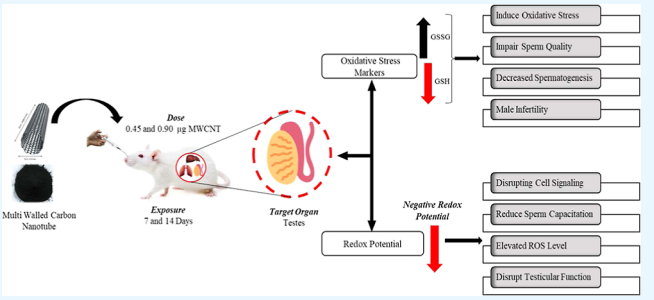
ABSTRACT: Carbon nanotubes (CNTs) possess remarkable properties that make them valuable for various industrial applications. However, concerns have arisen regarding their potential adverse health effects, particularly in occupational settings. The main aim of this research was to examine the effects of short-term exposure to multiwalled carbon nanotube nanoparticles (MWCNT-NPs) on testicular oxidative stress in *Swiss albino* mice, taking into account various factors such as dosage, duration of exposure, and particle size of MWCNT-NP. In this study, 20 mice were used and placed into six different groups randomly. Four of these groups comprised four repetitions each, while the two groups served as the vehicle control with two repetitions each. The experimental groups received MWCNT-NP treatment, whereas the control group remained untreated. The mice in the experimental groups were exposed to MWCNT-NP for either 7 days or 14 days. Through oral administration, the MWCNT-NP solution was introduced at two distinct dosages: 0.45 and 0.90 μg, whereas the control group was subjected to distilled water rather than the MWCNT-NP solution. The investigation evaluated primary oxidative balance indicators—glutathione (GSH) and glutathione disulfide (GSSG)—in response to MWCNT-NP exposure. Significantly, a noticeable reduction in GSH levels and a concurrent increase in GSSG concentrations were observed in comparison to the control group. To better understand and explore the assessment of the redox status, the Nernst equation was used to calculate the redox potential. Intriguingly, the calculated redox potential exhibited a negative value, signifying an imbalance in the oxidative state in the testes. These findings suggest that short-term exposure to MWCNT-NP can lead to the initiation of testicular oxidative stress and may disrupt the male reproductive system. This is evident from the alterations observed in the levels of GSH and GSSG, as well as the negative redox potential. The research offers significant insights into the reproductive effects of exposure to MWCNTs and emphasizes the necessity of assessing oxidative stress in nanomaterial toxicity studies.



1. INTRODUCTION

The rapidly progressing field of nanotechnology holds significant promise for influencing the economy, society, and the environment. However, prior to widespread adoption, it is essential to conduct thorough assessments of the possible health and environmental consequences linked to nanomaterials. Among these materials, carbon nanotubes (CNTs), a class of carbon-based nanomaterials, have gained considerable attention and practical use, particularly in the field of biomedicine. To optimize their applicability in biomedicine, CNTs have been modified with polymers, proteins, nucleic acids, and lipids.[1] While nanoparticles (NPs) hold substantial potential in the medical domain, it is worth noting that the characteristics that render them promising for various applications can also contribute to their potential toxicity within biological systems. Factors such as NP size, shape, and chemical composition play a critical role and must be carefully considered during their production.[2] Issues are being raised regarding how interactions with cellular networks, the endocytic pathway, and the process of absorption might lead to cytotoxicity and disturbances in equilibrium within cells. The size of NPs plays a pivotal role in their interaction with biological systems, and it is intricately tied to their potential

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lethal effects.[3] Significantly, smaller NPs possess a larger surface area in relation to their mass, which gives them the capability to adsorb a higher quantity of chemical molecules.

CNTs are nanomaterials characterized by their one-dimensional structure, comprised primarily of single-walled (SWCNTs) and multiwalled (MWCNTs). SWCNTs have micrometer sizes, with diameters ranging from 0.40 to 2.00 nm, while MWCNTs have larger dimensions, with diameters ranging from 60 to 100 nm.[4] Human exposure to CNTs can occur unintentionally in industrial settings or intentionally when CNTs are utilized for therapeutic and diagnostic purposes in treating human diseases.[5] Owing to their smaller dimensions compared to cellular organelles, CNTs can permeate cells and organelles during production, therapeutic usage, or diagnostic applications, potentially causing toxicity. As a result, understanding the potential cellular toxicity of CNTs is crucial.[6] Research concerning the potential risks associated with CNTs has mainly focused on inhalation and skin contact, as these are the possible routes of exposure, but where CNTs are utilized in various applications, the potential for oral ingestion of CNTs among workers cannot be dismissed.[7] Despite inhalation being a primary route of exposure to consider, accidental ingestion can also occur due to the nature of the work environment. Industrial workers often handle CNT-containing materials and may inadvertently transfer these nanomaterials onto their hands. In the absence of stringent hygiene practices or adequate personal protective equipment, there is a tangible risk of CNTs being transferred from contaminated hands to the mouth during routine activities such as eating or drinking.[8] As the health implications of CNT exposure continue to be a subject of research, recognizing the plausibility of oral ingestion underscores the importance of robust safety protocols, proper training, and effective risk management strategies in industry to safeguard the well-being of workers in this evolving technological landscape. In order to evaluate the impacts, toxicological investigations on CNTs have been conducted both in vivo and in vitro. These studies have revealed that human exposure to CNTs can lead to genetic damage, apoptosis, impaired cell function, heightened membrane disruption, reduced cell adhesion, and induced oxidative stress.[9]

