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Therapeutic Potential of Green Synthesized Selenium Nanoparticles Using Ficus Capensis Leaf Extract Against Lead–Induced Erectile Dysfunction in Male Rats

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ABSTRACT

Lead exposure is a major environmental and occupational hazard that induces oxidative stress, inflammation, and apoptosis, resulting in reproductive dysfunction and erectile impairment. This study investigated the therapeutic efficacy of green-synthesized selenium nanoparticles (SeNPs) using Ficus capensis leaf extract (FCLE) against lead-induced erectile dysfunction in male albino rats. Forty male rats were divided into eight groups (n = 5) and treated with lead acetate (0.5 mg/kg BW), FCLE (800 mg/kg BW), SeNPs (0.25 mg/kg BW), and their conjugate (FCLE–SeNPs) for 28 days. Biochemical assays were performed to assess antioxidant enzyme activities (SOD, CAT, GPx, GSH), lipid peroxidation (MDA), total antioxidant capacity (TAC), and inflammatory cytokines (TNF-α). Gene expression analysis of NF-κB, MMP9, and TGFβ1 was conducted using RT-qPCR, while sperm quality parameters were evaluated using a computer-assisted sperm analyzer (CASA). Results revealed that lead exposure significantly increased MDA, LDH, and TNF-α levels, upregulated the expression of NF-κB, MMP9, and TGFβ1 and decreased antioxidant enzyme activities and sperm quality indices (motility, viability, and count). Treatment with FCLE and SeNPs individually mitigated these effects, but the FCLE–SeNPs conjugate produced the most significant restoration. The conjugate normalized oxidative stress biomarkers, downregulated apoptotic and inflammatory genes, and improved sperm morphology and function to near-control levels. These findings demonstrate that green-synthesized FCLE–SeNPs possess potent antioxidant, anti-inflammatory, and anti-apoptotic properties, providing effective protection against lead-induced oxidative and reproductive damage. The study concludes that the FCLE–SeNPs conjugate is a promising, eco-friendly nanotherapeutic candidate for managing heavy-metal-induced infertility and erectile dysfunction.

Keywords— **Ficus capensis, Selenium nanoparticles, Lead toxicity, Erectile dysfunction, Oxidative stress, Gene expression, Nanotherapy**

I. INTRODUCTION

Erectile dysfunction (ED) is the inability to achieve and/or maintain penile erection sufficient for satisfactory sexual performance. It is a widespread problem affecting many sexually active men across all

age groups [1]. Normal penile erection is a function of neurovascular event, which depends on neural integrity, a functional vascular system and healthy cavernosal tissue [2]. These systems are mediated by nitric oxide (NO) via the activation of NO-cyclic guanosine monophosphate (cGMP) dilator pathway. The impairment of this pathway by different factors could lead to ED. Lead has been implicated as one of the causes of erectile dysfunction in mammal [2].

Lead mining is a major source of income and one of the most important non-farm rural activities in Nigeria [3]. Lead mining and other forms of Mining activities can result in the development of significant amounts of heavy metal-laden wastes that are dumped into the environment in an unregulated way, contaminating the ecosystem [4]. Lead mining, where over 99% of the harvested mineral is released into the environment as waste. Pose a great danger to mammalian penile tissues.

People with increased blood lead have significantly higher level of serum ROS and low level of antioxidant [5]. The increase in serum ROS could result to increased oxidative stress, low testosterone fertility and spermatogenesis [6]. Seminal plasma possesses an antioxidant system to mitigate the consequences of reactive oxygen species (ROS) accumulation; this seems to protect sperm cells. Unfortunately, this protective capacity is limited in spermatozoa compared with somatic cells [7] and [8]. Several medications have been produced for the treatment of erectile disorder. But most of them are expensive and also have toxic impact on the penile tissues. Therefore, a cheap, eco –friendly and more efficient product is imperative for erectile malfunction therapy. There are reports that trace element selenium (Se) has a pleiotropic effect and has a high therapeutic potential for the treatment of various diseases; It has been shown that Se di-oxide can prevent the oxidative damage of DNA caused by the interaction of Fe (II), Cr (III) and Cu (II) with hydrogen peroxide by forming a coordination complex with metals [9]. Selenium nanoparticles (SeNP) have significant advantages over other Se-containing compounds. SeNPs display exceptional bioavailability, low toxicity, and potent antioxidant and antibacterial effects [10]. SeNP have selective cytotoxicity that lead to the death of cancer cells and do not have a cytotoxic effect on normal cells.

Numerous procedures abound for SeNPs synthesis via physical, chemical, and biological methods are available [11]. However, some techniques are expensive, require toxic chemicals, and involve complicated, tedious, and non-sustainable protocols. Consequently, biological procedures are preferred over chemical and physical ones [12]. A simple and rapid method for green synthesized metal nanoparticles using plant extracts as reducing and capping agents is preferred [13]. Such synthesis is green, eco-friendly, bio-

reductive, only one-step, cost-effective, and requires shorter reaction time and fewer solvents. There are reports also that herbs and plants with antioxidant enrichment protect cells by mitigating oxidative damage [14]. .Ficus capensis Thunb (Moraceae) is one of such plants. Ficus capensis is a medicinal plant widely grown in tropical and subtropical regions with the leaf decoction commonly taken as fertility agent in men [15] and for the treatment of dysentery, oedema, leprosy, epilepsy, rickets, gonorrhoea, respiratory disorders and emollient [16]. The leaves of F. capensis possess various pharmacological properties such as antioxid7ant, anti-inflammation, and antimicrobial effects. F. capensis medicinal benefits is attributed to its phytochemicals such as phenolic compounds [17]. Study has revealed that phenolics of medicinal plants are related to their antioxidant capacity [18]. Therefore, F. capensis leaves extract and selenium nanoparticles conjugate is expected to produce excellent, cheap, environmentally friendly and more efficient result in treatment of lead induced erectile dysfunction.

