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Biogenic Selenium Nanoparticles Mitigate Arsenic-Induced Testicular Dysfunction and Steroidogenic Gene Dysregulation in Mice

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Abstract

Arsenic poisoning of drinking water is a global environmental health problem, and prolonged exposure has been increasingly linked to male reproductive damage. The present work was aimed to evaluate the protective effects of biogenic selenium nanoparticles (SeNPs) synthesised utilising Moringa oleifera leaf extract against sodium arsenite (NaAsO2)-induced testicular injury and steroidogenic gene dysregulation in adult male Swiss albino mice. The SeNPs were characterised by UV–Vis spectroscopy, TEM, FTIR, XRD and dynamic light scattering (DLS) and confirmed spherical morphology with an average size of 45–65 nm. Forty mice were randomly assigned to five groups (n = 8): control, SeNP control (0.5 mg/kg b.w.), arsenic alone (5 mg/kg b.w.), arsenic + low-dose SeNPs (0.25 mg/kg), and arsenic + high-dose SeNPs (0.5 mg/kg). All therapies were given orally for 35 consecutive days. Arsenic exposure resulted in significant disruption of serum testosterone, luteinizing hormone and follicle-stimulating hormone levels and an increase in oestradiol. Increased morphological defects significantly reduced sperm count, motility and viability. MDA of testicular tissue was markedly increased, while SOD, CAT, GPx and GSH were depleted in concert. RT-qPCR showed significant down-regulation of steroidogenic acute regulatory protein (StAR), CYP11A1, CYP17A1, 3β-HSD, and 17β-HSD mRNA expression, as well as up-regulation of the pro-apoptotic Bax/Bcl-2 ratio in arsenic-treated testes. Co-administration of biogenic SeNPs dose-dependently corrected these aberrations, returning the hormonal balance, sperm quality, antioxidant defences, and steroidogenic gene expression to normal levels. Histopathological study revealed the retention of seminiferous tubular architecture in SeNPs treated groups. These findings suggest that phytochemically capped SeNPs represent a viable chemoprotective strategy against arsenic-mediated male reproductive toxicity.

Keywords: *Selenium Nanoparticles; Green Synthesis; Arsenic; Testicular Toxicity; Steroidogenesis; Star; CYP11A1; Oxidative Stress; Apoptosis; Male Fertility.*

I. INTRODUCTION

Arsenic is a ubiquitous metalloid contaminant that ranks among the top environmental hazards according to the Agency for Toxic Substances and Disease Registry [1]. Tens of millions of people across South and Southeast Asia, Latin America, and parts of Africa are chronically exposed to inorganic arsenic through contaminated groundwater, with concentrations frequently exceeding the WHO guideline of 10 µg/L [2,3]. While the hepatotoxic, nephrotoxic, and carcinogenic effects of arsenic have been extensively documented, its reproductive toxicity particularly its impact on male fertility has received comparatively less attention until recent years [4]. Accumulating data now suggests that chronic arsenic exposure perturbs the hypothalamic–pituitary–gonadal (HPG) axis, affects spermatogenesis, compromises sperm quality and disturbs the expression of steroidogenic enzymes in the testes [5,6]. Mechanism of action is based on the overproduction of reactive oxygen species (ROS), which overwhelms the endogenous antioxidant defences, resulting in lipid peroxidation of Leydig and Sertoli cell membranes, mitochondrial malfunction, and finally germ cell death [7,8].

Selenium (Se) is an essential trace element and plays a crucial role in male reproductive physiology. It is a cofactor of glutathione peroxidase (GPx) and thioredoxin reductase, both abundantly produced in the testis [9]. Dietary supplementation with selenium has been found to

have potential in reducing heavy metal-induced oxidative damage; nevertheless, its narrow therapeutic window and toxicity at higher dosages remain major limits [10,11]. The attractive option is selenium in the form of nanoscale formulations that exhibit better bioavailability and lower acute toxicity than inorganic or organic selenium compounds [12,13]. The green synthesis of selenium nanoparticles (SeNPs) by plant extracts or microbial systems has gained momentum since it circumvents hazardous chemicals, produces biocompatible particles and introduces additional bioactive phytochemical coatings that may synergise with the inherent properties of elemental selenium. [14,15].

Moringa oleifera Lam. (Moringaceae), widely known as the "miracle tree," is exceptionally rich in polyphenols, flavonoids, ascorbic acid, and glucosinolates, all of which exhibit strong reducing capacity and antioxidant potential [16,17]. Several groups have successfully synthesized metallic nanoparticles using *M. oleifera* extracts, but reports on its use for selenium nanoparticle fabrication and their application in reproductive toxicology are scarce [18]. The steroidogenic pathway is governed by a cascade of enzymes and regulatory proteins most notably StAR, which mediates cholesterol transport across the mitochondrial membrane, followed by CYP11A1, CYP17A1, 3β-hydroxysteroid dehydrogenase (3β-HSD), and 17β-HSD all of which are vulnerable to oxidative and electrophilic insult [19,20]. Despite the known sensitivity of these genes to arsenic-induced oxidative stress, there are no published studies evaluating whether biogenic SeNPs can rescue steroidogenic gene expression under arsenic challenge. The present work was therefore designed to green-synthesize SeNPs using *M. oleifera* leaf extract, characterize the nanomaterial, and systematically assess its chemoprotective efficacy against arsenic-induced testicular dysfunction, hormonal imbalance, oxidative stress, steroidogenic gene dysregulation, and germ cell apoptosis in mice.

