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Impact of Selenium nanoparticles on Biochemical parameters of Doxorubicin induced hepatotoxicity in Rat model

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Abstract: This study investigates the mitigative effects of Selenium (Se) against Doxorubicin (DOX)-induced hepatotoxicity, particularly focusing on gene expressions of BCL2 and BAX as markers of cellular survival and apoptosis. Chemotherapy, notably with DOX, while effective against various malignancies, poses risks of hepatotoxicity, characterized by hepatocyte necrosis and fibrosis. The study explores Se's role, a vital trace mineral integral to Selenium nanoparticles, in combating oxidative stress induced by DOX. Employing a controlled experimental design, gene expressions in four groups (Control, Se, DOX, and DOX + Se) were studied using quantitative real-time PCR. Results showed that Se supplementation alone had minimal impact on BCL2 and BAX expressions. However, DOX treatment significantly decreased BCL2 levels and increased BAX levels, underscoring its hepatotoxic potential. The combined DOX+Se treatment moderated these effects, partially restoring BCL2 levels and reducing BAX expression, suggesting Se's protective capability against DOX-induced gene expression dysregulation. These results highlight the potential of Se in ameliorating the adverse effects of DOX, providing insights into protective strategies against chemotherapy-induced hepatotoxicity.

keywords: Doxorubicin – hepatotoxicity-selenium nanoparticles- BAX- BCL2

1.Introduction

The two primary forms of cancer treatment, chemotherapy, and radiotherapy are not without risk to normal tissues. Their anti-tumor benefits are often outweighed by detrimental side effects **[1]**. Among chemotherapeutic agents, Doxorubicin (DOX), an anthracycline drug, is extensively used for treating a plethora of malignancies, from solid tumors and soft tissue sarcomas to various hematological cancers [2]. One of the well-documented adverse effects of DOX is hepatotoxicity, which presents as hepatocyte necrosis, sinusoidal dilatation, and fibrosis [3]. The extent and the distribution of DOX-induced damage in the liver are critical areas of investigation due to their implications for patient outcomes.

DOX-induced hepatotoxicity, which can occur at both acute and sub-acute dosages due to its biphasic nature, is attributed to the drug's metabolism in the liver, resulting in reactive oxygen species (ROS) production. This in turn causes an imbalanced redox potential, leading to oxidative stress and consequent mitochondrial dysfunction, apoptosis, and inflammation [4]. These molecular mechanisms underscore the hepatotoxic potential of DOX, necessitating research into protective strategies and mitigation measures.

In the context of mitigating such adverse effects, Selenium (Se), identified in 1818, emerges as an essential trace mineral, initially misunderstood as toxic but now recognized for its vital roles in the body.

Due of selenium's strong reactivity with a wide range of compounds, scientists have been working to transform selenium into significant

selenium nanomaterials like ZnSe and CdSe [5]. These nanoparticles with selenium have strong biological activity, superior photoelectrical features, and semiconductor characteristics [6]. Integrating into Selenium nanoparticles Se is instrumental for metabolism and combating oxidative stress. It exists in both organic forms like Selenium nanoparticles and selenocysteine, and inorganic substances, like selenite and selenium. The intake and levels of Se show considerable variation across different populations, particularly in the Middle East. Selenium nanoparticles, discovered in 1973, are integral to cellular protection against oxidative damage and are pivotal in maintaining Se homeostasis. Selenium nanoparticles (SeNPs), a major Selenium nanoparticles plays a significant role in Selenium metabolism and transport, indicative of Se's importance in the regulation of physiological processes [7].

The aim of this study is to study the protective effects of selenium (Se) on DOX-induced hepatotoxicity, focusing specifically on the levels of gene expression of BCL2 and BAX as markers of cellular survival and apoptosis, respectively.

2. Materials and methods

Selenium nanoparticles Preparation

Zhang et al prepared selenium NPs using reducing selenite with limited adjustment. They dissolved 1 gram of chitosan and 0.8 gram of Vitamin C in 100 mL of 1% (w/w) acetic acid to get chitosan Vitamin C solution. Next that, 5 mL of selenite aqueous solution including 0.2 gram of sodium selenite was drop wisely combined with the chitosan/ Vitamin C solution and vigorously mixed (500–600 rpm) [8].

Characterization of Selenium nanoparticles

Ultraviolet-visible spectroscopy

Absorption of ultraviolet visible was evaluated in the local wavelength from 200 to 1100 nm by using a spectrophotometer (Cary series UV-Vis- NIR, Australia) to study the optical properties of specimens and their structure [9].

Transmission Electron Microscopy

A 200 kV acceleration voltage is applied to a CCD camera using transmission electron microscopy (JEOLJEM-2100, Japan). Before TEM testing, the samples will be diluted. A copper grid will be first applied on the wax plate. After drying in the air, a drop of diluted nano colloidal preparation for TEM measurements will be put to the copper grid's surface [10].

