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RESEARCH ARTICLE

Potency of Moringa Leaves Extract against Testicular Dysfunction Induced by Nanosilver and Sodium Arsenite in Mammalian Model

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ABSTRACT

The present study evaluated the potency of *Moringa oleifera* leaves extract against testicular dysfunction caused by chronic exposure to nanosilver and sodium arsenite. Sixty-three male rats were divided into seven groups (n=9) each. Group 1 served as the control. Animals of groups 2 and 3 were administered with low (2g/kg body weight) and high (3g/kg BW) dosages of Moringa extract, respectively. Groups 4 and 5 rats received combined low (100 mg/kg BW and 10.25mg/kg BW) and combined high doses (150 mg/kg BW and 16.4 mg/kg BW) of nanosilver and arsenite, respectively. Animals in groups 6 and 7 received combined (low and high) doses of nanosilver, arsenite, and Moringa extract daily and orally for 3 months, as previously described. Three animals from each group were dissected to collect blood and tissue samples at 4, 8 and 12 weeks. Exposure to nanosilver and arsenite at low and high doses resulted in decreased testis weight, a substantial decline in FSH and testosterone activity, but a considerable increase in LH levels. Histochemical analysis revealed reduced activities of antioxidant enzymes (GPx, GST, GSH, CAT and SOD) and elevated MDA levels. Histological examination presented vacuolation, hemorrhage, interstitial tissue edema, and seminiferous tubular deterioration. Administration of Moringa extract improved reproductive hormonal concentrations, reduced MDA, increased antioxidant enzymes activities, and partially mitigated histological alterations in a dose-dependent manner over three months of therapy. *Moringa oleifera* extract has proven effective as a detoxification strategy and serves as a natural, accessible intervention capable of alleviating the toxic effects of contaminants.

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INTRODUCTION

The testes are fundamental organs within the male reproductive system tasked with producing sperm and synthesizing steroid hormones. However, testes are susceptible to impairment from environmental pollutants, including heavy metals (Gautam *et al.,* 2024), ultrafine particles, and chemicals from different sources. These contaminants can damage the testicles by damaging sex cells or indirectly by destroying Sertoli and interstitial cells (Maciejewski *et al.,* 2022). Nanosilver (NS), also known as silver nanoparticles, is a structure with dimensions between 1 and 100 nm, and is recognized for its biomedical and antimicrobial properties. Nevertheless, nanosilver can exhibit toxicity by interacting with biological macromolecules and releasing toxic components, such as metal ions (Abbas *et al.,* 2024). Arsenic (As) and its derivatives, like sodium arsenite, are notorious and widespread ecological pollutants, originating from natural and anthropogenic sources. Due to its electrophilic nature, arsenic can bind to sulfhydryl groups on proteins, altering their function and activity (Ganie *et al.,* 2024).

These contaminants can pass into animals and humans through various ways like air, water and food chain besides dermal, subcutaneous and intraperitoneal routes (Jomova *et al.,* 2025; Wadhawan *et al.,* 2025). These pollutants can then penetrate the blood-testicular barrier, leading to harmful effects in animals, including DNA and cell membrane damage, cytotoxicity, suppression of the antioxidant enzyme system, inflammation, and oxidative stress (Naeem *et al.,* 2023; Santana *et al.,* 2023; Shakra *et*

al., 2024). Nanoparticles and heavy metals may cause testicular toxicity by disturbing the pituitary-testicular axis and creating a disparity in reproductive hormone levels, which can have adverse effects on the maturation of sexual organs, hinder germ cells' growth, and disturb testicular function by producing reactive oxygen species and inducing apoptosis (Samrot and Noel Richard Prakash, 2023; Fan *et al.,* 2024).

Native plants have become increasingly popular worldwide in recent years to treat various ailments because of their relative availability and negligible adverse effects. *Moringa oleifera* (MO) belongs to the Moringaceae family and has been used as food and medicine (Klimek-Szczykutowicz *et al.,* 2024). *M. oleifera* leaves are rich in riboflavin, protein, folic acid, Vitamins A, C, B, E, minerals, sterols, nicotinic acid, β-carotene, phenolic compounds, and are well known for their immune-stimulating and antioxidant properties (Barkat *et al.,* 2025). Moringa was suggested as a beneficial adjuvant to treatments as it significantly boosted antioxidant enzyme status and reduced organ damage caused by contaminants (Bashir *et al.*, 2024).

Although several studies have demonstrated the individual protective potential of *M. oleifera* extract against nanoparticles and heavy metals, few have investigated its efficacy against the combined toxicity of environmental toxicants, with limited insights into its long-term (chronic) synergistic effects on the reproductive parameters of mammalian models (Abbas *et al.,* 2024). Therefore, the goal of this study was to figure out how *M. oleifera's* antioxidant properties helped to protect male gonads against nanosilver and arsenite-induced testicular dysfunction via assessment of reproductive hormones status, oxidative stress parameters, and histopathological observations of the testicles.

MATERIALS AND METHODS

Chemicals: Sodium arsenite ($NaAsO_2$) and nanosilver (NS) powder with a diameter of 20nm without capping were purchased from reputable firms. Deionised water was used to prepare the arsenite solution and for the dispersion of AgNP, following the procedure outlined by Erhirhie *et al.* (2014). This involved vigorous vortexing, followed by sonication of the AgNP suspension for 5 minutes to prevent agglomeration. The doses of chemicals were prepared daily. The selection of low and high dosages in this study was based on previously published toxicological data and preliminary dose-response assessments, aiming to capture both sub-threshold (non-toxic or minimally toxic) and overtly toxic effects of the test chemicals.