II. MATERIALS AND METHODS

➢ *Chemicals and Reagents*

Sodium selenite (Na_2SeO_3, purity ≥ 99%), sodium arsenite ($NaAsO_2$, ≥ 90%), ascorbic acid, thiobarbituric acid, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), reduced glutathione, trichloroacetic acid, and bovine serum albumin were obtained from Sigma-Aldrich (St. Louis, MO, USA). ELISA kits for testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol were purchased from Cusabio Biotech (Wuhan, China). TRIzol reagent, RevertAid First Strand cDNA Synthesis Kit, and Maxima SYBR Green qPCR Master Mix were from Thermo Fisher Scientific (Waltham, MA, USA). All other chemicals were of analytical grade.

➢ *Preparation of Moringa Oleifera Leaf Extract*

Fresh *Moringa oleifera* leaves were collected from a local nursery in Al-Diwaniyah, Iraq, and taxonomically authenticated at the Department of Biology, College of Education, University of Al-Qadisiyah. Leaves were washed thoroughly under running tap water followed by three rinses with distilled water, shade-dried for 5 days at room temperature, and ground to a fine powder using a mechanical blender. 20g of the powder were added to 200mL of double distilled water and heated at 80°C with continuous magnetic stirring for 20min. The resultant decoction was cooled to room temperature and filtered under vacuum through Whatmann No. 1 filter paper. The clear yellowish green filtrate was kept at 4 °C and used within 48 h [21].

➢ *Green Synthesis of SeNPs*

The SeNPs were synthesised by reduction of sodium selenite using M. oleifera leaf extract according to the procedure published by Menon et al. [22] with modifications. In brief, 50 mL of freshly generated 10 mM sodium selenite aqueous solution was heated to 60 °C on a magnetic stirrer and 20 mL of the leaf extract was added dropwise for 10 min under vigorous stirring. The colour gradually changed from colourless to brick-red within 30–45 min, due to the reduction of Se4+ to Se0 and the nucleation of elemental selenium nanoparticles. To guarantee complete conversion, an additional reducing agent, ascorbic acid (30 mM, 5 mL), was added. The reaction was then kept at 60 °C for another 3 h. The obtained SeNP suspension was centrifuged at 12,000 rpm for 20 min and the pellet was washed three times with deionised water and once with 100% ethanol. The pure nanoparticles were freeze-dried (-50 °C, 0.01 mbar) for 24 h and kept as lyophilised powder at 4 °C in amber vials until use [23].

➢ *Characterization of SeNPs*

The generation of SeNPs was tracked by UV–Vis. spectrophotometry (Shimadzu UV-1800) in the range of 200–800 nm. The morphology and particle size were investigated by transmission electron microscopy (TEM; JEOL JEM-2100F) at 200 kV and the size distribution was obtained from at least 150 individual particles using ImageJ. Fourier transform infrared spectroscopy (FTIR; Bruker ALPHA II) was used to identify functional groups in the range 4000–400 cm−1 using KBr pellets.Crystallinity was evaluated by X-ray diffraction (XRD; Rigaku MiniFlex 600, Cu Kα, 2θ = 10–80°). The hydrodynamic diameter, polydispersity index (PDI), and zeta potential were determined by dynamic light scattering (DLS; Malvern Zetasizer Nano ZS) at 25 °C [24].

➢ *Experimental Animals*

Forty mature male Swiss albino mice (25–30 g) aged 8–10 weeks were purchased from the animal facility of the College of Veterinary Medicine, University of Al-Qadisiyah. Animals were housed in polypropylene cages under regulated circumstances (22±2 °C, 55±5% relative humidity, 12 h light/dark cycle) with free access to standard rodent chow and tap water. Treatments were initiated after a one-week acclimatisation period. All methods were authorised by the Institutional Animal Ethics Committee, University of Al-Qadisiyah (QAD/2/4/2233) in compliance with NIH criteria [25].

➢ *Experimental Design*

Mice were divided into 5 groups of 8 animals each and after acclimatisation were treated by oral gavage once daily for 35 consecutive days:

Table 1 Experimental Groups and Treatment Protocol.

Group	Designation	Treatment	Dose (oral gavage)
G1	Normal control	Distilled water	0.2 mL/day
G2	SeNP control	Biogenic SeNPs only	0.5 mg/kg b.w.
G3	Arsenic	$NaAsO_2$ alone	5 mg/kg b.w.
G4	As + Low SeNP	$NaAsO_2$ + SeNPs	5 mg/kg As + 0.25 mg/kg SeNPs
G5	As + High SeNP	$NaAsO_2$ + SeNPs	5 mg/kg As + 0.5 mg/kg SeNPs

First, sodium arsenite was dissolved in distilled water and given, and then SeNP suspension was given 1 h later. Prior to each dosage, SeNPs were newly distributed in distilled water by short sonication (5 min, 40 kHz). The dose of arsenic was chosen based on existing literature [26].

