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Paper

Exploring the potential of selenium nanoparticles and fabricated selenium nanoparticles @vitamin C nanocomposite in mitigating nicotine-induced testicular toxicity in rats

Rabab F. Hindawy[1], Rana M.M. Refaat[1], Atef E. Fouda[1], Mohamed A. El-Shishtawy[1], Adarsh Kumar[2], Nagi M. El-Shafai[3], Eman M. Faruk[4,5], Ola E. Nafea[6,*]

[1]Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Benha University, Al Nadi Al Ryadi, Qism Benha, Al-Qalyubia Governorate, Benha 13518, Egypt,
[2]Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences (AIIMS), AIIMS Campus, Ansari Nagar East, New Delhi, India,
[3]Nanotechnology Center, Chemistry Department, Faculty of Science, Kafrelsheikh University, Qism Kafr El-Shaikh, Kafr Al Sheikh First, Kafr El-Sheikh Governorate, Kafrelsheikh 33516, Egypt,
[4]Anatomy Department, College of Medicine, Umm Al-Qura University, College of Medicine, Umm Al-Qura, University, Al Abidiyah, Makkah, Saudi Arabia,
[5]Department of Histology and Cytology, Faculty of Medicine, Benha University, Al Nadi Al Ryadi, Qism Benha, Al-Qalyubia Governorate, Benha, Egypt,
[6]Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Zagazig University, Zagazig Rd inside Zagazig University, Shaibet an Nakareyah, Al-Sharqia Governorate, Zagazig 44519, Egypt
[*]Corresponding author: Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt.
Email: olanafea@zu.edu.eg

Background: The tobacco epidemic signifies a major public health threat. Nicotine (NIC), a major active constituent in tobacco, impedes male fertility and semen quality. This work is implemented to explore the potential of selenium nanoparticles (SeNPs) and the newly fabricated SeNPs @vitamin C (SeNPs@VITC) nanocomposite in mitigating testicular toxicity induced by NIC.
Materials and methods: The six groups of 48 adult Wistar rats were designed as follows: the control group injected intraperitoneally with normal saline, the SeNPs group treated orally with 2 mg/kg of SeNPs, the SeNPs@VITC nanocomposite group treated orally with 2 mg/kg of SeNPs@VITC nanocomposite, the NIC group injected intraperitoneally with 1.25 mL/kg of NIC, the NIC+ SeNPs group received SeNPs plus NIC, and the NIC+ SeNPs@VITC nanocomposite group received SeNPs@VITC nanocomposite plus NIC. Treatments were administered over a 28-day period.
Results: NIC treatment significantly caused poor sperm quality, decreased serum testosterone, increased follicle-stimulating hormone (FSH), luteinizing hormone (LH) concentrations, reduced hemoglobin levels, leukocytosis, disrupted testicular oxidant/antioxidant balance, and disorganized testicular structure. The construction of the novel SeNPs@VITC nanocomposite, compared to NIC plus SeNPs alone, demonstrated a more potent ameliorative effect on NIC-induced reproductive toxicity in adult rats. The SeNPs@VITC nanocomposite significantly increased sperm count, reduced the percentage of sperm head abnormalities, lowered both serum FSH and LH concentrations, and improved the hemoglobin response.
Conclusions: Both SeNPs and SeNPs@VITC nanocomposite alleviated the testicular toxicity induced by NIC, but the SeNPs@VITC nanocomposite exhibited superior efficacy. The SeNPs@VITC nanocomposite could be employed to advance enhanced therapeutic strategies for addressing male infertility.

Key words: nanocomposite; nicotine; reproductive toxicity; selenium nanoparticles; testicular toxicity; Vitamin C.

Introduction

The tobacco epidemic signifies a major public health threat, affecting more than 8 million people annually worldwide, and causing more disability and adverse health outcomes compared to other known behavioral risk factors.[1] Nicotine (NIC) is a major active constituent in tobacco, considered a primary alkaloid found in nearly all tobacco products.[2] NIC can be consumed in various forms and can be delivered via a broad range of methods.[3] For instance, individuals commonly consume NIC through smoking traditional cigarettes, using smokeless tobacco products like snuff or chewing tobacco, or utilizing newer electronic NIC delivery systems such as e-cigarettes or vape pens.[4,5]

Tobacco use is strongly associated with deleterious effects on public health. Moreover, NIC impedes male fertility and semen quality. Prior studies have demonstrated that NIC adversely affect sperm characteristics, seminal plasma, and many other factors related to fertility. Furthermore, NIC was identified in both blood and semen of smokers and its concentration was positively correlated with the number of cigarettes consumed daily.[6–8]

Selenium (Se) is a trace element essential for a wide range of physiological processes. For instance, Se acts as antioxidant defender through maintaining of cellular integrity and hampering oxidative stress. Moreover, Se has an immune-regulating effect through reduction oxidation (redox)-mediated reactions of

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selenoproteins that can protect immune cells against oxidant injury.[9,10]

Phospholipid hydroperoxide glutathione peroxidase (PHGPx) and selenoprotein P are two selenoproteins responsible for maintaining normal mammalian spermatogenesis. The primary selenoprotein expressed by germ cells in the testis is PHGPx, which serves as a fundamental link between Se, healthy sperm, and men fertility. Se supplementation can significantly improve sperm quality, restore ovarian function, and reduce pregnancy-related complications. In addition, the antioxidant potential of Se is critical for maintaining human reproductive health.[11,12]