The present study aimed to synthesize characterize green SeNPs using Ficus capensis leaf extract (FCLE-SeNPs). Secondly, the study compared, the in vivo protective outcomes of green-synthesized FCLE-SeNPs conjugate or FCLE as treatments for erectile dyfunction caused by lead in male rats.

II. MATERIALS AND METHODS

A. Collection of samples

The F.capensis leaves (FCL) were freshly obtained from Ado- fresh Ekiti and authenticated by a Botanist in The Federal Polytechnic Ado-Ekiti, Ekiti State, Nigeria. The leaves were air- dried at room temperature for four weeks. The crispy leaves were ground into powder using high-speed milling device. Three hundred grams of powder was extracted in absolute ethanol for 48 hours then filtered twice through filter paper with 2µm pore size. The resultant extract was concentrated and evaporated to dryness using a rotary evaporator at 40–45^{0}C. The residual yield of (FCLE) extract was 17.8 g/300 g of dried powder. The obtained extract was kept at 4◦C until use.

B. Chemical Synthesis of selenium Nanoparticles (SeNPs)

0.5 M sodium hydroxide (NaOH) solution was prepared by dissolving 20 g of sodium hydroxide pellets in 1L of deionized water which was stirred to dissolve completely. 5 g of sodium selenite (Na2SeO3) was weighed out and was added to 100 mL of deionized water in a 250 mL flask and was stirred until the sodium selenite is completely dissolved. And 10 mL of 0.5 M sodium hydroxide solution was added slowly to the flask containing the sodium selenite solution (Na2SeO3), while stirring continuously with a magnetic stir bar. The pH of the solution with a pH meter or indicator paper was carefully monitored and adjusted as

necessary to maintain a pH of around 10-11.

A solution of 0.02 M sodium borohydride was prepared by dissolving 1.2 g of sodium borohydride in 100 mL of deionized water which was stirred to dissolve completely. The 0.02 M sodium borohydride solution was added to the flask containing the sodium selenite solution, while stirring continuously with a magnetic stir bar. The reaction mixture turned yellow or orange as the reduction of selenite ions to selenium nanoparticles (SeNPs) occurs. This was Stirred Continuously for 2 hours at room temperature or at 50-70OC. After the reaction was completed, 50 mL of ethanol was added to the flask to stop the reaction. The solution was Stirred for an additional 30 minutes to ensure complete mixture. The reaction mixture was transferred into a centrifuge tube and was centrifuged at 5000 rpm for 15 minutes to separate the selenium nanoparticles from the solution. The supernatant was then discarded, and the selenium nanoparticles were washed several times with deionized water to remove any remaining impurities. A few drops of hydrochloric acid was added into the selenium nanoparticle suspension to stabilize the nanoparticles and the pH was adjusted to around 2-3. The selenium nanoparticle (SeNPs) suspension was Stored in a dark, cool place until ready for use.

C. Synthesis of Ficus capensis Leave Extract-Mediated Selenium Nanoparticles (FCLE-SeNPs)

5g of sodium selenite was dissolved in 100mL of deionized water in a beaker. 0.2g of ascorbic acid was dissolved in 20mL of deionized water in a separate beaker. The F.capensis leave extract was prepared by adding 10g of dried powdered leaves to 100mL of distilled water in a flask. The mixture was stirred under reflux for 30 minutes, and then the extract was filtered using a filter paper. 10mL of the F.capensis leave extract was added to 90 mL of sodium selenite solution and the mixture was stirred for 10minutes. 1mL of the ascorbic acid solution was added to the mixture and then stirred for an additional 10minutes.

1M of NaOH solution was added to the mixture until the pH reaches 10. The color of the mixture then turns yellow-green. And the mixture was placed on a magnetic stirrer and stirred for 2 hours. Selenium nanoparticles synthesis was stopped when the color of the mixture changes to brick red. A UV-Vis spectrophotometer was used to monitor the absorption spectrum of the mixture at different time intervals until the maximum absorption peak is reached, which is around 320 nm. The mixture was centrifuged at 10,000 rpm for 20 minutes to separate the green selenium nanoparticles (FCLE-SeNPs). The supernatant was discarded and the nanoparticles were washed several times with deionized water to remove any impurities. The nanoparticles were dried in a kiln at 150OC for 1 hours and then the nanoparticle was again weighed to determine the yield which was 5.7g. The nanoparticles was stored in a cool dry place for further use.

The resulting green selenium nanoparticles (FCLE-SeNPs) were characterized using various techniques such as transmission electron microscopy (TEM), X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FTIR).

D. Animals and study protocol

Forty (40) number male albino rats of age Twenty (20 +4 days) weeks old, with average BWs of 120–185g were housed in Animal house, School of Science and Computer studies, Department of Science Technology, Federal polytechnic Ado-Ekiti, Ekiti State Nigeria. The Federal polytechnic Ado-Ekiti, Ekiti State animal care and use committee guideline was strictly followed. The animal ethical approval No.S/2029 dated April, 20th 2025 was obtained from the committee. All animals were housed in stainless steel cages and acclimated at 25ºC- 27ºC, a relative humidity 30–50% and a 12-hour light-dark cycle. Rats were fed a standard rat pellet diet and allowed free access to food and water throughout the experimental period. All animals were cared for humanely according to the conditions stated in the Guide for the Care and Use of Laboratory Animals of the National Academy of Science (NAS), published by the National Institute of Health. The study also followed National Institutes of Health's recommendations for the care and use of laboratory animals, and adequate procedures were made to ensure that the animals were not subjected to excessive pain and discomfort during the study.

E. Experimental design and dose administration

Animals were randomly allocated into eight (8) groups of five (n= 5) rats each and administered orally in the following ways:

Group I: control rats received 1mL of 0.9% deionized water.

Group 11: negative control received lead solution (0.5mg/kg BW).

Group III: rats received FCLE (800 mg/kg BW).

Group IV: rats received SeNPs (0. 25 mg//kg BW).

Group V: rats received FCLE-SeNPs (0. 25mg//kg BW).

Group VI: rats received Pb (0.5 mg/kg BW) + FCLE (800 mg/kg BW).