➢ *Sample Collection, Analyses, and Statistics*

Sample collection [27], assessment of sperm quality [28,29], serum hormone by ELISA [30], testicular oxidative stress biomarkers (MDA [31], SOD [32], CAT [33], GPx [34], GSH [35], NO [36]), protein quantification [37], RT-qPCR for steroidogenic and apoptosis genes with $2^{-\Delta\Delta Ct}$ method [38], histopathological examination with Johnsen's scoring [39], and statistical analysis by one-way ANOVA/Tukey's test [40] were performed as described in the full methods section.. Primer sequences for StAR, CYP11A1, CYP17A1, 3β-HSD, 17β-HSD, Bax, Bcl-2, and β-Actin are listed in Table 2.

Table 2 Primer Sequences Used for RT-qPCR Analysis.

Gene	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	bp
StAR	CAGGGAGAGGTGGCTATGCA	CCGTGTCTTTTCCAATCCTCTG	128
CYP11A1	AGGTCCTTCAATGAGATCCCTT	TCCCTGTAAATGGGGCCATAC	136
CYP17A1	GCCCAAGTCAAAGACACCTAAT	GTACCCAGGCGAAGAGAATAGA	145
3β-HSD	TGGACAAAGTATTCCGACCAGA	GGCACACTTGCTTGAACACAG	118
17β-HSD	GATGTGGCTGTCAACTGTGC	CCAAATCCTCAGGCTCTTGC	132
Bax	TGAAGACAGGGGCCTTTTTG	AATTCGCCGGAGACACTCG	142
Bcl-2	ATGCCTTTGTGGAACTATATGGC	GGTATGCACCCAGAGTGATGC	151
β-Actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT	154

III. RESULTS AND DISCUSSION

➢ *Characterization of Biogenic SeNPs*

The UV–Vis absorption spectra of synthesised SeNPs exhibited a prominent surface plasmon resonance (SPR) peak at ~265 nm (Fig. 1). This correlates with the standard absorption of zero-valent selenium nanoparticles reported in the literature [22,41]. The colloidal suspension was brick-red in colour and remained stable for four weeks when stored at 4 °C without obvious precipitation suggesting strong colloidal stability.

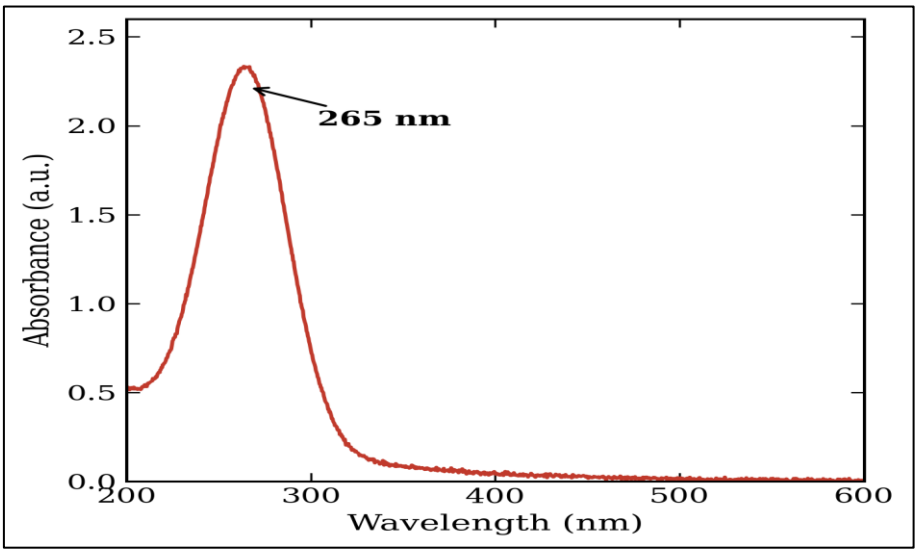

Fig 1 UV–Vis Absorption Spectrum of Biogenic SeNPs Synthesized using Moringa Oleifera Leaf Extract, Showing the Characteristic Absorption Maximum at ~265 nm.

TEM micrographs showed mainly spherical and highly distributed nanoparticles with a size of 45-65 nm and an average particle size of 52.3 ± 7.4 nm (Fig. 2a,b). The DLS measurement indicated a hydrodynamic diameter of 78.6 ± 3.2 nm (PDI = 0.21) and a zeta potential of −28.4 ± 1.6 mV, which is a sign of significant electrostatic stabilisation [42,43].