Oxidative stress, as originally conceptualized by Sies, signifies a significant imbalance between oxidative and antioxidant activities within cells. This imbalance, with pro-oxidants holding the upper hand, can potentially lead to oxidative damage and harm. While a certain level of oxidative stress is essential for cellular signaling and regulation, excessive levels can result in cellular damage. NPs have a well-documented propensity to induce oxidative stress within cells by disrupting the normal functioning of various organelles, including mitochondria, peroxisomes, lysosomes, and the Golgi apparatus. This disturbance culminates in the excessive production of reactive oxygen species (ROS). Consequently, the assessment of NP toxicity relies significantly on the use of biomarkers for oxidative stress. Significantly, oxidative stress induced by NPs can be neatly classified into two distinct types, each uniquely propelled by specific mechanisms. The first type, often termed primary oxidative stress, involves the direct initiation of oxidative stress through ROS generated on the surfaces of NPs. In contrast, the second mechanism, known as secondary oxidative stress, occurs when exposure to NPs triggers the generation of ROS as a result of mitochondrial dysfunction. CNTs pose particular challenges when it comes to their elimination from the body, increasing the risk of their accumulation in various organs. Some studies suggest that organs such as the spleen, kidneys, testes, and lungs are particularly vulnerable to oxidative stress induced by free radicals.[10]

The research objectives of this study aim to investigate the intricate dose-time relationship between exposure to MWCNT-NPs and its consequential effects on oxidative stress levels within the testes of *Swiss albino* mice. This research seeks to systematically uncover how varying doses and durations of MWCNT-NP exposure influence the extent of oxidative stress in this pivotal reproductive organ. The significance of this research is underscored by its direct implications for reproductive health. Understanding how MWCNT-NP exposure influences testicular oxidative stress and histopathology, which create an intimate link between testicular health and male fertility. The findings have the potential to inform risk assessment for individuals exposed to MWCNT-NP in various occupational or environmental contexts, contributing to the establishment of safety guidelines and exposure limits. Furthermore, the research's novelty lies in its exploration of the dynamic dose-time relationship, a crucial aspect of NP toxicity, and its focused investigation into the specific impact of MWCNT-NP on the testes. Ultimately, this study not only addresses immediate health concerns but also advances our understanding of nanotoxicology by uncovering mechanisms that may have broader implications for managing oxidative stress induced by various nanomaterials.

2. MATERIALS AND METHODS

2.1. Preparation of Multiwalled Carbon Nanotube Nanoparticles. MWCNT-NPs were purchased from Sigma-Aldrich (CAS #308068-56-6). MWCNT-NP was a black, dry powder with >98% carbon. The average mean diameter of MWCNT-NP was 50−90 nm, and its length was > 6.5 μm according to the manufacturer. The size and morphology of NPs were characterized by a scanning electron microscope (SEM).

2.2. Animal Specifications. Twenty adult male *S. albino* mice (7−8 weeks old) were utilized in this study, in which mice were kept in cages (n = 4/cage and n = 2 control/cage) in a typical laboratory setting with temperatures between 20 and 24 °C. The mice ranged in body weight from 30 to 37.5 g. Before the trial began, the animals were given a day to acclimatize and were given unlimited access to supplemental food and water.

2.3. Experimental Design. The study consisted of four distinct groups, each comprising four mice, for a total of 16 mice under the treatment. Among these groups were two vehicle groups, each containing two mice. The experimental design also encompassed two different time points for exposure, specifically 7 and 14 days. In addition, two varying doses of exposure were employed on the basis of LD_{50} 1.8 μg, equivalent to 25% of LD_{50} considered as MWCNT-NP low dose (MWC-L) and 50% of LD_{50} considered as MWCNT-NP high dose (MWC-H). The first group received treatment with a MWC-L dose (0.45 μg) for 7 days, the second group received the same treatment for 14 days of exposure, and the third group served as the vehicle control with no treatment. The fourth and fifth groups were exposed to the MWC-H dose (0.90 μg) for 7 and 14 days, respectively, and the sixth group remained as the vehicle control with no treatment. All treatments were administered through oral administration, as

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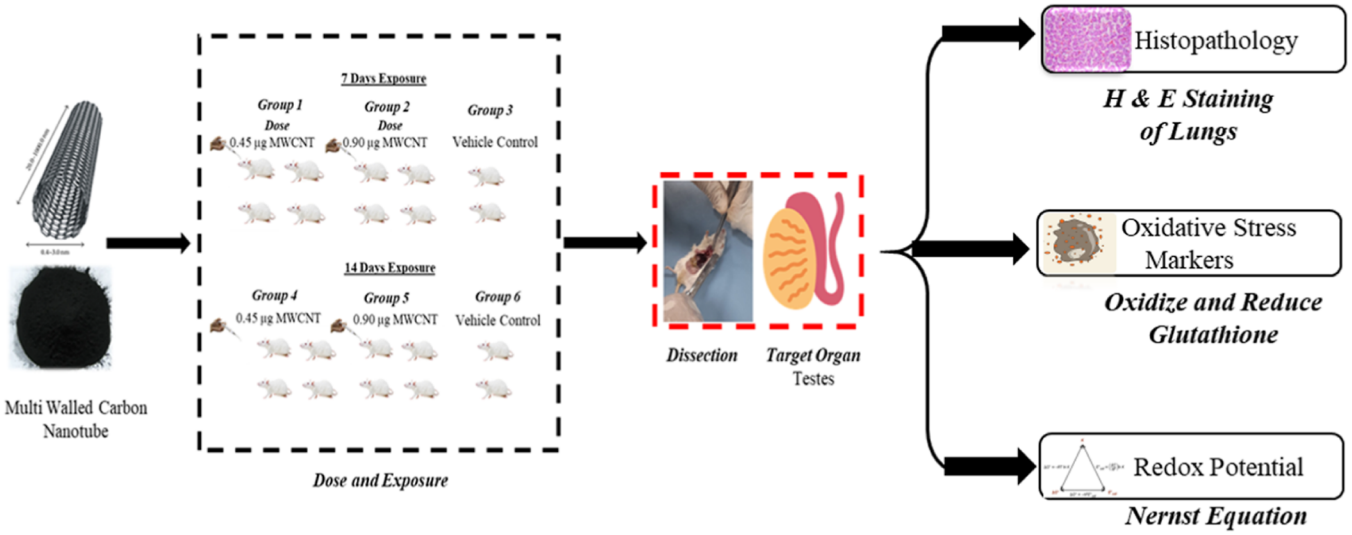