Preparation of Moringa extract: The *M. oleifera* leaves were gathered, dried, homogenized into a fine powder, and packed into airtight bags. 40 and 60g of Moringa powder were separately mixed with 100mL of distilled water. Each mixture was kept on a hot plate magnetic stirrer for half an hour and then placed overnight at room temperature. Moringa leaves extract was then filtered and kept at 4°C and administered to rats according to the established protocol developed by Erhirhie *et al. (*2014).

Experimental design: Sixty-three Sprague Dawley rats, 5 weeks old, were categorized into seven groups. Each group has nine animals. The study was conducted in the animal facility of the Zoology Department, Lahore College for Women University, Lahore, Pakistan, under standard housing conditions. Group 1 was kept as control. The rats in groups 2 and 3 were administered 2g/kg BW and 3g/kg BW of Moringa leaves extract, respectively. Group 4 animals received low doses of nanosilver (100mg/kg BW) and arsenite (10.25mg/kg BW). However, animals in group 5 were given high doses of nanosilver (150 mg/kg BW) and arsenite (16.4mg/kg BW). The groups 6 and 7 animals received low and high dosages of nanosilver, arsenite, and Moringa extract, as defined previously. The doses were administered orally to animals and given daily for 12 weeks (Fig.1).

Blood sampling and hormone estimation: Blood samples were collected in vials without EDTA through cardiac puncture. After being kept for 30 minutes at room temperature, the blood samples were centrifuged for 15 minutes at 4000 rpm to collect serum (Shahin *et al.,* 2018). The serum luteinizing hormone (LH); Ref no. ER1123, follicle-stimulating hormone (FSH); Ref no. ER0960, and testosterone (Ref no. ER1462) activity were measured using the Rat ELISA Kits following the guidelines of the Fine Test manufacturer in China. The absorbance of hormones for ELISA was measured using a Medical Pro reader-96 (Germany) ELISA plate reader.

Organ collection: Three rats were chloroformed and dissected after 4, 8, and 12 weeks of the experiment. Testes were carefully removed, immediately washed with phosphate buffer saline (PBS), and weighed separately. These tissue samples were then wrapped in aluminum foil and frozen at -40°C until used. The right testicular tissue (200mg) was processed in 1mL 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) extract buffer. Tissue samples were centrifuged at 12000rpm for 15min at 4°C to collect supernatant for histochemical analysis.

Estimation of antioxidant parameters: Lipid peroxidation marker, malonaldehyde (MDA), was determined in testes through the technique of Iqbal *et al.* (1996). The antioxidant enzyme catalase (CAT) level was determined utilizing an amended procedure of Chance and Maehly (1955). Superoxide dismutase (SOD) concentration was evaluated following the protocol of Kakkar *et al.* (1984). Glutathione-S-transferase (GST) activity was evaluated by adopting the procedure of Habig *et al.* (1974). Glutathione peroxidase (GPx) level was estimated using the technique of Flohé and Günzler (1984). Meanwhile, reduced glutathione (GSH) level was assessed using the protocol of Ellman (1959).

Histological examination: Following 4, 8, and 12 weeks of the experiment, tissue samples from the left testis of each animal were harvested and processed for histological examination, adopting the technique explained by Cardiff *et al.* (2014). Testis samples were immediately fixed in a 10% formalin solution to maintain tissue integrity. The fixed tissues were then exposed to an ascending grade of ethanol for dehydration, followed by xylene treatment to achieve tissue clearing. Subsequently, the samples were embedded in paraffin wax. Thin sections of the testicular tissue, approximately 5 to 6 micrometers thick, were obtained using a microtome. These sections were stained