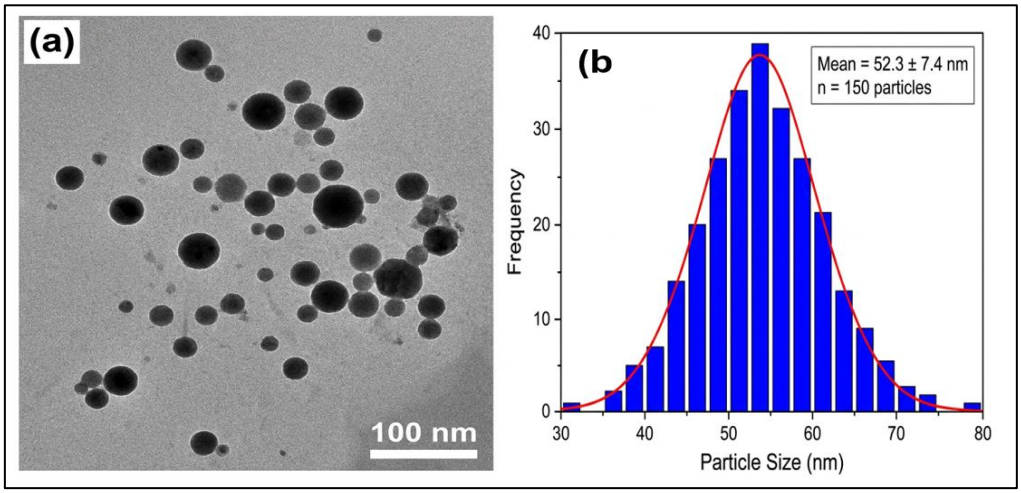

Fig 2 Morphological Characterization of Biogenic SeNPs: (a) Schematic Representation of Spherical SeNPs and (b) Particle Size Distribution Histogram from TEM Analysis (Mean = 52.3 ± 7.4 nm, n = 150 Particles).

The FTIR spectrum (Fig. 3) showed broad absorption bands at 3380 cm^{-1} (O–H stretching), 2935 cm^{-1} (C–H stretch), 1645 cm^{-1} (amide I/C=O stretching), 1420 cm^{-1} (C–N stretching), 1045 cm^{-1} (C–O stretching), and 690 cm^{-1} (Se–O stretching), confirming the presence of *M. oleifera* phytochemicals as capping agents [44,45].

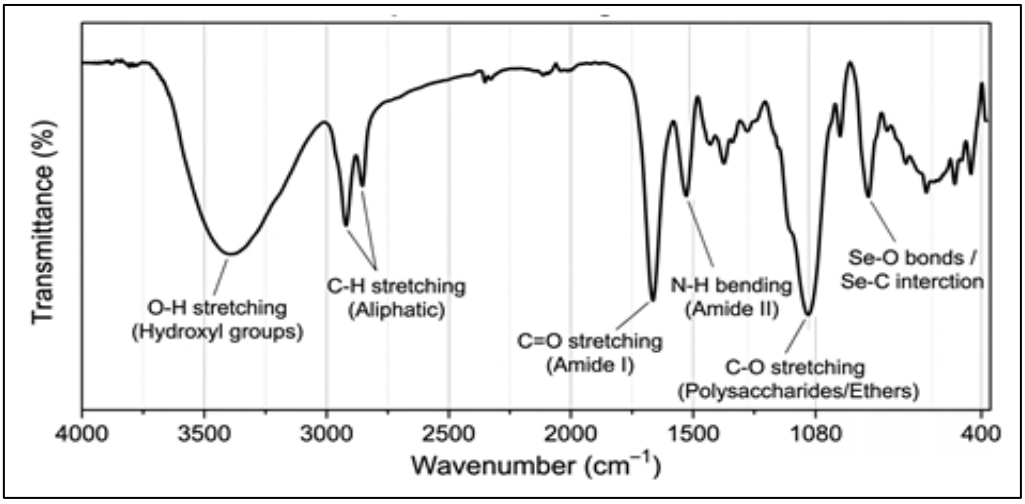

Fig 3 FTIR Spectrum of Biogenic SeNPs Showing Functional Group Signatures from the Moringa Oleifera Extract Capping Layer.

➢ *Body Weight and Reproductive Organ Weights*

Arsenic intoxication causes systemic catabolic effects, which is reflected by considerable reduction in ultimate body weight and reproductive organ weights of arsenic treated mice (G3) as compared to controls (Table 3) [46,47]. Organ weights were partially to totally restored in a dose-dependent manner with G5 showing statistically identical values from control.

Table 3 Effect of Biogenic SeNPs on Body Weight and Reproductive Organ Weights in Arsenic-Treated Mice.

Parameter	G1 (Control)	G2 (SeNP)	G3 (Arsenic)	G4 (As+Low Se)	G5 (As+High Se)
Initial BW (g)	27.4 ± 1.8^{a}	27.1 ± 1.6^{a}	27.6 ± 1.9^{a}	27.3 ± 1.7^{a}	27.5 ± 1.8^{a}
Final BW (g)	35.8 ± 2.1^{a}	35.2 ± 2.3^{a}	29.4 ± 2.5^{c}	32.1 ± 2.0^{b}	34.5 ± 1.9^{ab}
BW gain (g)	8.4 ± 1.2^{a}	8.1 ± 1.3^{a}	1.8 ± 0.9^{c}	4.8 ± 1.1^{b}	7.0 ± 1.0^{a}
Rel. testis (mg/g)	7.85 ± 0.42^{a}	7.92 ± 0.38^{a}	5.12 ± 0.55^{c}	6.38 ± 0.48^{b}	7.41 ± 0.40^{a}
Rel. epididymis (mg/g)	2.45 ± 0.18^{a}	2.51 ± 0.20^{a}	1.62 ± 0.22^{c}	1.98 ± 0.19^{b}	2.31 ± 0.17^{a}
Rel. sem. vesicle (mg/g)	3.18 ± 0.25^{a}	3.24 ± 0.28^{a}	1.85 ± 0.30^{c}	2.42 ± 0.24^{b}	2.95 ± 0.22^{ab}

Values are expressed as mean ± SD (n = 8). Different superscript letters within the same row indicate significant differences ($p < 0.05$, one-way ANOVA/Tukey's test).