Figure 1. Schematic diagram for representing the dose administration of MWCNT-NP in male *S. albino* mice for 7 and 14 days of exposure. Targeted organ was obtained after 7 and 14 days to determine the oxidative stress markers and histological evaluation.

shown in Figure 1. This comprehensive experimental design enabled the assessment of the effects of MWCNT-NP at different doses and exposure durations, utilizing vehicle groups as essential controls to contextualize the observed outcomes.

2.4. Dose Preparation. The dispersion of MWCNT-NP was achieved using an ultrasonic liquid processor at 4 °C and 30% amplitude, with pulse readings of 1 s on and 1 s off, for a total of 30 min. Prior to dispersion, MWCNT-NP was suspended and sonicated in a sterile saline solution (0.9% NaCl) containing 1% tween-80. The control group was administered only distilled water as their treatment. Before the oral administration, the suspensions of MWCNT-NP underwent a 15 min sonication process to prevent aggregation. These MWCNT-NP suspensions were then administered daily to mice through gavage for durations of 7 and 14 consecutive days. The selection of dosage and the method of administering MWCNT-NP were informed by earlier studies.[1,11]

2.5. Samples Collection. At the end of the study (days 7 and 14), mice were exsanguinated, and blood was taken from the heart and collected in sterile, closed blood collection systems, 4.5 mL, which were left for 30 min upright. After dissection, the testes were immediately removed, cleaned with PBS to remove any blood, and stored in 10% formalin for histological analysis.

2.6. Histopathology. Tissues were fixed in 10% buffered formalin for 24 h and subsequently processed using an automated tissue processor. The processing involved gradual dehydration through ascending concentrations of ethyl alcohol (ranging from 80 to 99.8%), followed by clearing with xylene and ultimately embedding in paraffin. For histological evaluation, 5 μm-thick sections were obtained from the paraffin-embedded tissue using a rotary microtome (LEICA RM 2125). The sections were affixed onto slides, subjected to staining with hematoxylin and eosin, and subsequently analyzed by using a digital optical microscope. This examination aimed to capture and record any discernible histopathological changes that were observed.

2.7. Assessment of GSH and GSSG Levels. To quantify the levels of reduced glutathione (GSH) and glutathione disulfide (GSSG), high-performance liquid chromatography (HPLC) analysis was conducted on deproteinized testicular tissue samples. Initially, 200 mg of testicular tissue was homogenized in 250 mM Tris−HCl buffer (pH = 7.4) at 4 °C. Following this, 500 μL of 50% w/v trichloroacetic acid (TCA) solution, also kept at 4 °C, were added to each homogenized sample. The specimens were gently mixed and subsequently subjected to centrifugation at 15,000*g* for 15 min at 4 °C. To 1 mL of the supernatant, a mixture of 2 M NaOH and 2 M $NaHCO_3$ (400 μL) was slowly added until gas production ceased. Next, iodoacetic acid (100 μL at a concentration of 1.5% w/v in water) was introduced, and the samples were incubated for 60 min at 4 °C in the absence of light. After incubation, 500 μL of 1.5% w/v 1,5-dinitrofluorobenzene (DNFB) in absolute ethanol was added, and the samples were incubated in darkness for 48 h at 25 °C. Finally, 25 μL of these prepared samples was injected into the HPLC system, with the UV detector set at a wavelength of λ = 252 nm for analysis, as a similar methodology was previously reported by ref 12.

2.8. Determination of Redox Potential. The redox potentials (E_h) of the GSH redox couple were calculated by applying the Nernst equation to the experimentally determined concentrations of GSH and GSSG in eq 1. This computation followed the methodology described by ref 13

$$E_h = E^0 - (2.303RT/nF)\log([GSH]^2/[GSSG]) \quad (1)$$

This calculation involves E^0, which represents the standard potential for the GSH/GSSG redox couple (−288 mV at pH 7 in tissue), R denoting the gas constant (8.314 J K^{-1} mol^{-1}), T representing the absolute temperature, F standing for Faraday's constant (9.6485 × 104 C mol^{-1}), and n indicating the number of electrons transferred. The Nernst equation was applied under T = 25 °C (298.15 K), with the conversion factor of 2.303 used to convert ln to log10. More negative values indicate a heightened state of reduction, while more positive values signify the converse—a heightened state of oxidation.