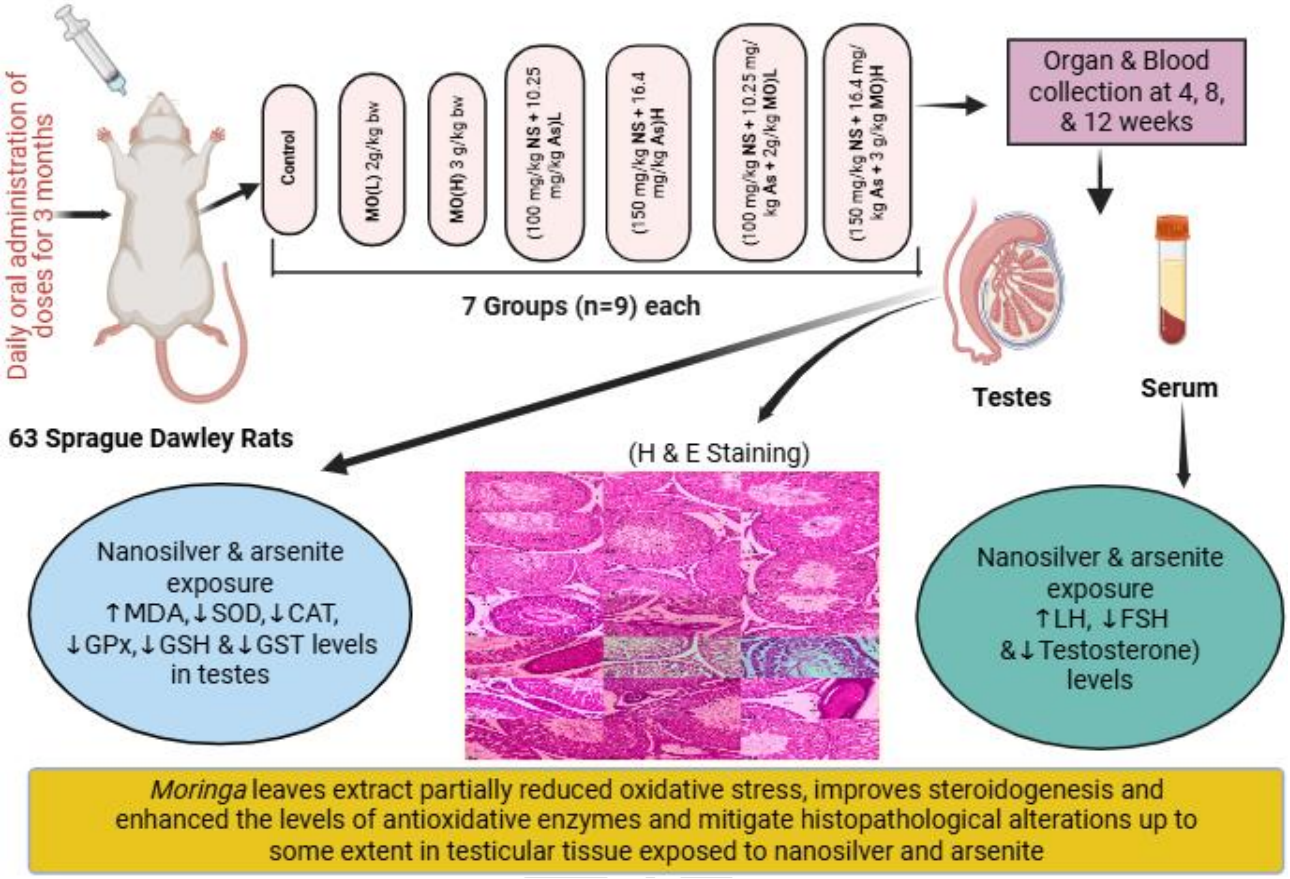

Fig. 1: Experimental design for evaluating the potency of Moringa leaves aqueous extract in mitigating the nanosilver and arsenite-induced testicular dysfunction in rats.

using hematoxylin and eosin (H&E). Microscopic examination was conducted at 40X magnification using a camera-fitted Optika B-150 microscope. Images were captured and processed using the Future Winjoe software platform.

Statistical analysis: Data were displayed as Mean±standard error (SEM). Statistical analysis was carried out through two-way ANOVA to assess variations between the groups by using post hoc Tukey and Dunnett's test for further pairwise comparison, all performed with GraphPad Prism version 8.0. A P-value of 0.05 was considered significant, whereas a P-value of 0.01 was interpreted as highly significant.

RESULTS

Impact of Moringa, nanosilver, and arsenite on male reproductive hormones: There was a gradual rise (P<0.01) in the hormonal activity of animals in all experimental groups over time. The levels of serum reproductive hormones (testosterone and FSH) were substantially (P<0.01) decreased by nanosilver and arsenite (NS+As) co-exposure, besides increased LH activity at both doses (low and high) in comparison to Moringa-supplemented animals at both doses and control rats. However, co-supplementation of Moringa with nanosilver and arsenite (NS+As+MO) to rats at both dose levels remarkably (P<0.01) regularizes the hormonal activity in a dose-dependent way, contrary to the chemicals-intoxicated rats. Moreover, the animals treated with alone at both dose levels sustained consistent male reproductive hormone activity similar to that of the control group. Additionally, exposure to nanosilver and arsenite at high doses caused more harmful (P<0.01) effects on the activity of hormones compared to rats that received a low dose of chemicals across each successive (4, 8, and 12) week of the trial, as illustrated in Fig. 2.

Impact of Moringa, nanosilver and arsenite on lipid peroxidation: A gradual rise (P<0.01) in MDA activity was observed in all experimental groups over time. A dose-dependent prominent upsurge (P<0.01) was observed in testis MDA activity in nanosilver and arsenite co-exposed (NS+As) animals at both doses (low and high) compared to Moringa-treated groups and control animals. However, co-administration of Moringa with nanosilver and arsenite (NS+As+MO) at both dosages considerably drops (P<0.01) lipid peroxidation marker (MDA) activity in contrast to chemicals-intoxicated rats, but the MDA activity of animals treated simultaneously with Moringa, nanosilver, and arsenite remains higher than the control and Moringa-treated groups. However, Moringa extract alone administration at both dose levels (low and high) substantially (P<0.01) lessened the activity of lipid peroxidation marker in the testes of animals relative to the control at 4, 8, and 12 weeks of the trial in a dose-dependent manner. Additionally, co-exposure to nanosilver and arsenite at high doses led to a more (P<0.01) deleterious effect (increase) in oxidative stress marker compared to the animals receiving low dose over a three-month experimental period, as shown in Table 1.

Table 1: Potency of *Moringa oleifera* (MO) leaves extract against Nano silver (NS) and arsenite (As) induced changes in antioxidant parameters of the testes in rats during three months (4, 8, and 12 weeks) of the experiment.