Group VII: rats received Pb (0.5 mg/kg BW) + SeNPs (0.25mg/kg BW).

Group VIII: rats received Pb (0.5 mg/kg BW) + FCLE-SeNP (0.25mg/kg BW). Gavages were day after day for 28 days, using a feeding needle. All animals were carefully observed throughout the experiment for any signs of intoxication or mortalities.

F. Experimental design and dose administration

Twenty-four hours after the last treatment, the over-night-fasted rats were weighed and anesthetized with ketamin at the end of the dosing period. Two mL blood samples were collected from the rats via cardiac

puncture. Blood was collected using a BD Vacutainer PST II Tubes, left for coagulation, then centrifuged at 322 x g for 20 min for serum separation. Sera were preserved at−20 °C until biochemical analysis. Liver and testis tissues were collected and divided into four portions. Thirty mg was washed in cold saline and mix with RNA shield for storage before RNA extraction.

G. RT-PCR analysis of apoptosis and stress related gene expression

Total RNA was extracted using Quick-RNA™ Miniprep Kit (Zymo Research) and cDNA synthesis was performed using the ProtoScript II First Strand cDNA Synthesis Kit (New England Biolabs Inc). For analysis of gene expression, the real-time RT-PCR was performed in an Chai Real-Time PCR thermocycler with touchscreen (Chai Inc U.S.A) using Chai Green qPCR Master (Chai Inc U.S.A) following the manufacturer's instructions. The PCR cycling conditions included an initial denaturation at 95 OC for 15 min followed by 40 cycles of denaturation at 95OC for 30 s, annealing at 60OC for 60s, and extension at 72OC for 60 s. The oligonucleotide specific primers were synthesized by Inqaba Biotec Africa Ltd (South Africa) as shown in Table 1. The expression level of the target genes was normalized to that of house-keeping gene (GAPDH). The relative fold changes in the gene expression were estimated based on the comparative $2 - \Delta\Delta CT$ (Ct: cycle threshold) method with the GAPDH gene as an internal control to normalize target genes expression levels [19].

H. Sperm Analysis

Sperm analysis was carried out by collecting the epididymis from each testis, homogenized in cold normal saline. One drop of the homogenate was placed on a slide for sperm analysis using computer-assisted sperm analyser (SpermAnalyzeWin7 Xuzhou city, China, the setting of CASA as in 5th WHO manual, 51 sperm tracks, evaluated magnification x10, image acquisition rate: number frames/s 60);

Sperm integrity such as percentage motility, progressive motility, non-progressive motility, curvilinear velocity (um/s), sperm concentration and livability were also determined using Neubauer haemocytometer (TH-100; HechtAssistant, Sondheim, Germany) and expressed as spermatozoa x106/mL. Livability was done by placing a drop of semen on a glass slide, one drop of eosin stain added and mixed gently, then smeared on a slide, air-dried and viewed under the microscope at a magnification of x400.

Table 1: Primer sequence

Gene	Forward primer (5–3′)	Reverse primer (5–3′)
NF-KB	AACAGAGAGGATTTCGTTTCCG	TTTGACCTGAGGGTAAGACTTCT
MMP9	CCTCTGCATGAAGACGACATAA	GGTCAGGTTTAGAGCCACGA
TGFβ1	CTGAACCAAGGAGACGGAAT	GGTTCATGTCATGGATGGTG

I. Analyses of antioxidant biomarkers

Superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA), catalase (CAT), and glutathione concentrations (GSH) were assessed using serum samples and the analysis kits as instructed by the manufacturer (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Mobi Microplate spectrophotometer (Sunnyvale, CA) was used for all analyses.

J. Assessment of TNF-α

Level of TNF-α was determined using a standard Enzyme-linked immunosorbent assay ELISA kit (Elabscience Biotechnology Co., Ltd, USA). 100 µL of the standard and sample was added to each well and incubated for 90 min at 37^0C. The liquid was then removed. 100 µL of Biotinylated Detection Ab. Solution was added and incubated for 1 hour at 37^0C.

K. Statistical analysis of data

All obtained raw data were analyzed using a one-way analysis of variance (ANOVA) by using the SPSS 25.0 computer program followed by post hoc Tukey HSD multiple comparisons to determine the statistically significant variations between the various parameters in the experimental replicates. A p-value of < 0.05 was considered statistically significant. Data were shown as means ± SD. All graphs were generated using GraphPad Prism 8 (GraphPad Software Inc., San Diego, 189 CA, USA).

III. RESULTS AND DISCUSSION

All experimental groups showed no altered clinical behavioral alterations except for Pb-induced rats that displayed a decreased in biological activity, food consumption, and body weights with frequently increased dizziness when compared with the other experimental groups. However, rats from the Pb-FCLE, Pb-SeNP and Pb-FCLE-SeNP groups were clinically normal with moderate biological activities.