2.9. Statistical Analysis. The gathered GSH and GSSG data were subjected to statistical analysis using analysis of variance (ANOVA) at a significance threshold of $p \leq 0.05$. Subsequent posthoc comparisons were conducted using the least significant difference (LSD = 0.05). Graphical representations incorporated alphabetical labels (e.g., a, b, and c) on

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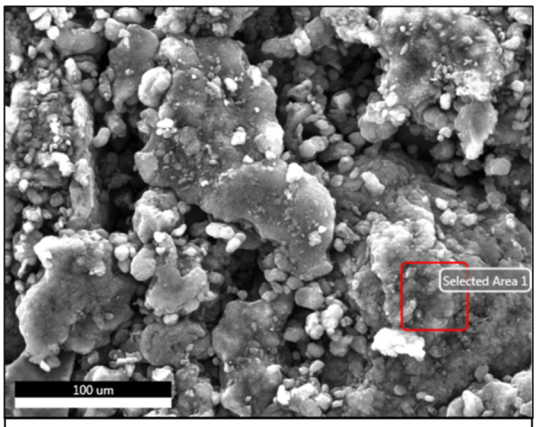
the bars, indicating significant differences across various dosages and exposure durations.

2.10. Institutional Ethical Statement. The research was conducted in adherence to established guidelines and received approval from the Ethical Committee of the Department of Environmental Sciences at Lahore College for Women University.

3. RESULTS AND DISCUSSION

3.1. Morphology and Shape of MWCNT-NP. The surface morphology and structural properties of MWCNT-NP were examined using SEM (FEI brand model Inspect S50) analysis. As shown in Figure 2, the results disclosed that the



Ezaf Smart Quant Results			
Elements	Weight%	Atomic%	Net Int.
C K	100	100	5069.19

Figure 2. Surface morphology of MWCNT-NP utilizing SEM analysis.

MWCNT-NP exhibits a spherical shape and tends to aggregate into larger clusters. Similar findings were also observed by ref 14. The average MWCNT-NP diameter was 50−90 nm with 100% carbon.

3.2. Histological Evaluation of Testes. The observed histological disruptions in the testes of mice exposed to MWCNT-NP underscore the potential reproductive toxicity associated with nanomaterial exposure. It emphasizes the importance of investigating the effects of different doses and durations of exposure to better understand the dose−response relationship and potential long-term consequences for male reproductive health. Figure 3 provides informative visual representations through hematoxylin and eosin (H&E)-stained testicular sections, showcasing the effects of exposure to varying doses (0.45 and 0.90 μg) of MWCNT-NP for 7 and 14 days. In contrast, Figure 3a,d depicts the normal histological appearance of testicular tissues in the control group after 7 and 14 days, respectively. Figure 3b illustrates mild degeneration of seminiferous tubules resulting from exposure to the lower dose (0.45 μg) of MWCNT-NP, while Figure 3c shows severe degeneration of seminiferous tubules with evident black aggregates of MWCNT-NP following 7 days of exposure. Similarly, Figure 3e demonstrates cell lining rupture and mild Leydig cell hyperplasia, while Figure 3f exhibits Leydig cell hyperplasia after 14 days of exposure. These visual depictions provide valuable insights into the direct interactions of MWCNT-NP with testicular tissues. The subsequent histopathological evaluation underscores significant alterations induced by differing doses and durations of MWCNT-NP exposure compared to the control group. These modifications encompass a spectrum of histopathological changes, including instances of seminiferous tubule degeneration and Leydig cell hyperplasia, all of which have the potential to exert adverse effects on male reproductive health.

The observed reduction in reduced GSH and concurrent increase in GSSG levels within the testes of *S. albino* mice exposed to MWCNTs may have significant implications for testicular histopathology. Histological examination provides valuable insights into the structural alterations that can occur as a result of these changes in the GSH system. The observed disruptions in testicular histology were attributed to several factors associated with MWCNT-NP exposure. First, MWCNT-NP is known to induce oxidative stress in testicular tissues. This oxidative stress can lead to the peroxidation of lipids, protein damage, and DNA fragmentation within testicular cells. These cellular alterations can manifest histologically as changes in the architecture of the testicular tissue.[15]

The integrity of seminiferous tubules is pivotal for sperm production and exceptionally susceptible to oxidative harm. Disturbances in these tubules can lead to the loss of germ cells, disarray in the epithelium, and compromised spermatogenesis, which manifest histologically as altered tubular structure, diminished germ cell populations, and cell sloughing into the tubular lumen.[16] Moreover, oxidative stress has the capacity to trigger an inflammatory response within the testes, characterized by the infiltration of immune cells, notably macrophages and neutrophils.[17] This immune reaction can exacerbate tissue damage and further contribute to irregularities in the histological patterns. The observed disruptions in testicular histopathology among mice exposed to MWCNT-NP underscore the potential hazards associated with nanomaterial exposure in terms of reproductive toxicity. This underscores the critical necessity to delve into the effects of various dosages and exposure durations, facilitating a comprehensive grasp of the dose−response relationship and potential long-lasting implications for male reproductive health.

3.3. Oxidative Stress Markers. *3.3.1. Reduced Glutathione.* The GSH system serves as a pivotal defense mechanism, guarding against the harmful impacts of xenobiotics and free radicals within the testes. To assess the organism's overall antioxidant protection status, the study scrutinized the levels of both reduced GSH and GSSG. The results uncovered significant variations in mice exposed to higher doses (0.90 μg) of MWCNT-NP for 7 and 14 days, showing substantial reductions of 22 and 49% in GSH concentration, respectively. On the contrary, exposure to lower MWCNT-NP doses (0.45 μg) for the same time duration resulted in noteworthy reductions of 12 and 35% in GSH levels in comparison to the control group, as shown in Figure 4. This decline in GSH content triggered oxidative stress within the testicular tissues, disturbing the delicate redox balance and shifting it toward an oxidizing environment. The subsequent oxidative imbalance not only underscores potential testicular damage but also raises concerns about adverse impacts on sperm quality and reproductive health in exposed individuals.