Treatments	MDA (nmol/min/mg Protein)	SOD (Unit/mg Protein)	CAT (unit/mg/min)	GPx (U/g tissue)	GSH (µmol/g tissue)	GST (U/g tissue)
4 Weeks						
Control	0.24±0.02	28.89±0.59	7.16±0.16	7.76±0.17	34.85±0.79	1.82±0.01
MO(L)	0.18±0.00**	31.77±1.00**	7.85±0.08**	8.20±0.04**	36.43±1.03**	1.95±0.02**
MO(H)	0.12±0.03**	33.01±1.64**	8.27±0.13**	8.82±0.11**	37.45±0.49**	2.00±0.04**
(NS+As)L	0.70±0.06**	21.48±0.51**	4.83±0.11**	5.42±0.15**	20.52±0.84**	1.54±0.01**
(NS+As)H	0.81±0.03**	18.59±0.46**	4.13±0.10**	4.93±0.15**	18.85±0.90**	1.35±0.03**
(NS+As+MO)L	0.58±0.02**	24.07±0.70**	5.50±0.27**	5.91±0.05**	24.81±0.61**	1.58±0.01**
(NS+As+MO)H	0.69±0.05**	21.40±0.71**	5.32±0.14**	5.31±0.18**	21.96±1.41**	1.46±0.02**
8 Weeks						
Control	0.35±0.04	31.29±0.88	8.15±0.15	8.54±0.13	39.67±0.34	2.36±0.10
MO(L)	0.22±0.02**	34.29±0.92**	8.74±0.11**	9.19±0.14**	41.13±0.52**	3.08±0.13**
MO(H)	0.14±0.03**	37.33±0.44**	8.83±0.10**	9.80±0.15**	41.67±0.71**	3.37±0.09**
(NS+As)L	0.89±0.08**	24.14±0.78**	5.80±0.15**	5.93±0.16**	24.55±1.45**	1.79±0.12**
(NS+As)H	0.96±0.02**	21.09±1.01**	5.04±0.10**	4.82±0.16**	22.32±0.84**	1.15±0.07**
(NS+As+MO)L	0.77±0.00**	26.15±0.46**	6.47±0.03**	6.75±0.11**	30.27±1.50**	1.50±0.10**
(NS+As+MO)H	0.76±0.03**	24.63±0.55**	5.91±0.08**	5.84±0.10**	26.48±0.74**	1.90±0.07**
12 Weeks						
Control	0.44±0.01	34.17±0.35	8.89±0.07	9.71±0.14	41.36±0.51	2.78±0.11
MO(L)	0.34±0.04**	36.44±0.51**	9.14±0.06**	10.22±0.14**	42.04±0.25**	3.55±0.15**
MO(H)	0.28±0.01**	38.89±0.67**	9.82±0.06**	10.47±0.12**	43.32±1.07**	3.78±0.10**
(NS+As)L	0.94±0.03**	26.00±0.59**	6.24±0.10**	6.23±0.11**	26.77±0.76**	2.15±0.15**
(NS+As)H	1.10±0.05**	24.59±0.71**	5.78±0.12**	6.16±0.31**	28.93±0.53**	2.01±0.10**
(NS+As+MO)L	0.83±0.01**	29.10±0.80**	6.85±0.06**	7.80±0.13**	31.51±1.27**	2.39±0.10**
(NS+As+MO)H	0.86±0.02**	27.03±1.41**	6.31±0.12**	6.90±0.37**	31.20±0.74**	2.17±0.26**

L and H referred to low and high doses. Each value represents mean ± SEM, n=3/week. P<0.01= **highly significant and P<0.05= *significant.

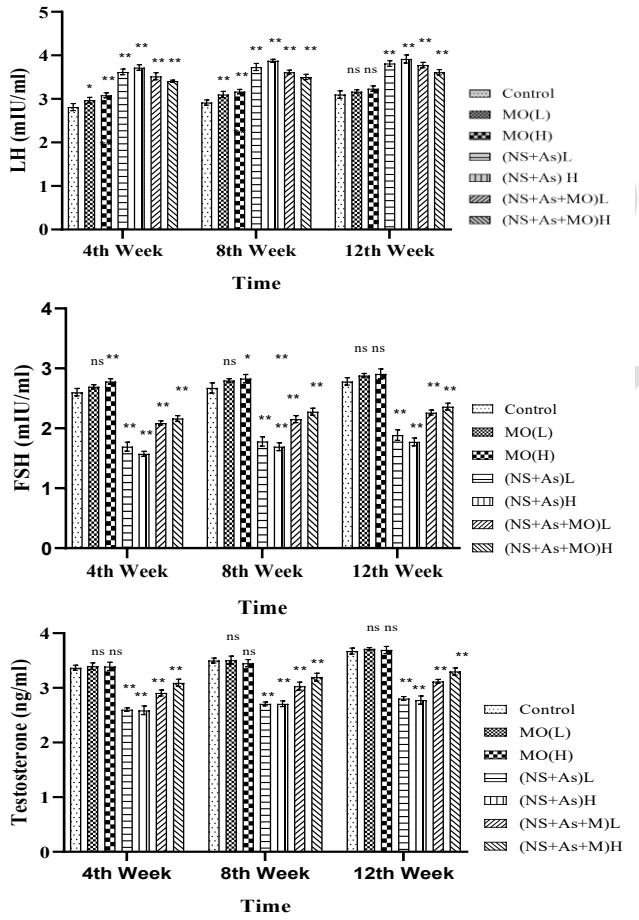

Fig. 2: Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and Testosterone levels during three months (4, 8, and 12 weeks) exposure of Nanosilver (NS), arsenite (As) and *Moringa oleifera* (MO) leaves extract treatment, where ns non-significant; * significant; ** highly significant (P<0.01) difference between control and treated groups. L and H referred to low and high doses.