➢ *Sperm Quality Parameters*

Table 4 summarises the sperm parameters in the epididymis. The arsenic-exposed mice showed a significant decrease in sperm count (~56 % decrease), progressive motility (~51 % decrease) and viability (~43 % decrease) with an approximate 3.5-fold increase in morphological abnormalities which corroborates the findings of Sarkar et al. [48] and Jahan et al. [49]. The spermatoprotective effect of SeNPs likely reflects selenium's role as a cofactor for GPx4/PHGPx and selenoprotein P [50,51,52].

Table 4 Effect of Biogenic SeNPs on Epididymal Sperm Parameters in Arsenic-Treated Mice.

Parameter	G1 (Control)	G2 (SeNP)	G3 (Arsenic)	G4 (As+Low Se)	G5 (As+High Se)
Sperm count (×10⁶/mL)	38.5 ± 3.2^{a}	39.2 ± 3.5^{a}	16.9 ± 2.4^{c}	26.4 ± 2.8^{b}	35.1 ± 3.0^{a}
Motility (%)	78.4 ± 5.1^{a}	80.2 ± 4.8^{a}	38.5 ± 5.6^{c}	56.8 ± 4.9^{b}	72.3 ± 4.5^{a}
Viability (%)	85.6 ± 4.2^{a}	86.8 ± 3.9^{a}	48.9 ± 5.8^{c}	64.5 ± 5.0^{b}	79.2 ± 4.1^{a}
Abnormalities (%)	8.5 ± 1.4^{a}	7.8 ± 1.2^{a}	29.4 ± 3.8^{c}	18.6 ± 2.9^{b}	11.2 ± 1.8^{a}

Values are expressed as mean ± SD (n = 8). Different superscript letters denote significant differences at $p < 0.05$.

➢ *Serum Reproductive Hormones*

The hormonal profiles are given in Table 5. Serum testosterone was dramatically suppressed (~65% decrease), paralleled by significant reductions in LH and FSH, while estradiol was elevated approximately 1.8-fold [53,54,55]. SeNP co-treatment dose-dependently ameliorated these disturbances, consistent with previous reports that selenium protects Leydig cell steroidogenesis [56].

Table 5 Effect of Biogenic SeNPs on Serum Reproductive Hormone Levels in Arsenic-Treated Mice.

Parameter	G1 (Control)	G2 (SeNP)	G3 (Arsenic)	G4 (As+Low Se)	G5 (As+High Se)
Testosterone (ng/mL)	4.85 ± 0.45^{a}	5.02 ± 0.48^{a}	1.68 ± 0.28^{c}	3.12 ± 0.35^{b}	4.15 ± 0.38^{ab}
LH (mIU/mL)	3.42 ± 0.32^{a}	3.55 ± 0.35^{a}	1.85 ± 0.25^{c}	2.58 ± 0.28^{b}	3.18 ± 0.30^{a}
FSH (mIU/mL)	2.95 ± 0.28^{a}	3.08 ± 0.30^{a}	1.52 ± 0.22^{c}	2.15 ± 0.25^{b}	2.72 ± 0.26^{a}
Estradiol (pg/mL)	18.5 ± 2.1^{a}	17.8 ± 1.9^{a}	33.2 ± 3.5^{c}	25.4 ± 2.8^{b}	20.1 ± 2.3^{a}

Values are expressed as mean ± SD (n = 8). Different superscript letters indicate $p < 0.05$ by one-way ANOVA/Tukey's test.

➢ *Testicular Oxidative Stress and Antioxidant Status*

The oxidative stress landscape in testicular tissue is depicted in Table 6 and Fig. 4. MDA increased ~3.2-fold in arsenic-treated mice; NO was elevated ~2.4-fold. SOD, CAT, GPx, and GSH were markedly suppressed [57,58,59]. Co-administration of SeNPs reversed these perturbations dose-dependently, mechanistically coherent with selenium's role as a GPx cofactor [60,61,62].

Table 6 Effect of Biogenic SeNPs on Testicular Oxidative Stress Markers in Arsenic-Treated Mice.