The observed significant reduction in GSH levels in the testes following MWCNT-NP exposure indicates the induction

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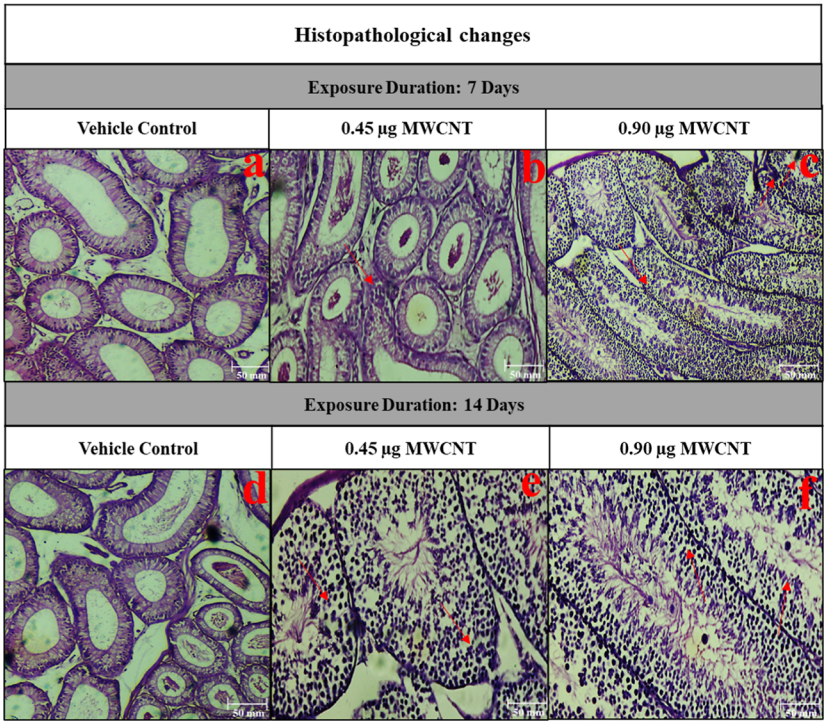

Figure 3. Histological changes in mice testes after oral administration of MWCNT-NP. (a,d) Normal tissue of vehicle control mice of 7 and 14 days of exposure. After 7 days of exposure: (b) the treated group with MWCNT-NP low dose (0.45 μg) has mild degeneration of seminiferous tubules and (c) treated group with MWCNT-NP high dose (0.90 μg) shows severe degeneration of seminiferous tubules and the presence of black aggregates of MWCNT-NP. After 14 days of exposure: (e) the treated group with MWCNT-NP low dose (0.45 μg) ruptured cell lining and mild ledge cell hyperplasia seen and (f) the treated group with MWCNT-NP high dose (0.90 μg) showed Leydig cell hyperplasia.

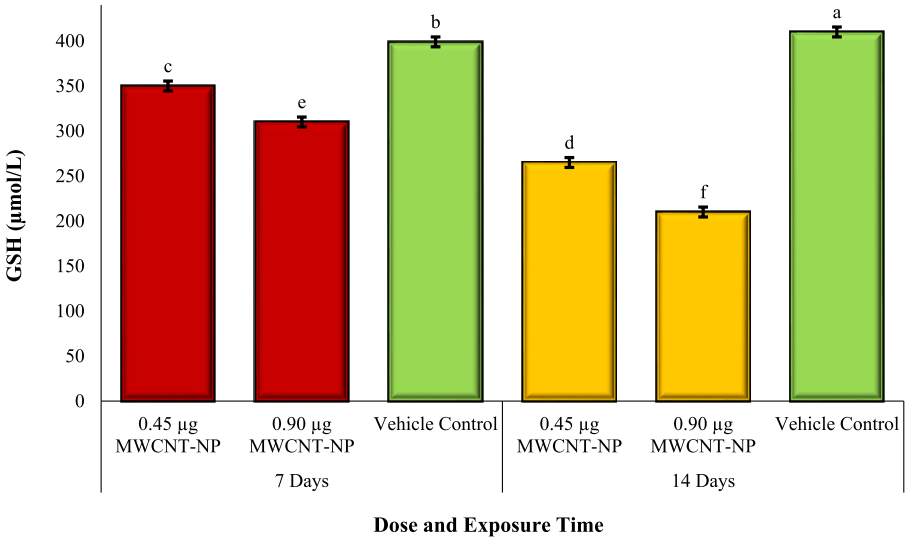

Figure 4. Reduced GSH in the testicular tissues of *S. albino* mice. GSH values were experimentally determined. All values represent the mean standard deviation of $n = 6$ groups, with four animals pooled in each group. Letters (e.g., a, b, c, d, e, and f) show that values are significantly different.

of oxidative stress within the testicular microenvironment. This outcome is consistent with existing literature,[18−20] which underscores the potential of CNTs to generate ROS, thereby triggering oxidative stress responses. Consequently, our study highlights the susceptibility of testicular tissues to oxidative damage, which could have far-reaching implications for testicular health and male reproductive outcomes.