Impact of Moringa, nanosilver and arsenite on antioxidant enzymes: Antioxidant enzymes activity in rats of all groups showed a gradual (P<0.01) rise over time. A dose-dependent prominent decrease (P<0.01) was observed in testicular GPx, GSH, GST, CAT, and SOD activity in nanosilver and arsenite (NS+As) co-exposed groups at both dosages (low and high) compared to control and Moringa *extract* administered groups. Furthermore, co-supplementation of Moringa with nanosilver and arsenite (NS+As+MO) at low and high doses significantly elevated (P<0.01) the antioxidant enzymes (GPx, GSH, GST, CAT, and SOD) activity contrary to nanosilver and arsenite co-exposed rats but the antioxidant enzyme activity of the animals treated concurrently with chemicals and Moringa, persisted lower from the Moringa-treated groups and control animals. However, animals treated with Moringa alone at both doses revealed a considerable (P<0.01) increase in the concentration of antioxidant enzymes compared to control animals in a dose-dependent way across each successive month of the treatment (4, 8, and 12 weeks). Additionally, exposure to nanosilver and arsenite in high doses led to more harmful (P<0.01) effects (decline) in the antioxidant enzyme activities relative to the rats of the low-dose groups over a three-month experimental period, as presented in Table 1.

Impact of Moringa, nanosilver and arsenite on testicular histology: The histoarchitecture of control and Moringa-extract treated testes at both dose levels appeared normal with regular spermatogenesis. A basement membrane enclosed the seminiferous epithelium, which consisted of sertoli and various spermatogenic cells. Leydig cells and blood vessels were present in the interstitium between the tubules, indicative of characteristic testicular morphology at 4, 8, and 12 weeks of the experiment (Fig. 3: 4a, 8a, 12a, 4b, 8b, 12b, 4c, 8c, and 12c). Histological investigations, however, demonstrated that nanosilver and arsenite co-exposure (NS+As) caused serious testicular injury at both dose levels and induced degenerative changes, like the deterioration of the seminiferous tubules, congestion of seminiferous epithelium with enlarged lumen, the halo

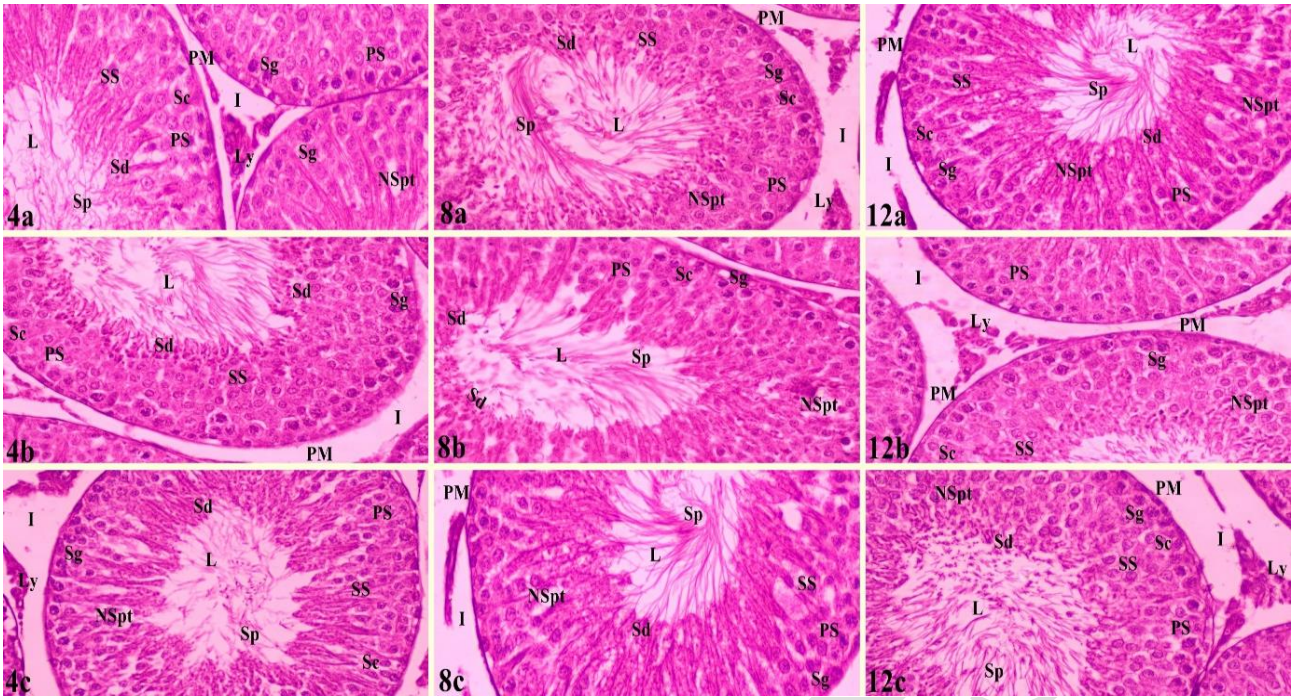

Fig. 3: Potency of *Moringa oleifera* (MO) leaves extract against nanosilver (NS) and arsenite (AS) induced histopathological alterations in rat testes at 4, 8 and 12 weeks of the experiment. L and H referred to low and high doses. Control; (4a, 8a, 12a, 40X), MO(L); (4b, 8b, 12b, 40X), MO(H); (4c, 8c, 12c, 40X) showed normal spermatogenesis (NSpt). The seminiferous tubules consisted of the lumen (L) and all cell types like Sertoli cells (Sc), Spermatogonia (Sg), Primary Spermatocytes (PS), Secondary Spermatocytes (SS), Spermatid (Sd), and spermatozoa (Sp). The peritubular membrane (PM) surrounds the seminiferous tubule. Interstitium (I) containing Leydig cells (Ly), Blood Vessel (BV). (H&E) staining.