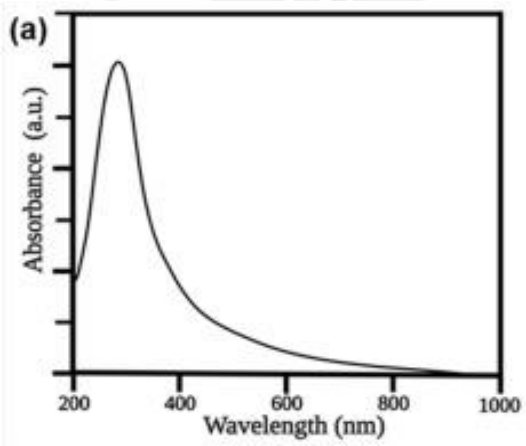

Figure 1: The UV–visible spectrum

Figure 2: Green synthesized SeNPs

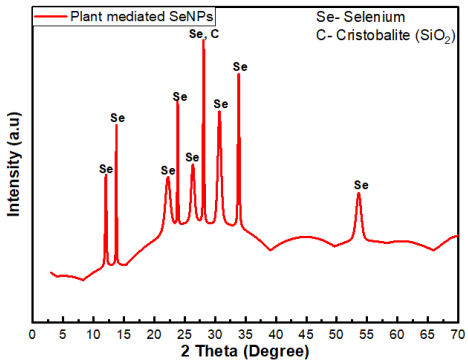

Figure 3: XRD Spectra of the green SeNPs

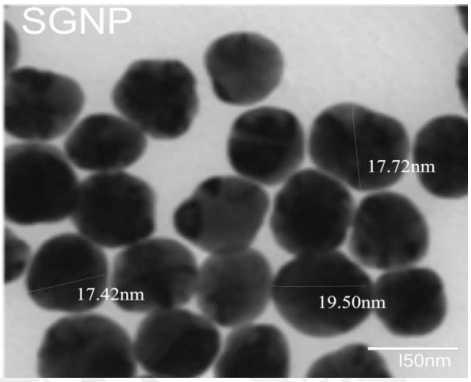

Figure 4: TEM Analysis

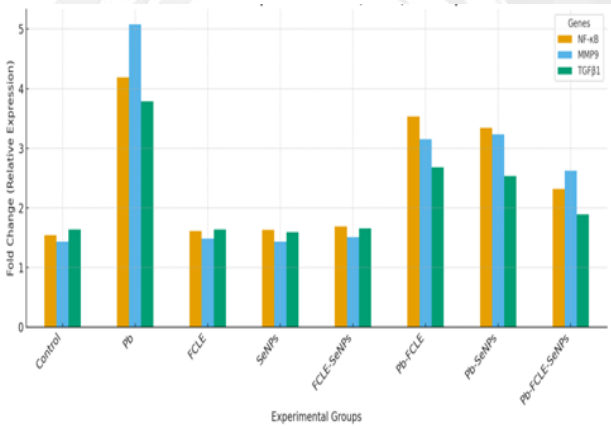

Figure 5: Relative Gene Expressions of NF - κβ, MM9 and TGFβ1

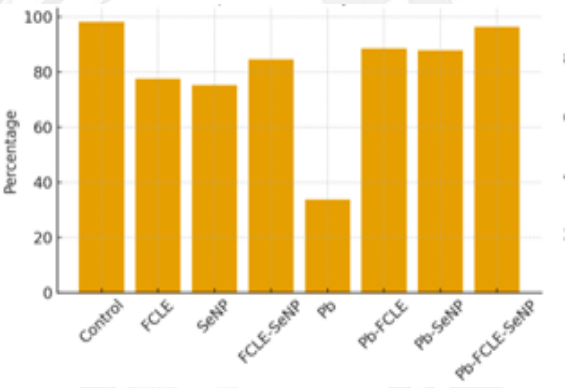

Figure 6: Percentage sperm mortility

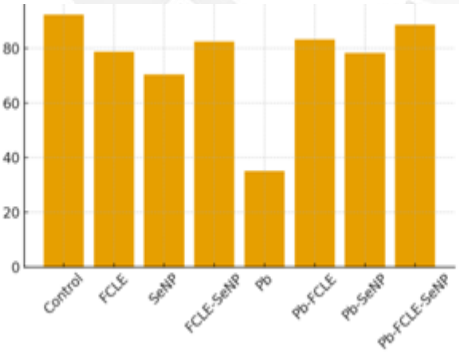

Figure 7: Percentage sperm viability

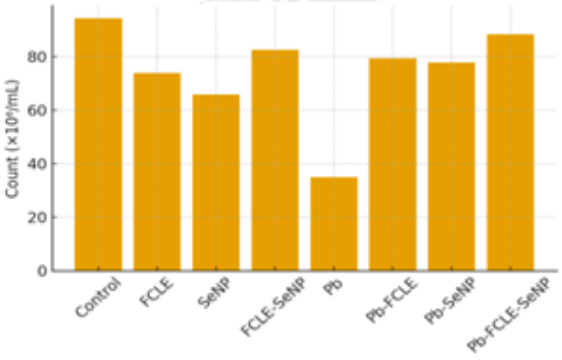

Figure 8: Sperm Count (10^0/mL)

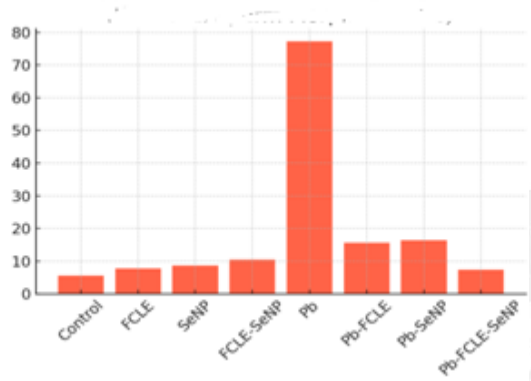

Figure 9: Percentage Abnormal Sperm Morphology

Table 2. Relative gene expression (mean ± sd)

Group	NF-κB	MMP-9	TGF-β1
Control	1.545 ± 0.016	1.432 ± 0.009	1.634 ± 0.002
Pb	4.187 ± 0.002	5.087 ± 0.011	3.788 ± 0.002
FCLE	1.616 ± 0.005	1.477 ± 0.013	1.640 ± 0.002
SeNPs	1.620 ± 0.020	1.431 ± 0.003	1.592 ± 0.002
FCLE-SeNPs	1.650 ± 0.053	1.512 ± 0.002	1.653 ± 0.001
Pb-FCLE	3.518 ± 0.017	3.151 ± 0.002	2.676 ± 0.004
Pb-SeNPs	3.345 ± 0.010	3.231 ± 0.003	2.534 ± 0.003
Pb-FCLE-SeNPs	2.327 ± 0.012	2.621 ± 0.002	1.890 ± 0.004

NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells), **MMP9** (Matrix Metalloproteinase 9) and **TGFβ1** (Transforming Growth Factor Beta 1)

Table 3. Relative Gene Expression one –way ANOVA

Parameter	F-value	p-value	Significance
NF-Kb	6645.68	<0.001	Significant
MMP9	105714.14	<0.001	Significant
TGF-β1	284148.84	<0.001	Significant