Parameter	G1 (Control)	G2 (SeNP)	G3 (Arsenic)	G4 (As+Low Se)	G5 (As+High Se)
MDA (nmol/g)	15.2 ± 1.8^{a}	14.6 ± 1.6^{a}	48.5 ± 4.2^{c}	31.8 ± 3.1^{b}	19.4 ± 2.3^{a}
SOD (U/mg)	14.5 ± 1.3^{a}	15.0 ± 1.4^{a}	5.8 ± 0.8^{c}	9.4 ± 1.0^{b}	13.2 ± 1.1^{a}
CAT (U/mg)	42.3 ± 3.8^{a}	43.8 ± 3.5^{a}	16.5 ± 2.2^{c}	28.6 ± 2.9^{b}	38.2 ± 3.2^{ab}
GPx (U/mg)	9.6 ± 0.9^{a}	10.1 ± 1.0^{a}	3.2 ± 0.5^{c}	6.1 ± 0.7^{b}	8.5 ± 0.8^{a}
GSH (µmol/g)	7.2 ± 0.7^{a}	7.5 ± 0.8^{a}	3.0 ± 0.4^{c}	4.8 ± 0.5^{b}	6.5 ± 0.6^{a}
NO (µmol/g)	3.8 ± 0.5^{a}	3.5 ± 0.4^{a}	9.1 ± 1.0^{c}	6.2 ± 0.7^{b}	4.3 ± 0.5^{a}

Values are expressed as mean ± SD (n = 8). Different superscript letters indicate $p < 0.05$.

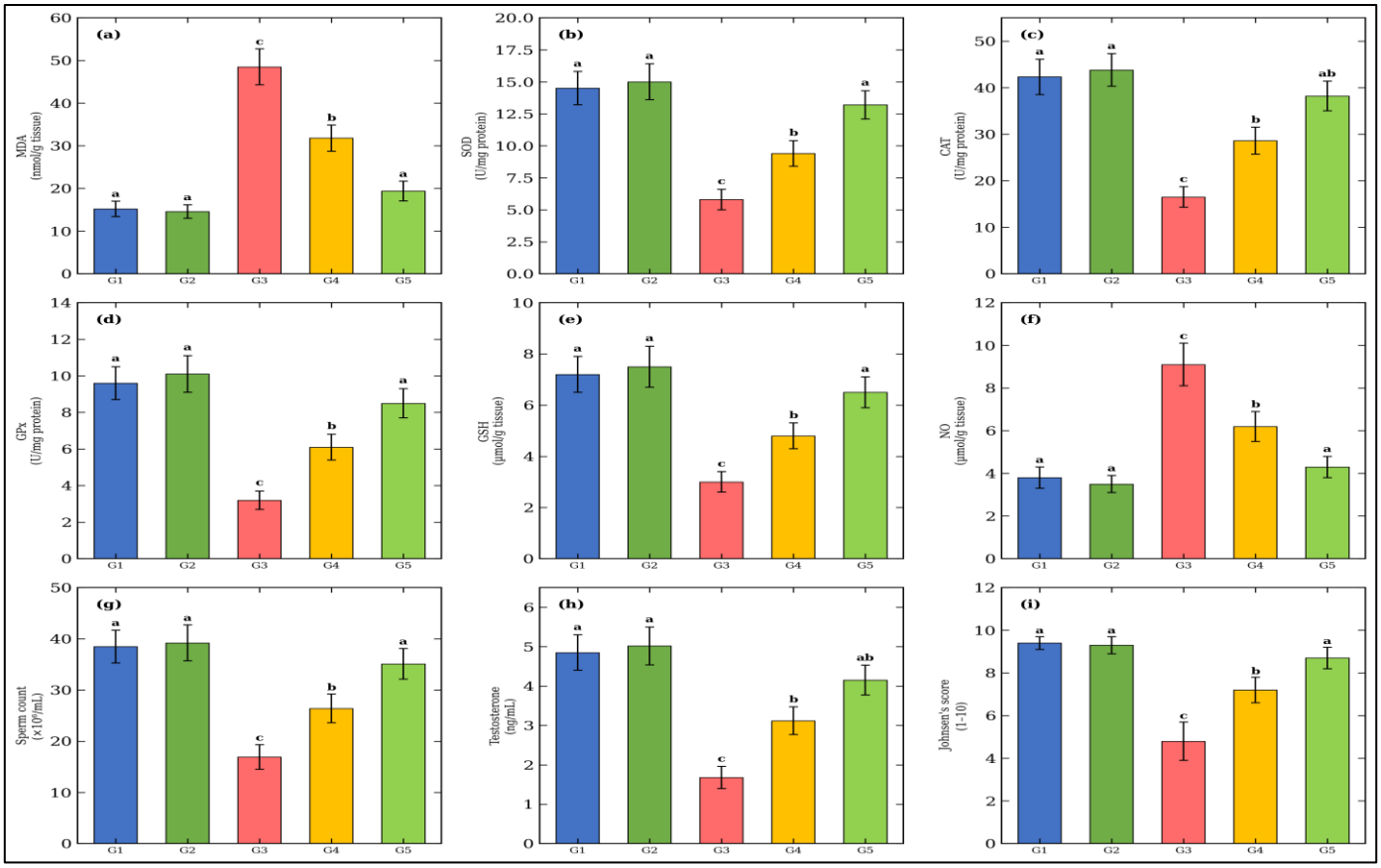

Fig 4 Comprehensive Bar Chart Presentation of (a–f) Testicular Oxidative Stress Markers (MDA, SOD, CAT, GPx, GSH, NO), (g) Epididymal Sperm Count, (h) Serum Testosterone, and (i) Johnsen's Spermatogenic Score Across Experimental Groups. Different Letters Indicate Statistically Significant Differences ($p < 0.05$).