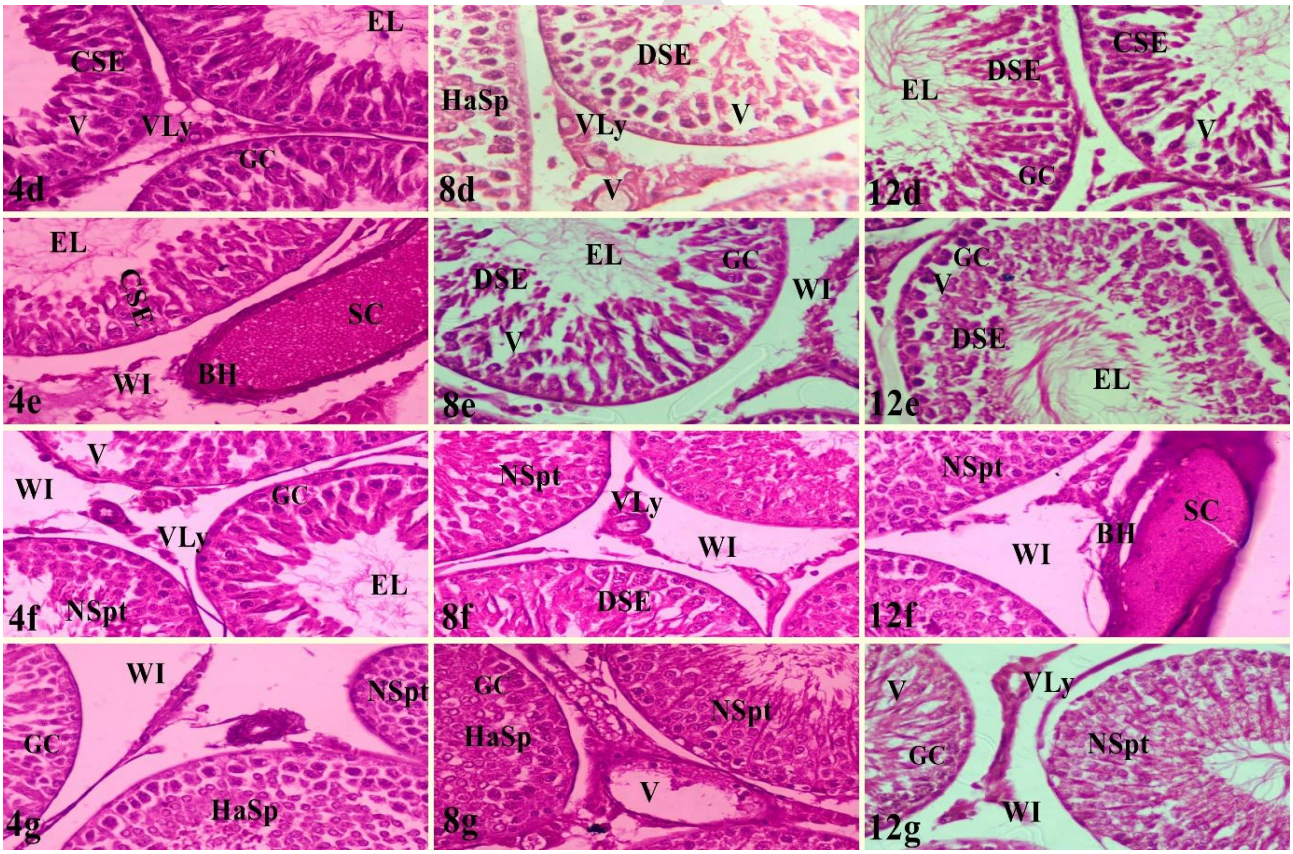

Fig. 3: Potency of *Moringa oleifera* leaves extract against nanosilver (NS) and arsenite (AS) induced histopathological alterations in rat testes at 4, 8 and 12 weeks of the experiment. L and H referred to low and high doses. (NS+As) L; (4d, 8d, 12d 40X) and (NS+As) H; (4e, 8e, 12e, 40X) dose groups exhibited degenerative changes like Deterioration of Seminiferous Epithelium (DSE), Congestion of Seminiferous Epithelium (CSE), Enlarge Lumen (EL), Giant Cells formation (GC), Vacuolation (V), halo-like appearance (HaSp) of spermatocytes. Blood Hemorrhage (BH)with Severe Congestion (SC) was observed in the Wide Interstitium (WI) with vacuolated Leydig cells (VLy). (NS+As+MO) L; (4f, 8f, 12f, 40X) and (NS+As+MO) H; (4g, 8g, 12g, 40X) groups consisted of histological alterations hemorrhage (BH), vacuolation (V), and partial disorganization of spermatogenic cells and deterioration of seminiferous epithelium. due to exposure to chemicals that were mitigated in terms of the normal spermatogenic (NSpt) process to some extent after Moringa leaves extract administration, (H&E) staining.

appearance of germ cells with subsequent vacuolation, giant cell formation along with apoptosis and necrosis. Furthermore, male germ cells became disorganized and exfoliated into the lumen of the tubule, besides enlargement of interstitial spaces and disruption of peritubular membrane compared to control and Moringa extract-treated groups across each successive month of the treatment **(**Fig. 3: 4d, 8d, 12d, 4e, 8e and 12e). The morphology of seminiferous tubules in the nanosilver and arsenite co-exposed groups was comparable to the control group regarding the organization of spermatogenic cells, except for incredibly depressed spermatogenesis. The histological changes induced in the testes by nanosilver and arsenite exposure at low and high dose levels were partially alleviated in groups that were given Moringa extract (NS+As+MO). However, some deteriorating alterations such as vacuolation, hemorrhage, and partial disorganization of germ cells) were still pragmatic in each successive month of the experiment (4, 8, and 12 weeks) **(**Fig. 3: 4f, 8f, 12f, 4g, 8g and 12g).