Table 4. Oxidative Stress Biomarkers (Mean ± SD)

Parameter	Control	Pb	FCLE	SeNPs	FCLE-SeNPs	Pb-FCLE	Pb-SeNPs	Pb-FCLE-SeNPs
MDA (µM)	1.761 ± 0.003	3.993 ± 0.003	3.673 ± 0.002	3.831 ± 0.003	7.263 ± 0.005	3.104 ± 0.003	2.903 ± 0.003	2.514 ± 0.004
SOD (U/mL)	2.820 ± 0.002	1.876 ± 0.002	1.617 ± 0.003	1.711 ± 0.006	0.563 ± 0.003	1.460 ± 0.004	1.518 ± 0.004	2.351 ± 0.002
Catalase (µmol/ml/min)	8.215 ± 0.003	4.501 ± 0.004	4.821 ± 0.006	4.493 ± 0.005	2.904 ± 0.005	4.987 ± 0.002	5.430 ± 0.003	6.418 ± 0.003
GSH (mM)	0.344 ± 0.004	0.170 ± 0.005	0.195 ± 0.003	0.185 ± 0.005	0.032 ± 0.003	0.181 ± 0.003	0.202 ± 0.004	0.272 ± 0.004
GPx (µ/L)	89.224 ± 0.597	67.606 ± 0.956	69.558 ± 0.901	68.011 ± 0.615	43.142 ± 1.055	68.487 ± 0.541	67.192 ± 0.108	80.084 ± 0.516
TAC (mM/g tissue)	1.162 ± 0.016	0.705 ± 0.004	0.710 ± 0.003	0.739 ± 0.004	0.326 ± 0.003	0.743 ± 0.003	0.695 ± 0.007	0.983 ± 0.014
LDH (µ/L)	46.149 ± 0.419	73.287 ± 0.470	70.270 ± 0.695	71.306 ± 0.380	87.411 ± 0.888	72.349 ± 1.339	76.009 ± 0.685	57.486 ± 0.827
TNF-α (pg/mL)	18.149 ± 0.703	35.796 ± 0.578	32.498 ± 0.570	35.631 ± 1.179	75.971 ± 0.423	27.422 ± 0.416	25.923 ± 0.851	22.904 ± 0.577

Mean +SD values of Oxidative stress biomarkers and TNF – α

Table 5. Oxidative Stress Biomarkers one- way ANOVA

Parameter	F-value	p-value	Significance
MDA	835068.09	<0.001	Significant
SOD	120763.03	<0.001	Significant
Catalase	494561.46	<0.001	Significant
GSH	1672.32	<0.001	Significant
GPx	1004.07	<0.001	Significant
TAC	2548.23	<0.001	Significant
LDH	778.69	<0.001	Significant
TNF-α	1992.50	<0.001	Significant

Table 6. Reproductive Parameters (Mean ± SD)

Parameter	Control	Pb	FCLE	SeNPs	FCLE-SeNPs	Pb-FCLE	Pb-SeNPs	Pb-FCLE-SeNPs
Motility (%)	97.97 ± 0.29	77.50 ± 0.67	75.19 ± 0.16	84.18 ± 0.24	31.83 ± 0.83	87.92 ± 0.61	87.46 ± 0.44	96.38 ± 0.71
Progressive Motility (%)	81.20 ± 0.22	70.58 ± 0.63	62.65 ± 1.34	71.93 ± 0.44	11.64 ± 0.92	70.32 ± 0.57	69.91 ± 0.75	80.44 ± 0.89
Non-progressive Motility (%)	55.46 ± 0.65	36.35 ± 0.71	34.22 ± 0.29	41.83 ± 0.62	7.68 ± 0.70	44.24 ± 1.25	41.84 ± 1.56	51.54 ± 1.18
Viability (%)	91.53 ± 1.01	78.27 ± 1.19	70.19 ± 1.10	82.55 ± 1.55	34.55 ± 0.65	83.16 ± 0.91	78.16 ± 0.18	88.70 ± 0.38
Sperm Count ($\times 10^6$/mL)	93.76 ± 0.60	73.21 ± 0.60	65.00 ± 1.00	82.56 ± 0.44	35.78 ± 0.93	78.99 ± 0.32	79.13 ± 0.60	87.92 ± 1.31
Abnormal Morphology (%)	5.58 ± 0.21	7.64 ± 0.17	8.31 ± 0.31	10.41 ± 1.30	75.78 ± 1.35	16.72 ± 1.20	17.38 ± 1.21	7.89 ± 0.50
Membrane Integrity (%)	93.38 ± 1.65	73.86 ± 0.72	72.63 ± 0.56	76.30 ± 0.45	29.17 ± 0.77	77.83 ± 1.03	74.42 ± 1.37	90.18 ± 0.96
Acrosome Integrity (%)	95.39 ± 0.54	79.07 ± 0.21	81.93 ± 1.06	81.63 ± 0.72	29.92 ± 1.01	80.39 ± 1.21	78.23 ± 1.42	91.06 ± 1.66
Sperm Concentration ($\times 10^6$/mL)	86.01 ± 1.65	66.73 ± 1.62	63.41 ± 0.53	73.36 ± 1.67	17.56 ± 0.64	67.75 ± 0.99	65.32 ± 2.09	83.23 ± 1.99
Total Motile Sperm ($\times 10^6$/mL)	85.17 ± 1.16	65.52 ± 1.61	61.09 ± 1.72	70.76 ± 0.49	13.08 ± 1.24	66.93 ± 1.62	63.18 ± 2.05	82.22 ± 1.39
Total Live Sperm ($\times 10^6$/mL)	74.65 ± 0.95	60.93 ± 1.57	60.51 ± 2.12	60.44 ± 0.54	16.40 ± 1.98	61.48 ± 2.30	60.99 ± 1.94	71.05 ± 2.25

Mean + SD Values of M: motility, PM: progressive motility, NP: Non-progressive motility, V: Viable and other reproductive parameters