The testes, by virtue of their high metabolic activity and the continuous production of sperm, are particularly vulnerable to oxidative stress-induced damage.[21,22] Oxidative stress to the testicular microenvironment can adversely affect sperm quality and fertility, as reported in previous studies.[23−25] Our findings, therefore, raise concerns regarding the potential compromise of testicular function and the quality of sperm produced in response to MWCNT-NP exposure. Given that MWCNT-NP-induced oxidative stress has been linked to detrimental effects on various biological systems, including DNA damage and

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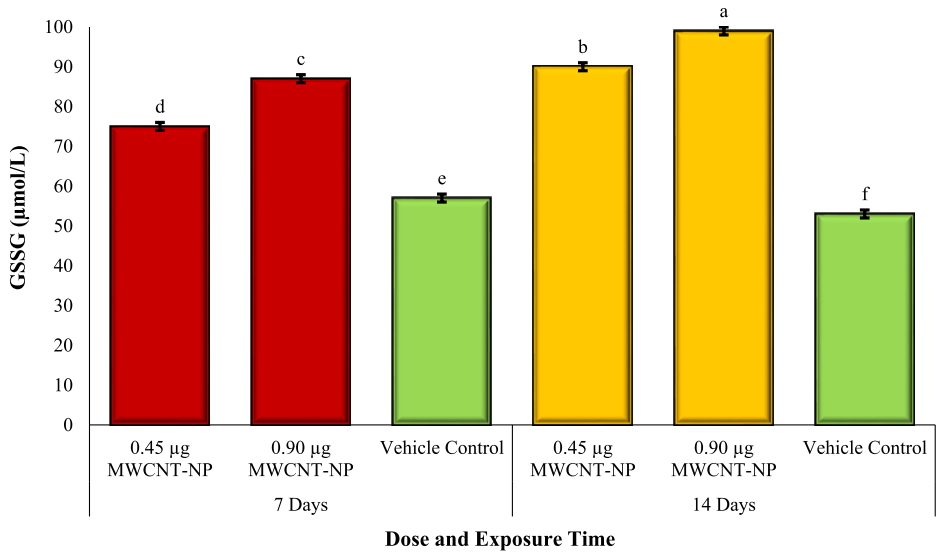

Figure 5. Oxidized GSSG in the testicular tissues of *S. albino* mice. GSSG values were experimentally determined. All values represent the mean standard deviation of $n = 6$ groups, with four animals pooled in each group. Letters (e.g., a, b, c, d, e, and f) show that values are significantly different.

protein oxidation, it is plausible that similar mechanisms may be at play in the testes.[26,27]

Additionally, it is imperative to conduct a comprehensive exploration into the role played by the generation of ROS induced by MWCNT-NP in the depletion of reduced GSH levels. This is particularly crucial, as the excessive production of ROS can actively deplete the GSH reservoir while it acts to neutralize these highly reactive species. Importantly, this phenomenon has garnered supporting evidence from previous research, with the study by ref 10 yielding consistent findings. This collective body of evidence underscores the robustness and relevance of understanding the interplay between MWCNT-NP-induced ROS and GSH depletion, contributing to a deeper comprehension of the oxidative stress mechanisms induced by nanomaterial exposure.

3.3.2. Oxidized Glutathione. Significant and statistically meaningful differences in GSSG levels were observed in mice exposed to MWCNT-NP. Specifically, when exposed to a higher dose concentration of 0.90 μg for durations of 7 and 14 days, there were remarkable increases of 53 and 87% in GSSG concentration, respectively. In contrast, exposure to MWCNT-NP at a lower dose concentration of 0.45 μg for the same 7 and 14 day durations resulted in substantial increases of 32 and 70% in GSSG levels compared to the control group treated with the vehicle control, as shown in Figure 5. These findings underscore the significant impact of MWCNT-NP exposure on the accumulation of GSSG in the testicular tissues of mice.

Our investigation into the effects of MWCNT-NP exposure on testicular GSSG levels in *S. albino* mice has yielded intriguing results. We observed a significant increase in GSSG levels within the testicular tissue following exposure to MWCNT-NP. This elevation in GSSG, the oxidized form of glutathione, reflects a perturbation in the redox balance of the testicular microenvironment and raises several important implications for testicular health and male reproductive outcomes, as similarly reported by ref 28.

The testes are a highly dynamic and metabolically active organ responsible for the production of spermatozoa, a process that relies on a delicate redox balance to ensure the integrity of sperm DNA and cellular components.[25] The observed increase in GSSG levels suggests the induction of oxidative stress within the testicular milieu. This oxidative stress is often attributed to the generation of ROS by MWCNT-NP, which can overwhelm the endogenous antioxidant defense systems, leading to the oxidation of GSH to GSSG.[29] Hence, this disturbance in the equilibrium of redox homeostasis can carry substantial implications for testicular function and overall male reproductive health, a trend corroborated by previous studies of various nanomaterials. Notably, research by refs 30−32 has consistently reported similar outcomes, emphasizing the relevance and broad applicability of these findings in the context of nanomaterial-induced effects on testicular physiology and male reproductive well-being.

Elevated oxidative stress within the testicular microenvironment can adversely affect sperm quality and function. The increased GSSG levels may lead to increased cellular damage, including lipid peroxidation, protein oxidation, and DNA fragmentation, all of which can compromise sperm motility, viability, and fertilization capacity. The repercussions extend beyond sperm quality, as oxidative stress-induced damage to the supporting cells and structures within the testes may disrupt the overall process of spermatogenesis, potentially leading to reduced sperm production.[33−36] These previous studies support our results, indicating that different nanomaterials have also shown similar effects, underscoring the consistency and broader implications of these findings in the context of nanomaterial-induced testicular oxidative stress and its impact on male reproductive health.