DISCUSSION

Testicular toxicity is influenced by several factors, including environmental, behavioral, hormonal, and nutritional inequities (Krzastek *et al.,* 2020; Sakar *et al.*, 2024). The current investigation demonstrated that serum levels of testosterone and FSH were dramatically reduced by nanosilver and arsenite (NS+As) co-exposure. Contrary to this, LH concentration was substantially raised at both doses (low and high) compared to Moringa extract-administered groups and control animals in all three months of the experiment. The coordinated action of LH, FSH, and testosterone is necessary for both the initiation and maintenance of spermatogenesis (Oduwole *et al.,* 2021). LH levels may arise as a compensatory response to reduced testosterone levels or increased concentrations of nitric oxide and cyclic GMP (cGMP), which influence protein kinase G activity and increase the release of luteinizing hormone-releasing hormone (LHRH) from hypothalamic axon terminals (Melis and Argiolas, 2021). Disruptions in reproductive hormone balance can adversely affect several stages of germ cell development, potentially leading to impaired spermatogenesis.

These disruptions align with observations by Jasem and Abas (2022), who found that chemical exposure may result in DNA damage, apoptosis of Leydig cells, and a decline in testosterone production, suggesting a detrimental anti-steroidogenic effect. This decrease in testosterone activity may be associated with a downregulation of steroidogenic acute regulatory (STAR) protein, which impairs the transference of cholesterol into the mitochondria and, consequently, inhibits the conversion of cholesterol to progesterone, a critical step in testosterone biosynthesis (Mohlala *et al*., 2023). The decrease in reproductive hormone activity may be attributed to the inefficiency of the endocrine pathway by obstructing the hypothalamic-hypophysial axis, or may be due to a decline in GnRH activity (Al-Suhaimi *et al.,* 2022). In contrast to the chemicals-exposed rats at low and high doses, the co-administration of nanosilver, arsenite, and Moringa extract (NS+As+MO) or Moringa alone administration at both doses significantly regularized the steroidogenesis indices. across each successive (4, 8, and 12) weeks of the trial compared to control animals. Moringa extract alone or in combination with chemicals lowered injury to the testicles and sperm production, likely because of its androgenic characteristics. These results are corroborated by those reported by El-Sheikh *et al.* (2016) and Mansour *et al.* (2020), who documented partially elevated concentrations of LH, FSH and testosterone in animals following Moringa administration. Previous study has verified that Moringa can help develop male germ cells and other reproductive functions owing to aphrodisiac and androgenic characteristics (Bashah and Noor, 2021). The occurrence of phytochemicals like terpenes, alkaloids, saponins, and xanthones helps in sustaining hormonal equilibrium by moderating several processes within the endocrine system and manipulating its feedback regulation. (Abd *et al.,* 2020)

The primary factor responsible for testicular toxicity and male infertility is oxidative stress. The male gonads (testes) have a delicate antioxidant enzyme system which work collectively to mitigate the generation of reactive oxygen species (Sengupta *et al.,* 2024). Malondialdehyde (MDA) is frequently employed to indicate oxidative and cellular injury, reflecting the extent of lipid peroxidation affecting essential biomolecules, including DNA, lipids, and proteins (Mas-Bargues *et al.,* 2021). In the present study, animals that received a combined exposure of chemicals (NS+As) at both doses exhibited a substantial decline in testicular antioxidant enzymes including GST, CAT, GPx, SOD and GSH, accompanied by a noteworthy rise in MDA activity, in comparison to Moringa-treated groups and control animals in all three months of the experiment. This disturbance in antioxidant homeostasis endorses the disproportionate production of ROS, contributing to an array of pathological effects on sex cells (Drevet *et al.,* 2022; Elsayed *et al.,*2024).

Comparable findings were observed in antioxidant assessments of groups exposed to nanoparticles (Behairy *et al.,* 2022) and heavy metals (Raeeszadeh *et al.,* 2021), which exhibited clear signs of oxidative damage, evidenced by decreased activities of antioxidant enzymes, alongside elevated ROS production. These disparities frequently lead to inflammation, cytotoxicity, and initiation of apoptosis (Dianová *et al.,* 2022). However, supplementation of Moringa (MO) extract alone or with the chemicals (NS+As+MO) at both low and high dose levels revealed noteworthy protective effects by reinstating antioxidant enzyme levels and lowering MDA activity in rats in comparison to animals exposed only to the chemicals (nanosilver and arsenite) across 4, 8 and 12 weeks. The outcomes showed a dose-dependent effect, indicating that higher doses of Moringa may provide greater protection against oxidative stress induced by these toxic agents.