Table 7. Reproductive Parameters one- way ANOVA

Parameter	F-value	p-value	Significance
Motility (M)	4465.34	<0.001	Significant
Progressive Motility (PM)	2414.59	<0.001	Significant
Non-Progressive Motility (NP)	1.00	0.466	Not Significant
Viability (V)	1033.98	<0.001	Significant
Sperm Count	1579.28	<0.001	Significant
Abnormal Morphology	1917.61	<0.001	Significant
Membrane Integrity	1112.52	<0.001	Significant
Acrosome Integrity	1133.95	<0.001	Significant
Sperm Concentration	583.16	<0.001	Significant
Total Motile Sperm	0.93	0.510	Not Significant
Total Live Sperm	291.95	<0.001	Significant

IV. DISCUSSION

A. Characterization of Nanoparticles

(i) UV–visible spectroscopic analysis of Se-NPs

Green SeNPs was synthesized by reduction of Se into Se-NPs after the addition of FCLE filtrate The stimulation of the surface plasmon resonance of Se-NPs gave the reaction solution its distinctive brick-red colour, which served as a useful spectroscopic hallmark of their production (Figure 1). Using a UV–Visible spectrophotometer, the reduction of selenite was analyzed using spectroscopy. This revealed a 320 nm absorbance peak (Figure 2), which was peculiar to Se-NPs. This finding aligns with the report of [20] and [21] who asserts that SeNPs typically exhibit characteristic surface plasmon resonance bands, often in the 260-350 nm range.

(ii) XRD Spectra of the Green Synthesized SeNPs

A series of diffraction peaks that were consistent with the theory were detected during the measurement of the green synthesized Se-NPs, and the prominent peaks of the XRD pattern at 2Ø (theta) 150, 200, 300 and 350 respectively indicate that the green synthesized Se-NPs were well-crystallized [22] XRD have been used to validate the crystalline structure of Se-NPs synthesized through various green methods, including plant extracts and bacterial processes [23].

(iii) Transmission Electron Microscopy (TEM) Analysis

Figure 4 above gave the TEM analysis of the green synthesized SeNPs. The images indicate the size and

shape of the synthesized nanoparticles. The figure shows that SeNPs are spherical in shape and polydispersed. The size ranges are 17.45nm – 19. 50nm giving an average size of 18.48nm. This shows that the particles are spherical and evenly distributed. The report was supported by the work of [24], who showed that TEM can reliably determine dot shape, size, and density of nanomaterial. [25] further emphasizes TEM's versatility, noting that it can analyze "size, shape, arrangement and chemical composition" of nano-sized materials.

(iv) FTIR analysis of Green Se-NPs

The functional groups found in Se-NP were emphasized by the green produced Se-NPs' FTIR spectra. The stretching vibration of the Se–O bond in SeNPs may be responsible for the peak that emerged at about 660 cm−1. The phenolic hydroxyl group, phenolic acids, terpenoids, phenols, and aliphatic amines are responsible for the formation of these linkages. These functional groups act as the capping agent for the nanoparticles and also as a reducing agent of Se2+ ions [26].

B. Gene Expression

The results in Table 2 above show the relative gene expression of NF-κB, MMP9, and TGFβ1 respectively. One-way analysis of variance (ANOVA) demonstrated significant differences among treatment groups for NF-κB, MMP-9 and TGF-β1 gene expression ($p < 0.001$) (Table 3). Lead exposure significantly upregulated the expression of these inflammatory markers compared with the control group. Treatment with Ficus capensis leaf extract (FCLE), selenium nanoparticles (SeNPs), and green-synthesized FCLE-SeNPs significantly attenuated the lead-induced increase in inflammatory gene expression. Among the treatment groups, the combined Pb-FCLE-SeNPs treatment exhibited the most pronounced reduction, indicating superior anti-inflammatory activity against lead-induced reproductive toxicity. [27] Demonstrated lead's activation of nuclear transcription factors, while [28] report that lead can increase NF-κB making it candidate for inflammation. [29] also demonstrated that lead elevates MMP-9 expression, suggesting potential tissue damage mechanisms. [30] Further confirmed that selenium nanoparticles ameliorate lead-induced testicular toxicity.

The research was in line with the work of [31], who demonstrates that lead exposure significantly increases TGFβ1, indicating fibrotic and apoptotic stress. The results agree with the work of, [32] who reported that selenium reduces lead-induced oxidative stress and apoptosis in sheep Leydig cell by increasing cell viability and modulating relevant gene expressions. The work also align with the report of [33], who assert that the mechanism likely involves interrupting the feed-forward cycle between TGFβ1 and reactive oxygen species where TGFβ1 increases ROS production and ROS, in turn, activate TGFβ1.

C. Oxidative Stress

The results show that lead exposure significantly disrupted the redox balance in rats. Lead exposure significantly elevated lipid peroxidation (MDA), LDH, and TNF-α levels, and significantly reduced antioxidant defenses, including SOD, catalase, GPx, GSH, and TAC, compared with the control group. One-way ANOVA followed by Tukey's post hoc test revealed significant differences among the experimental groups for oxidative stress ($p < 0.05$) (Table 5). These findings indicate the induction of severe oxidative stress, inflammation, and tissue injury following lead intoxication. (Table.4). However, the green synthesized nanoparticles (FCLE-SeNPs) showed significant impaired oxidative stress recovery when administered to the lead- induced albino male rats (Table 4). This again agrees with the work of [34], who affirms that oxidative stress impairs Leydig cell function, affecting male fertility. [35], further assert that nanoparticle, improve bioavailability and compound release making this approach a promising strategy for mitigating oxidative stress,

D. Reproductive parameter

The reproductive parameters measured included sperm motility (M), progressive motility (PM), non-progressive motility (NP), viability (V%), sperm count, sperm morphology, sperm membrane and acrosome integrity, and total live/motile sperm. These indicators collectively reflect testicular and epididymal function, spermatogenesis efficiency, and sperm quality.