➢ *Steroidogenic and Apoptosis-Related Gene Expression*

The mRNA expression profiles of key steroidogenic genes are illustrated in Fig. 5. Arsenic exposure (G3) caused dramatic downregulation of all five steroidogenic transcripts: StAR (~72%), CYP11A1 (~64%), CYP17A1 (~58%), 3β-HSD (~61%), and 17β-HSD (~55%) [63,64,65,66]. Co-treatment with SeNPs substantially rescued gene expression, with G5 restoring StAR to ~82% of control values [67].

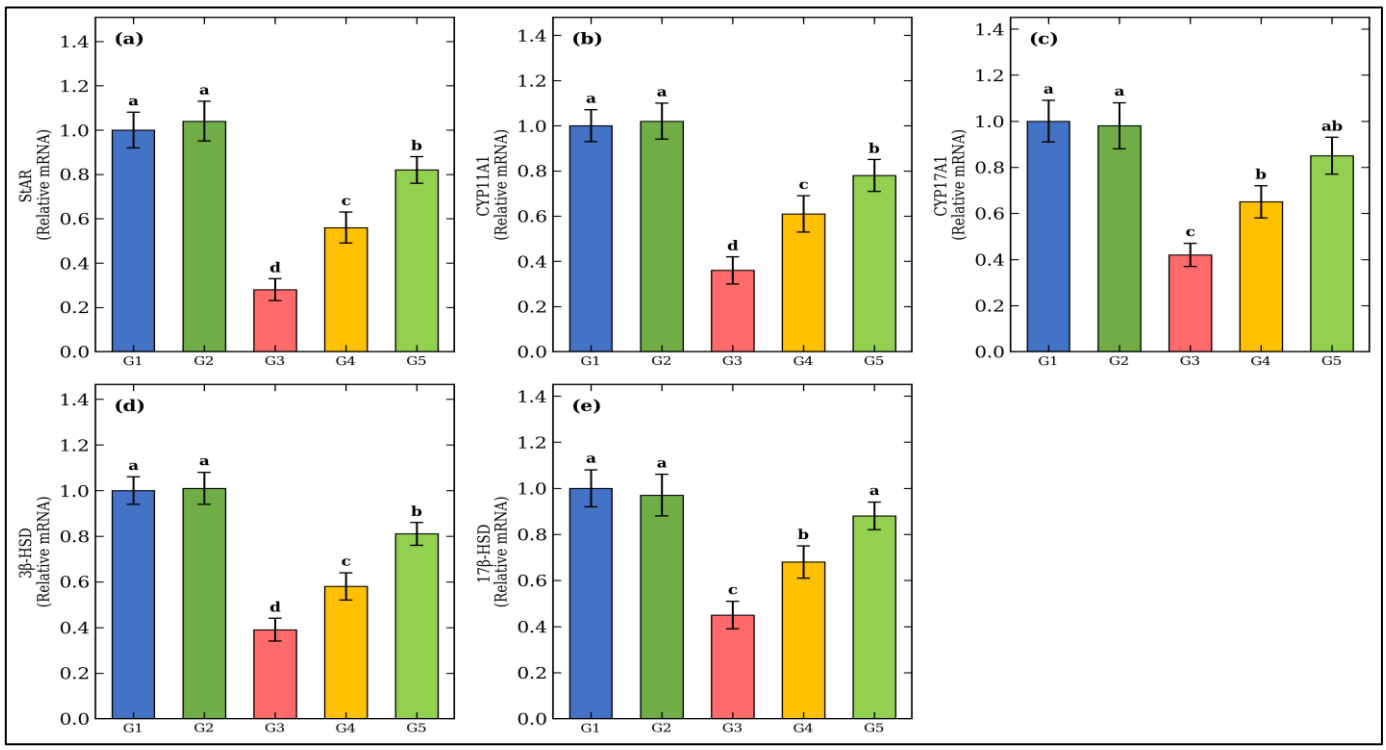

Fig 5 Relative mRNA Expression of Steroidogenic Genes in Testicular Tissue: (a) StAR, (b) CYP11A1, (c) CYP17A1, (d) 3β-HSD, and (e) 17β-HSD. Data Normalized to β-Actin by 2^{-DDct} Method. Different Letters Indicate $p < 0.05$ ($n = 8$).

Regarding apoptosis markers, the Bax/Bcl-2 mRNA ratio was elevated ~4.8-fold in G3, confirming a pro-apoptotic shift. SeNP co-treatment dose-dependently suppressed Bax while restoring Bcl-2 (Fig. 6) [68,69].