Zeta Potential Measurements

A zeta potential analyzer and particle sizing system (Malvern Zetasize Nano-Zs 90, USA) was utilized to measure the zeta potential of magnetite at 25 C. 90 degrees was the scattering angle. Prior to measurement, deionized water was added to the magnetic nanoparticle suspensions. Each sample is colloidally dispersed and put into a zeta cell, which is a glass cuvette with round holes, using the zeta potential measurement method. When charged particles are exposed to an electric field, they exhibit electrokinetic effects. This method utilizes the electrophoresis principle to estimate zeta potential [11].

Experimental Animals and Housing:

In this investigation, 70 fully grown female Sprague-Dawley rats, weighing 200 ± 250 gm and 6 months old, were employed. The controlled environment they were kept in had a 12-hour light-dark cycle, 24ºC temperature, and 50–70% humidity. Food and drink were freely available. The Mansoura University Institutional Animal Ethics Committee accepted rules for the care and methods that were used.

Ethical Consideration:

Research ethics declaration the experimental procedures would strictly adhere to the NIH guidelines for the Care and Routine of Laboratory Animals. All protocols are submitted by the ethical committee of Mansoura University, Urology and Nephrology Center (ID: MU-ACUC (SC.MS. 22.118)).

Experimental Strategy and Treatment Groups:

The rats were randomized into four main groups, each consisting of six rats:

Control group: Received injections of 0.9% saline intraperitoneally (i.p.).

1-Dox group: Administered DOX intraperitoneally as a only dose of 20 mg/kg[12].

2-SeNPs group: Received SeNPs i.p. at a dose

of 0.2 mg/kg for 4 weeks [13].

3-Dox + SeNPs group: Treated with the same DOX regimen as the Dox group and Se NPs as in the Se NPs group [14].

Collection of Sample:

Following the experiment, cardiac punctures were used to obtain blood samples for biochemical examination. The rats were then sacrificed under anesthesia, the liver tissues were removed for additional examination. After being separated, the serum was kept at -80ºC.

Liver tissues were divided into two parts: one preserved in 1 ml RNA later at -80ºC for molecular studies, and the other kept at -80ºC for oxidative stress measurements.

Gene Expression Investigation:

From the liver tissues, total RNA was isolated using TRIzol™ Reagent. The RNA quality and concentration were assessed using a Nano-Drop spectrophotometer. Reverse transcription was performed to synthesize cDNA. Quantitative real-time PCR analysis was then conducted for BCL2 and BAX gene expressions by using SYBR Green Master Mix on a Rotor-Gene real-time PCR system. The PCR conditions involved an initial denaturation step, followed by 40 cycles of denaturation, annealing, and extension. The housekeeping gene GAPDH was used to control the levels of gene expression, which were then expressed as fold changes utilizing the $2^{-\Delta\Delta Ct}$ method [15].

Superoxide Dismutase (SOD) Assay:

Superoxide Dismutase activity in liver tissue was assessed using a SOD Assay Kit that is sold commercially

[16]. This assay is based on the inhibition of the chromogenic reaction of superoxide radicals with a tetrazolium salt. Liver tissue homogenates were prepared in a specified buffer, and the mixture of reaction was incubated with the substrate solution delivered in the kit. At 450 nm, the absorbance was determined with a spectrophotometer. The amount of enzyme required to show 50% dismutation of the superoxide radical is one unit of SOD activity, which was expressed as units per milligram of protein.

Malondialdehyde (MDA) Assay:

Thiobarbituric Acid Reactive Substances (TBARS) assay was used to measure MDA levels, a sign of lipid peroxidation [17]. Liver tissues were homogenized and mixed with thiobarbituric acid. The mixture was then heated, leading to the formation of a thiobarbituric acid-reactive substance, which is a pink-colored complex with MDA. This complex's absorbance was measured at 532 nm. Using a standard curve, the concentration of MDA was determined and reported as nanomoles per milligram of tissue.

Statistical Analysis:

The data was displayed as mean ± standard deviation (SD) [18]. The significance of differences between the control group and treatment groups was analyzed using one-way ANOVA followed by post-hoc testing where assume. Statistical significance is described as a p-value <0.05.

3. Results and Discussion

Characterization of Selenium nanoparticles

Ultraviolet-visible spectroscopy of Selenium nanoparticles

UV–vis spectroscopy, the most simple and indirect technique, was used to determine the chemical reduction of selenium ions to Se nanoparticles (Se NPs). Strong absorption peaks for and Se NPs were seen at 260 nm, which is associated with the generation of Se NPs during the reduction process. UV–visible absorption spectra of selenium nanoparticles is shown in Figure 1.