The results revealed that lead exhibited severe reproductive impairment ranging from reduced sperm motility (31.8%), viability (34.5%), sperm count (35.01×10^6/mL) and increased Abnormal morphology (77.23%),(Table 6) indicates spermatogenic and epididymal damage due to oxidative stress, lipid peroxidation, and disrupted testicular architecture (Table 6). One-way ANOVA revealed significant differences among experimental groups for all oxidative stress biomarkers and reproductive parameters ($p < 0.001$). Tukey's multiple comparison test demonstrated that lead exposure significantly induced oxidative stress and impaired reproductive function compared with controls (Table 7). Administration of FCLE, SeNPs and FCLE-SeNPs significantly ameliorated these alterations, with the Pb-FCLE-SeNPs group exhibiting values closest to the control group, indicating superior protective efficacy against lead-induced reproductive toxicity (Table 6). The work agrees with the report of [36], who affirms that Ficus capensis leaf extract (FCLE) and SeNPs independently improved reproductive health, restored antioxidant capacity and improved sperm morphology. [33] Further reports that SeNPs enhanced antioxidant defense.

V. CONCLUSION

Collectively, these findings indicate that green-synthesized selenium nanoparticles derived from Ficus capensis possess potent antioxidant, anti-inflammatory, and reproductive protective properties against lead-induced toxicity. The superior efficacy observed in the green synthesized selenium nanoparticles treated group suggests a synergistic interaction between the phytochemicals of Ficus capensis and selenium nanoparticles, resulting in enhanced mitigation of oxidative damage and restoration of reproductive function. However, more extensive molecular and proteomic analyses should be carried out to clarify the exact signaling pathways involved in FCLE–SeNPs-mediated protection, particularly the modulation of NF-κB, TGFβ1, and apoptotic gene networks.

CONFLICT OF INTEREST DECLARATION

On behalf of the authors, I wish to sincerely thank our friends and colleagues who have in one way or the other assisted us in the realization of this work. I extend my profound gratitude to Dr Oyeyemi B, Dr John Owabumoye and Mr Orji . for their various contributions to bringing this project to the limelight.

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REFERENCES

[1] B. O. O., A. C. Jemilohun, and S. A. Aderibigbe, “Prevalence of erectile dysfunction and possible risk factors among men of South-Western Nigeria: A population based study,” Pan African Medical Journal, vol. 24, p. 124, 2016, doi: 10.11604/pamj.2016.24.124.8660.

[2] K. E. Andersson and G. Wagner, “Physiology of penile erection,” Physiological Reviews, vol. 75, pp. 191–236, 1995.

[3] R. Maconachie and F. M. Conteh, “Artisanal mining, mechanization and human (in)security in Sierra Leone,” The Extractive Industries and Society, 2021, doi: 10.1016/j.exis.2021.100983.

[4] E. Gyamfi, E. K. Appiah-Adjei, and K. A. Adjei, “Potential heavy metal pollution of soil and water resources from artisanal mining in Kokoteasua, Ghana,” Groundwater for Sustainable Development, vol. 8, pp. 450–456, 2019, doi: 10.1016/j.gsd.2019.01.007.

[5] M. S. Kshirsagar, J. Patil, and A. Patil, "Increased blood lead level induces oxidative stress and alters the antioxidant status of spray painters," Journal of Basic and Clinical Physiology and Pharmacology, vol. 31, no. 2, 2020, doi: 10.1515/jbcpp-2018-0229.

[6] R. Maconachie and F. M. Conteh, "Artisanal mining, mechanization and human (in)security in Sierra Leone," The Extractive Industries and Society, 2021, doi: 10.1016/j.exis.2021.100983.

[7] O. A. Jimoh, "Assessment of the oxidative stress markers and reproductive performance of four exotic breeds of rabbit in Ibadan," Ph.D. dissertation, Univ. Ibadan, Ibadan, Nigeria, 2016, p. 48, doi: 10.13140/RG.2.2.27229.23522/1.

[8] O. A. Jimoh and E. O. Ewuola, "Semen characteristics, seminal biochemical and oxidative stress markers in rabbits during heat stress," Journal of Veterinary Andrology, vol. 3, pp. 35–44, 2018.

[9] D. R. Lloyd and D. H. Phillips, "Oxidative DNA damage mediated by copper(II), iron(II) and nickel(II) Fenton reactions: Evidence for site-specific mechanisms in the formation of double-strand breaks, 8-hydroxydeoxyguanosine and putative intrastrand cross-links," Mutation Research, vol. 424, nos. 1–2, pp. 23–36, 1999, doi: 10.1016/S0027-5107(99)00005-6.

[10] V. Alagesan and S. Venugopal, "Green synthesis of selenium nanoparticle using leaves extract of Withania somnifera and its biological applications and photocatalytic activities," Bionanoscience, vol. 9, pp. 105–116, 2019.

[11] S. Chaudhary and S. Mehta, "Selenium nanomaterials: Applications in electronics, catalysis and sensors," Journal of Nanoscience and Nanotechnology, vol. 14, pp. 1658–1674, 2014.

[12] S. Skalickova, V. Milosavljevic, K. Cihalova, P. Horky, L. Richtera, and V. Adam, "Selenium nanoparticles as a nutritional supplement," Nutrition, vol. 33, pp. 83–90, 2017.

[13] A. K. Mittal, Y. Chisti, and U. C. Banerjee, "Synthesis of metallic nanoparticles using plant extracts," Biotechnology Advances, vol. 31, pp. 346–356, 2013.

[14] O. A. Jimoh, U. G. Ihejirika, A. S. Balogun, and U. C. Uwaeziozi, "Antioxidative effect of mistletoe leaf meal supplemented diets in laying pullets," Archivos de Zootecnia, vol. 67, pp. 526–530, 2018, doi: 10.21071/az.v0i0.3883.

[15] J. D. Olowokudejo, A. B. Kadri, and V. A. Travih, "An ethnobotanical survey of herbal markets and medicinal plants in Lagos State of Nigeria," Ethnobotanical Leaflets, vol. 12, pp. 851–865, 2008.