Elevating GSSG levels within the testes in response to MWCNT-NP involves a complex interplay of mechanisms, prominently featuring the MWCNT-NP-induced generation of ROS. When MWCNT-NP come into contact with biological systems, they initiate ROS production, including superoxide radicals ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), which are known to induce oxidative stress within cells. This oxidative stress disrupts the delicate balance between ROS generation and the body's antioxidant defenses.[10] Consequently, the primary antioxidant, reduced GSH, is called upon to counteract ROS, leading to its depletion as it reacts with these highly reactive molecules. This depletion drives a shift in

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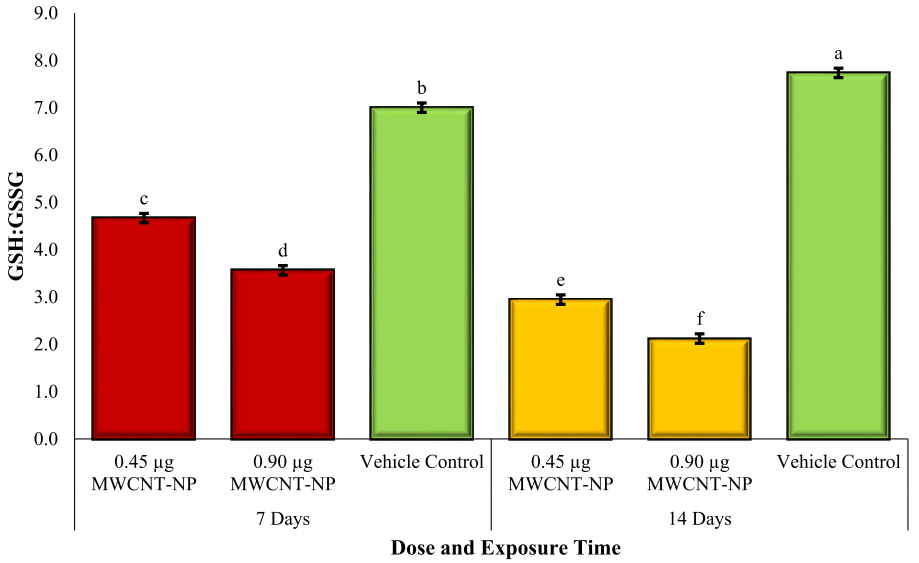

Figure 6. GSH/GSSG ratios in the testicular tissues of *S. albino* mice. GSH/GSSG values were experimentally determined. All values represent the mean standard deviation of $n = 6$ groups, with four animals pooled in each group. Letters (e.g., a, b, c, d, e, and f) show that values are significantly different.

Table 1. Oxidant Status (GSH and GSSG) after 7 and 14 Days of Exposure to Low and High Doses of MWCNT-NP[a]

sr. no.	parameters	7 days exposure			14 days exposure		
		MWC-L 0.45 µg	MWC-H 0.90 µg	control	MWC-L 0.45 µg	MWC-H 0.90 µg	control
1	GSH	350	310	399	265	210	410
2	GSSG	75	87	57	90	99	53
3	GSH/GSSG	4.7	3.6	7.0	2.9	2.1	7.7

[a]Results are presented as the mean values of GSH and GSSG expressed in µmol/L.

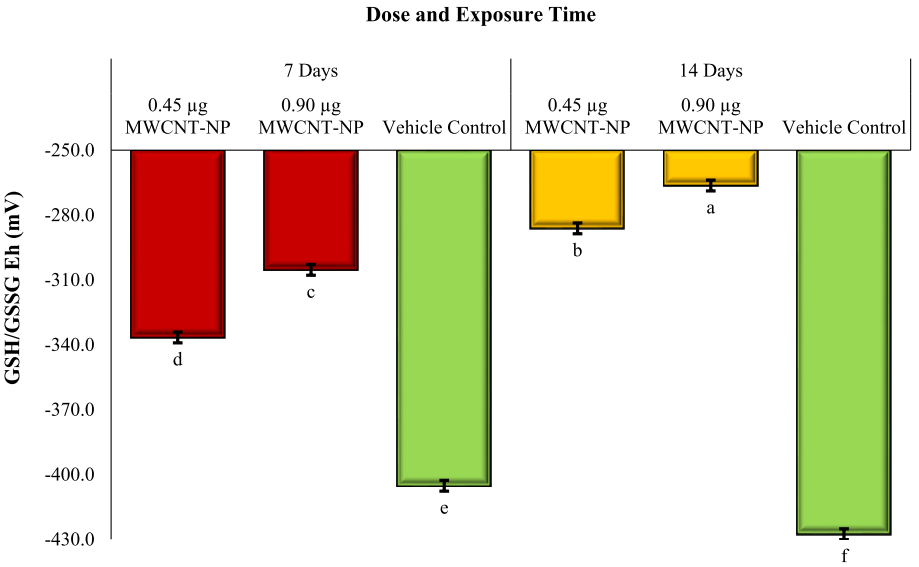

Figure 7. GSH redox potentials in the testicular tissues of *S. albino* mice. Redox values were determined using the Nernst equation and experimentally measured GSH and GSSG concentrations. Letters (e.g., a, b, c, d, e, and f) show that values are significantly different.

the redox state of the testes toward GSSG, the oxidized form of GSH. The accumulation of GSSG thus mirrors the increased demand for GSH in response to oxidative stress induced by MWCNT-NP, ultimately disrupting the redox homeostasis of testicular cells. Prolonged exposure to MWCNT-NP-induced oxidative stress and elevated GSSG levels may result in cellular damage and dysfunction within the testes, potentially affecting critical functions such as spermatogenesis and hormone production.[37,38]

3.3.3. GSH/GSSG Ratio. The GSH/GSSG ratio exhibited significant and noteworthy reductions in mice testes exposed to higher dose concentrations (0.90 µg) of MWCNT-NP over 7 and 14 days, with reductions of 49 and 73%, respectively. Similarly, exposure to lower MWCNT-NP doses (0.45 µg) for

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7 and 14 days resulted in notable decreases of 33 and 62% in the GSH/GSSG ratio compared to the healthy control group, as shown in Figure 6 and Table 1. These reductions can be attributed to MWCNT-NP-induced oxidative stress, inflammation, disruption of cellular redox homeostasis, potential direct interactions between MWCNT-NP and GSH, and impaired GSH biosynthesis, collectively highlighting the potential complications in the testicular tissues of exposed mice.