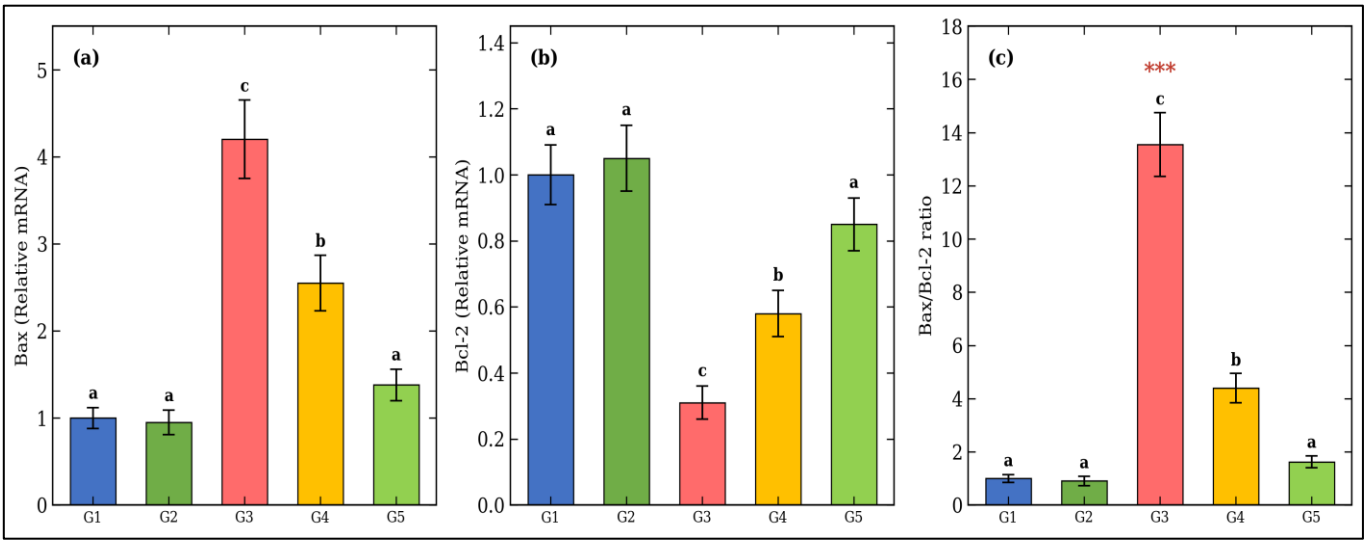

Fig 6 (a) Relative Bax mRNA Expression, (b) Bcl-2 mRNA Expression, and (c) Bax/Bcl-2 Ratio in Testicular Tissue. *** $p < 0.001$ vs. Control. Different Letters Indicate $p < 0.05$ ($n = 8$).

➢ *Histopathological Findings*

Control (G1) and SeNP-only (G2) groups displayed normal testicular architecture with Johnsen's scores of 9.4 ± 0.3 and 9.3 ± 0.4, respectively. Arsenic-treated testes (G3) presented severe histopathological lesions with Johnsen's score of 4.8 ± 0.9 [70,71]. The high-dose SeNP group (G5) showed substantial recovery (score: 8.7 ± 0.5). The semi-quantitative histopathological scores are presented in Fig. 4i.

The chemoprotective mechanism operates through three interconnected pathways: (1) selenoprotein-mediated ROS scavenging, (2) restoration of StAR/CYP11A1 and testosterone biosynthesis, and (3) anti-apoptotic Bax/Bcl-2 normalization [72,73,74,75]. The graphical abstract illustrates this multi-target mechanism.

IV. CONCLUSION

This study provides compelling evidence that biogenic selenium nanoparticles synthesized using *Moringa oleifera* leaf extract effectively mitigate arsenic-induced testicular dysfunction in adult mice through a multi-pronged mechanism encompassing antioxidant defense restoration, steroidogenic gene expression rescue, hormonal rebalancing, and anti-apoptotic activity. The Table 5 Hormonal profiles green-synthesized SeNPs showed spherical morphology (45–65 nm), high colloidal stability (ζ = -28.4 mV) and bioactive phytochemical corona. Oral co-administration of SeNPs at 0.25 and 0.5 mg/kg b.w. for 35 days dose-dependently normalised testicular MDA, NO, SOD, CAT, GPx, and GSH; restored serum testosterone, LH, and FSH; improved sperm count, motility, and morphology; up-regulated the mRNA expression of StAR, CYP11A1, CYP17A1, 3β-HSD, and 17β-HSD; and suppressed the arsenic-induced elevation of Bax/Bcl-2 ratio. Histopathology revealed preservation of the integrity of the seminiferous tubules. These results suggest biogenic SeNPs as potential nutraceutical intervention for populations chronically exposed to arsenic. Future studies should explore long-term safety, fertility outcomes in breeding trials, and epigenetic changes.

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➢ *Conflicts of Interest*

The authors declare no conflicts of interest.

➢ *Highlights*

- Biogenic SeNPs were synthesized using Moringa oleifera leaf extract, yielding spherical nanoparticles (45-65 nm).
- Chronic arsenic exposure severely impaired sperm parameters, serum reproductive hormones, and testicular antioxidant status.
- Arsenic downregulated steroidogenic genes (StAR, CYP11A1, CYP17A1, 3β-HSD, 17β-HSD) in testicular tissue.
- Co-treatment with biogenic SeNPs dose-dependently restored hormonal balance, gene expression, and spermatogenic function.
- The Bax/Bcl-2 apoptotic ratio was normalized by SeNP supplementation, confirming anti-apoptotic protection.

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