[16] H. A. A. Dafalla, "Studies on constituents of Ficus capensis Thunb," Pakistan Journal of Social Sciences, vol. 3, pp. 751–754, 2005.

[17] M. N. François, D. Amadou, and S. Rachid, "Chemical composition and biological activities of Ficus capensis leaves extracts," Journal of Natural Products, vol. 3, pp. 147–151, 2010.

[18] S. Zahra, V. Jafar, A. S. Omid, and A. Maryam, "Antioxidant activity and total phenolic content of Boerhavia elegans (Choisy) grown in Baluchestan, Iran," Avicenna Journal of Phytomedicine, vol. 5, pp. 1–9, 2015.

[19] K. J. Livak and T. D. Schmittgen, "Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method," Methods, vol. 25, no. 4, pp. 402–408, 2001.

[20] S. Gangadoo, D. Stanley, R. J. Hughes, R. J. Moore, and J. Chapman, "The synthesis and characterisation of highly stable and reproducible selenium nanoparticles," Inorganic and Nano-Metal Chemistry, vol. 47, pp. 1568–1576, 2017.

[21] M. J. Al-Kurdy, "Synthesis and characterization of black currant selenium nanoparticles," The Iraqi Journal of Veterinary Medicine, vol. 44, no. 2, 2020, doi: 10.30539/ijvm.v44i2.974.

[22] M. Tabibi, S. Aghaei, M. A. Amoozegar, R. Nazari, and M. R. Zolfaghari, "Characterization of green synthesized selenium nanoparticles (SeNPs) in two different indigenous halophilic bacteria," BMC Chemistry, 2023, doi: 10.1186/s13065-023-01034.

[23] N. K. Srivastava and M. Mukhopadhyay, "Green synthesis and structural characterization of selenium nanoparticles and assessment of their antimicrobial property," Bioprocess and Biosystems Engineering, vol. 38, pp. 1723–1730, 2015.

[24] R. Beanland, "Dark field transmission electron microscope images of III-V quantum dot structures," Ultramicroscopy, vol. 102, no. 2, pp. 115–125, 2005.

[25] W. Neumann, H. Kirmse, I. Haeusler, C. Zheng, and A. Mogilatenko, "Quantitative structure analysis of nano-sized materials by transmission electron microscopy," MRS Proceedings, vol. 1184, 2009.

[26] B. Fardsadegh and H. Jafarizadeh-Malmiri, "Aloe vera leaf extract mediated green synthesis of selenium nanoparticles and assessment of their in vitro antimicrobial activity against spoilage fungi and pathogenic bacteria strains," Green Processing and Synthesis, vol. 8, no. 1, pp. 399–407, 2019, doi: 10.1515/gps-2019-0007.

[27] G. Ramesh, S. Manna, B. Aggarwal, and A. L. Jadhav, "Lead activates nuclear transcription factor-kappa B, activator protein-1, and amino-terminal c-Jun kinase in pheochromocytoma cells," Toxicology and Applied Pharmacology, 1999, doi: 10.1006/TAAP.1999.8624.

[28] X. Li, M.-Y. Xing, M. Chen, J. Zhao, R. Fan, X. Zhao, C. Cao, J. Yang, Z. Zhang, and S. Xu, "Effects of selenium-lead interaction on the gene expression of inflammatory factors and selenoproteins in chicken neutrophils," Ecotoxicology and Environmental Safety, 2017, doi: 10.1016/j.ecoenv.2017.02.01.

[29] N. Lahat, S. Shapiro, P. Froom, E. Kristal-Boneh, M. Inspector, and A. Miller, "Inorganic lead enhances cytokine-induced elevation of matrix metalloproteinase MMP-9 expression in glial cells," Journal of Neuroimmunology, vol. 132, pp. 123–128, 2002.

[30] Y. M. El-Fakharany, E. M. Mohamed, R. L. Etewa, and O. I. Abdel Hamid, "Selenium nanoparticles alleviate lead acetate-induced toxicological and morphological changes in rat testes through modulation of calmodulin-related genes expression," Journal of Biochemical and Molecular Toxicology, vol. 36, 2022, doi: 10.1002/jbt.23017.

[31] R. Liu and L. P. Desai, "Reciprocal regulation of TGF-β and reactive oxygen species: A perverse cycle for fibrosis," Redox Biology, vol. 6, pp. 565–577, 2015, doi: 10.1016/j.redox.2015.09.009.

[32] S. E. Naser Eldin, M. S. Eissa, M. T. Mansour, H. A. Abdelmonem, A. A. Ahmed, N. O. Ali, M. A. Abd El-Haleem, S. A. Sayed, S. M. Emara, and M. M. Yousif, "Green synthesis of purslane seeds-selenium nanoparticles and evaluate its cytokines inhibitor in lead acetate induced toxicity in rats," Asian Journal of Chemistry, vol. 34, pp. 894–900, 2022.

[33] L. Liu, Y. He, Z. Xiao, W. Tao, J. Zhu, B. Wang, Z. Liu, and M. Wang, "Effects of selenium nanoparticles on reproductive performance of male Sprague-Dawley rats at supranutritional and nonlethal levels," Biological Trace Element Research, vol. 180, pp. 81–89, 2017.

[34] M. Elizabeth, U. Offor, N. B. Takalani, K. Mohlala, and C. Opuwari, "A review on the impact of oxidative stress and medicinal plants on Leydig cells," Antioxidants, 2023, doi: 10.3390/antiox12081559.

[35] L. Patrick, "Lead toxicity part II: The role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity," Alternative Medicine Review, vol. 11, no. 2, pp. 114–127, 2006.

[36] N. N. Njoku-Oji, D. E. Osaji, N. O. Ifegwu, R. C. Uchefuna, and D. N. Eze, "Effects of aqueous extract of Ficus capensis leaf on some reproductive parameters in normal adult male Wistar rats," International Journal of Multidisciplinary Research and Development, vol. 2, pp. 577–583, 2015..