The exposure of *S. albino* mice to MWCNT-NP has been associated with a noteworthy reduction in the GSH/GSSG ratio within the testes, which underscores the intricate interplay between these vital redox-active molecules and nanomaterial-induced oxidative stress. MWCNT-NP possesses the capacity to generate ROS upon interaction with biological systems, initiating a cascade of events within the testicular tissue. As ROS levels surge, the cellular antioxidant defense mechanisms are activated, with GSH serving as a key component in neutralizing these detrimental molecules.[39] Consequently, GSH molecules are gradually depleted as they engage in ROS detoxification, resulting in a decline in the level of the GSH pool. The diminished GSH levels, in conjunction with the sustained ROS onslaught, disrupt the balance of the GSH/GSSG ratio, favoring the accumulation of GSSG. This shift toward a more oxidized state of GSH reflects the oxidative stress imposed by MWCNT-NP exposure.[40] Such an alteration in the redox status can have profound implications for testicular function and health, as GSH is instrumental in maintaining the cellular redox homeostasis and protecting against oxidative damage.[41] Consequently, the reduction in the GSH/GSSG ratio observed in the testes of MWCNT-NP-exposed *S. albino* mice underscores the potential for nanomaterial-induced oxidative stress to perturb the delicate equilibrium of antioxidant defenses within this critical reproductive organ.

3.4. Redox Potential by the Nernst Equation. In the control group, the testicular tissues exhibited the most proreducing GSH/GSSG redox potential, with values of −405.3 mV after 7 days and −427.7 mV after 14 days. Following this, the high dose of 0.90 μg of MWCNT-NP for both 7 and 14 day exposures showed less negative potentials of −305.4 and −266.4 mV, respectively. The low dose of 0.45 μg of MWCNT-NP for 7 and 14 day exposures also displayed less negative potentials, measuring −336.6 and −286.2 mV, respectively, as shown in Figure 7. A negative redox potential due to dose-dependent exposure to MWCNT-NP in the testes of mice indicates a substantial disruption in the normal oxidative−reductive (redox) balance within the testicular tissue. Redox potential is a measure of the tendency of a biological system to undergo oxidation or reduction reactions, which are essential for maintaining cellular processes and homeostasis. The reduction in the negative redox potential ratio in the testes of *S. albino* mice following exposure to MWCNT-NP carries profound implications, not only for the redox balance but also for reproductive health and fertility.

The redox potential, mainly regulated by the balance between GSH and GSSG, stands as a crucial factor influencing the cellular redox status, and any disruption can carry significant implications for male fertility.

MWCNT-NP is well-established as a potent generator of ROS upon their interaction with biological systems. This redox imbalance has been previously elucidated in relation to male fertility by ref 42. The testes, being highly sensitive to oxidative stress, are particularly vulnerable to the detrimental effects of ROS, resulting in adverse outcomes such as DNA fragmentation, impaired sperm motility, and compromised sperm function, as noted in studies by refs 43 and 44. Additionally, the redox potential within the testicular microenvironment plays a pivotal role in governing various physiological processes critical to fertility, including spermatogenesis and hormone production, as highlighted in research by refs 45 and 46. Consequently, the reduction in the negative redox potential ratio has the potential to disrupt these essential processes, with possible consequences including impaired sperm production and function, hormonal imbalances, and ultimately compromise in male fertility.

4. CONCLUSIONS

In conclusion, this study investigated the dose-time relationship between MWCNT-NP exposure and its effects on testicular oxidative stress and histopathological transformations in *S. albino* mice. This study reveals significant insights into the potential health risks associated with nanomaterial exposure. The research highlighted the intricate interplay between varying doses and exposure durations of MWCNT-NP and their impact on testicular redox balance, emphasizing the vulnerability of the testes to oxidative stress. The observed histopathological alterations underscore the physical consequences of MWCNT-NP exposure, further supporting the notion that MWCNT-NP disrupts testicular morphology. The induction of oxidative stress and the concurrent accumulation of GSSG unveiled in this study align with previous research, affirming the consistency and broader applicability of these findings across nanomaterials. This study emphasizes the importance of comprehending the intricate connection between exposure to MWCNT-NP and the well-being of the testicular system. It offers valuable insights that can contribute to the protection of male reproductive health, particularly in light of the evolving landscape of nanotechnology and its expanding industrial applications.

■ ASSOCIATED CONTENT

Data Availability Statement

All the data is contained in the manuscript.

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Iqra Nasim: conceptualization, methodology, writing—original draft, validation, formal analysis, and investigation; **Nadia Ghani**: conceptualization, methodology, formal analysis, supervision, visualization, and writing-review and editing; **Rab Nawaz**: supervision, validation, visualization, and writing—review and editing; **Ali Irfan**: formal analysis, data curation, visualization, funding acquisition, and writing—review and editing; **Muhammad Arshad**: formal analysis, visualization, and writing—review and editing; **Maryam Nasim**: visualization, validation, and writing—review and editing; **Mohammad Raish**: conceptualization, data curation, formal analysis, and writing—review and editing; **Muhammad Atif Irshad**: visualization, investigation, and data curation; **Shazia Akram Ghumman**: data curation, methodology, and writing—review and editing; **Ajaz Ahmad**: validation, visualization, and writing—review and editing; and **Yousef A. Bin Jardan**: funding acquisition, project administration, formal analysis, and writing—review and editing.

Notes

The authors declare no competing financial interest.

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