Se is crucial for the normal thyroid gland function. The thyroid gland demonstrates a significant accumulation of Se within its tissues. Moreover, Se is required for the conversion of the thyroid hormone thyroxine into its active form (triiodothyronine), which is necessary for regulating metabolism, including chemical processes needed to maintain life, such as cardiac and nervous system functions, reproductive functions, development, and growth. Se is fundamental for healthy male and female reproductive functions. Earlier studies have underscored the correlation between Se intake and fertility. Additionally, Se is imperative in gestational health and during pregnancy. Unfavorable gestational outcomes, e.g. abortions, low birth weight, and damaging to fetal nervous and immune systems, have been reported in relation to Se deficiency. Reported animal and human studies clarified the vital role of Se in the process of spermatogenesis and male fertility.[10,13–15]

The health impact of Se is mainly dose-dependent, with a narrow window between its essential levels and the potentially associated toxicity.[16]

Nanoparticles (NPs) are minute clusters with either crystalline or amorphous structures, exhibiting distinct physical and chemical characteristics attributed to their size, which typically falls within the range of 1 to 100 nm.[17] Herein, selenium nanoparticles (SeNPs) offer advantages such as high safety, increased bioavailability, and unique biological activity compared to conventional Se forms.[18,19]

Vitamin C (VITC)/ascorbic acid has a multitude of metabolic functions in the human body. Its role as an electron donor is fundamental to all its known functions and properties. VITC is linked to the primary prevention of both common and complex diseases, including coronary artery diseases, stroke, and cancer. Scurvy is the hallmark of VITC deficiency; however, its deficiency has been associated with numerous illnesses, and in critically ill individuals, it may be connected to heightened disease severity and reduced survival rates. VITC plays a major role in maintaining male reproductive system functions as it serves as a significant testicular antioxidant, counteracting excessive reactive oxygen species (ROS), preventing sperm agglutination, inhibiting lipid peroxidation, safeguarding against DNA damage as well as facilitating the regeneration of vitamin E.[20–24]

Numerous nanoplatforms exist for the precise delivery of biomolecules, including antibodies, membrane receptors, nucleic acids, aptamers, proteins, peptides, as well as smaller entities like folates, vitamins, and carbohydrates.[25] Currently, in the realm of research and innovation, eco-friendly materials such as encompass nanocomposites. These materials demonstrate unique composite characteristics that render them appropriate for a wide range of industrial, medicinal, and energy-related applications.[26]

The novelty of the current study lies in the application of newly fabricated SeNPs and SeNPs@VITC nanocomposite to counteract the deleterious effects of NIC on rat testes. Consequently, this study was designed to explore the potential of the newly fabricated SeNPs and SeNPs@VITC nanocomposite in mitigating testicular toxicity induced by NIC. This could be achieved by investigating the effects of SeNPs and SeNPs@VITC nanocomposite on sperm quality, serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone hormones, hematological indices, testicular oxidant/antioxidant status, and concomitant histopathological lesions.

Materials and methods

Chemicals

Nicotine hydrogen tartrate (NHT) salt, CAS Number 65-31-6, ≥98% purity, was purchased from Sigma Chemical Company. Sodium selenite and L-ascorbic acid/VITC were purchased from Himedia Laboratories, Mumbai, India.

Synthesis of SeNPs

Based on the modified method of Ingole et al.,[27] SeNPs were prepared using 0.02 M of Sodium selenite in 100 mL double-distilled water. Next, 0.025 M of sodium citrate was slowly added to the solution while stirring and ultrasonication were performed for 30 min. The mixture was kept warm for 1 h until the selenium citrate complex solution was formed. Then, 0.5 mL of hydrazine hydrate was added drop by drop to the hot mixture until a red color appeared. The crystalline structure was centrifuged, washed several times with ethanol and water, and then dried at 50 °C.

Synthesis of SeNPs@VITC nanocomposite

One gram of VITC was dissolved in 100 mL of double-distilled water for 15 min. Then, 0.1 g of synthesized SeNPs was slowly added with vigorous stirring for 30 min. The mixture was then placed in an ultrasonication device (Prisma Tech) operating at a frequency range of 37 kHz to 80 kHz and a temperature of 20 °C for 15 min, followed by moderate stirring overnight. The product was washed several times with water and ethanol. After washing, the product was centrifuged at 6,000 rpm, and the final product was dried in a furnace at 45 °C under pressure.[28]

Characterization of SeNPs and SeNPs@VITC nanocomposite

X-ray diffractograms (XRD analysis)

XRD is a commonly used technique for characterization of nanomaterials.[29] XRD was performed using an X-ray diffractometer (Shimadzu XRD-6000, Japan). The data observed showed a single phase with a monoclinic structure, and lattice parameters were a = 4.688 A°, b = 3.423 Å, c = 5.132 Å, β = 99.506 Å and V = 82.31 Å. The characteristic patterns of SeNPs were appeared at 23.1°, 29.2°, 40.9°, 43.3°, 44.8°, 51.1°, 55.4°, 60.8°, 65.2°, and 71.1°. The patterns of VITC were observed at 2θ (degrees) = 12.1°, 18 A°, 21 A°, 22°, 24 °, 26.5°, 30°, 33°, 38°, and 41.8° (Kokila et al. 2017)[30]. All peaks can be readily assigned to those of crystalline SeNPs indicating the formation of a single-phase with a monoclinic structure. The main diameter of SeNPs has been calculated using the Debye Scherrer's equation ($D=K\lambda/\beta\cos\theta$).

K = shape factor (~ 0.9)
λ = X-ray wavelength (1.5405 Å),
β = full width at half maximum of the diffraction peak,
θ = Diffraction angle

Zeta potential/particle size

The zeta (ζ) potential is a measure of the surface charge and is commonly used to assess the strength of electrostatic interactions between particles and other biomolecules in a solution.[31]