The results align with the increasing evidence emphasizing Moringa*'s* potential as an anticipatory agent, capable of boosting the body's antioxidant defense system without causing harmful effects (Bashir *et al.,* 2024). Furthermore, Moringa's phytochemicals may regulate signalling cascades like pro-apoptotic proteins such as Bcl-2-associated X protein (Bax) by

elevating anti-apoptotic Bcl-2 activities, which alleviate mitochondrial structural and functional integrity and avert caspase-mediated cell death that affects gene expression related to antioxidant production (Kumar *et al.,* 2023). Additionally, time-related giving back of the results over 4, 8, and 12 weeks supports the ameliorative role of Moringa in the inhibition and suspension of oxidative stress in the future (Abdel-Daim *et al.,* 2020). Comparable outcomes were described by Alqahtani and Albasher (2021). Moringa's antioxidant characteristics may be ascribed to the presence of phenolic components such as flavonoids, quercetin, anthocyanins, and kaempferol, along with antioxidants like ɣ-tocopherol, α- and β-sitosterol, β-carotene, and vitamin A. These components can form soluble but poorly ionized complexes with toxic metals, which are then excreted from the body through urine or bile (Agarwal *et al.*, 2025).

The histological results of this study were correlated with the biochemical results. Throughout the three-month experimental period, the testicular architecture in the Moringa extract-treated (low and high dose) groups and control animals exhibited active spermatogenesis, with well-organized germ cell layers and intact interstitial tissue, indicative of healthy testicular function, consistent with observations reported by Mohamed (2024). In contrast, histological analysis revealed significant testicular damage in animals exposed to a combination of nanosilver and arsenite (NS+As), with the severity of degeneration in seminiferous tubules increasing in a dose- and time-dependent way relative to control and Moringa-treated low and high dose groups in all three months of the experiment. The chemical-exposed groups exhibited progressive degenerative alterations, including disruption of the seminiferous epithelium, vacuolation, exfoliation, and a characteristic halo-like appearance of germ cells, accompanied by evident signs of apoptosis, necrosis, and hemorrhage. Among these, vacuolation was identified as Sertoli cells' most prominent morphological response to damage, often accompanied by germ cell degeneration and exfoliation—findings that align with those of Ibrahim and Sadek (2022). The presence of large, multinucleated cells observed in this study and reported in previous research may result from the degeneration of spermatogenic cells due to the dysregulation of various proteins and the breakdown of intercellular bridges. This disruption eventually leads to the production of abnormal cells (Behairy *et al.,* 2024). Nanoparticles and heavy metal exposure have been shown to interfere with microtubule structures and other essential cellular components (Vassal *et al.,* 2021), significantly increasing germ cell sloughing in all treated groups compared to Moringa-alone-treated groups and control animals.

Previous studies have also indicated that the early stages of spermatogenic cell degeneration may compromise the integrity of the plasmalemma, contributing to cellular shedding (Boekelheide *et al.,* 2017). Moreover, rats exposed to a higher dose of the chemical combination (NS+As) exhibited more pronounced histopathological changes in the seminiferous tubules than those in the lower dose group, a pattern consistent with findings by Shangloo *et al.* (2022). Additionally, exposure to nanosilver and arsenite caused degenerative alterations in the basement membrane, a structure essential for preserving testicular tissue's functional and structural integrity (Assar *et al.,* 2023).

There was a marked reduction in spermatogenic activity in both the low and high-dose groups. Distraction of the peritubular basement membrane can result in significant functional impairments within the male gonads (testes). The results of the current investigation align with research work performed by Elsharkawy *et al.* (2019) and Massányi *et al.* (2020). The histological impairment caused by interactive exposure of nanosilver and arsenite at both doses was partially improved in the testicular tissue of rats co-treated with Moringa extract (NS+As+MO) when compared to the respective chemically exposed groups across 4, 8, and 12-week time points, along with some degenerative alterations remained evident during the same periods. These results align with earlier research that has established the protective role of Moringa extract against the toxic impacts of heavy metals (Al–Hadidy and Mostafa, 2022).

The histopathological alterations observed in this study are consistent with previous findings indicating that nanoparticle exposure leads to significant cytological damage in testicular tissue (Thakur *et al.,* 2014). Moringa appears to counteract such damage by stabilizing hormonal and histochemical parameters within the testes. It exerts this protective influence by maintaining cellular membrane integrity and neutralizing harmful free radicals and oxidative agents (Ragab *et al.,* 2024).

Conclusions: Recent research has highlighted the potential of herbal antioxidants in managing male infertility. This study found that suppression of the antioxidant defense system due to co-exposure to chemicals (nanoparticles and heavy metals) increases oxidative stress and disrupts reproductive hormones, thereby exacerbating reproductive damage in male rats. However, Moringa's antioxidant and anti-inflammatory effects mitigated this damage by improving steroidogenesis, normalizing testicular histopathology and antioxidative enzyme status, but only up to some extent, particularly during disease conditions. Moringa leaves extract alone supplementation did not show any adverse effects. Therefore, Moringa extract may potentially be used as a cost-effective herbal treatment due to its easy accessibility and cost-effectiveness, particularly in the prevention and treatment of diseases caused by exposure of chemicals.

Ethics approval: This research received ethical clearance from the Ethical Review Committee of the Department of Zoology at Lahore College for Women University, Lahore, Pakistan, under reference number (REF/NO/LCWU/ZOO/610A), dated 01-08-2022.

Conflicts of interest: The authors do not have any conflicts of interest to declare.

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Authors' Contribution: FB performed the research work, collected the tissue and blood samples, transcribed

the original draft, and conducted the analysis of data. SS perceived and planned the study design and assisted in the statistical analysis and script of the manuscript. SN assist in making the study design and organization of data. FM and AS swotted and revised the transcript. All authors agreed with the manuscript prior to submission.

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