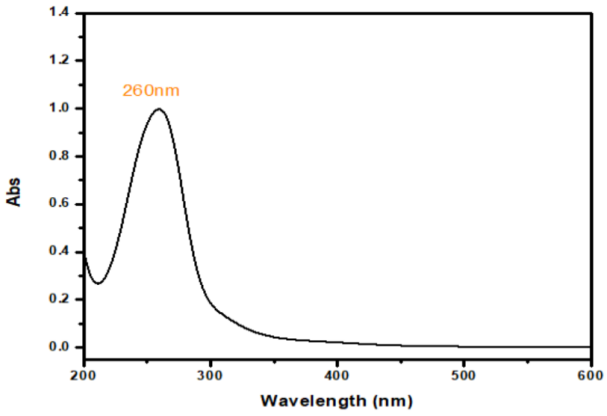

Figure 1. UV–vis spectra of Se NPs

Selenium nanoparticles: structural and morphological characteristics

The generated Se NPs' morphology and size were examined using Transmission Electron Microscopy (TEM), as seen in Figure 2. The

TEM pictures provided in Figures 2a and 2b clearly show the homogeneous sphere-shaped nature of the produced Se NPs. The production of Se nanoparticles using chitosan is confirmed by transmission microscopy examination, with most of the synthesized particles falling within the 100 nm range and the average size of the particles being 60±10 nm.

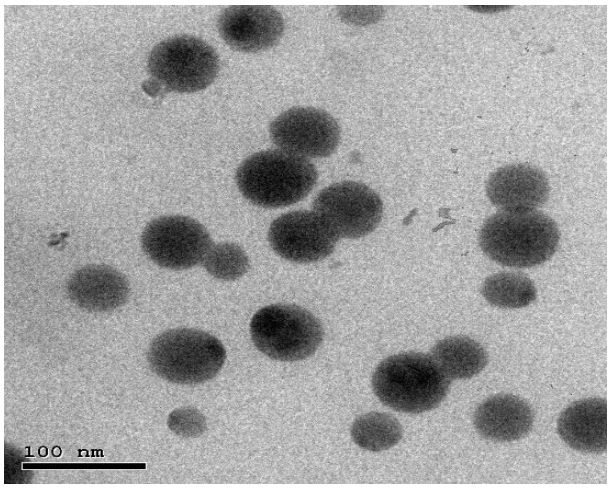

Figure 2. Shows the TEM image of the prepared sample

The zeta potential Measurements of Selenium nanoparticles

Using the Zetasizer Nano-ZS particle analyzer, the zeta potential of Se NPs was determined; the results of this experiment are illustrated in Figure 3. One important measure of the stability of the colloidal dispersion of nanoparticles is still the zeta potential. Se NPs exhibit positive zeta potential; the value of this potential was determined to be around 34.4 mV. The greater stability of these Se NPs is indicated by the bigger size of the zeta potential. The measurement of an effective electric charge on a nanoparticle's surface is called zeta potential. Higher zeta potential nanoparticles show more stability because of their stronger electrostatic attraction to one another.

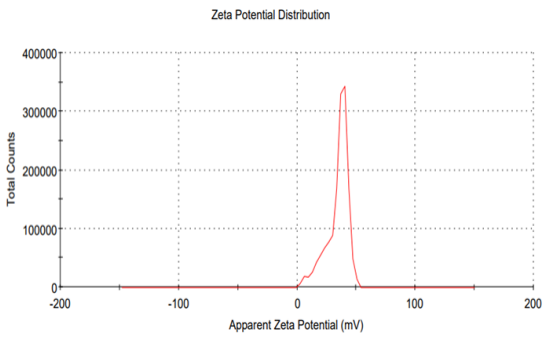

Figure 3. Shows the zetapotenial of SeNPs

Gene expression

In the analysis of gene expression as shown in Table 1, the control group served as the baseline for comparison, displaying mean expression levels of BCL2 at 1.02±0.06 and BAX at 0.98±0.03. Selenium supplementation alone did not significantly alter the expression of BAX, with mean levels at 0.93±0.05, and only slightly increased BCL2 expression to 1.08±0.07, suggesting minimal impact on these gene expressions by selenium alone.

Treatment with doxorubicin (DOX) resulted in a profound decrease in BCL2 expression, with mean levels plummeting to 0.01±0.004, which was significantly different from the control (denoted by 'a'). In stark contrast, BAX expression under DOX treatment surged to 7.7 ± 0.75, a substantial elevation indicating a significant response to DOX (indicated by 'a').

Table 1: Gene expression across treatment groups

Groups	BCL2	BAX
Control	1.02±0.06	0.98±0.03
Se	1.08±0.07	0.93±0.05
DOX	0.01±0.004 [a]	7.7±0.75 [a]
DOX + Se	0.08±0.02 [a]	5.52±0.19 [ab]

The combined treatment of DOX with selenium (DOX+Se) moderated the effects of DOX on gene expression. BCL2 levels were partially rescued to 0.08±0.02, although still significantly lower than control levels (noted by 'a'). BAX levels decreased to 5.52±0.19 compared to the DOX group, a significant decrease (indicated by 'b') but remained elevated in comparison to the control.

These results demonstrate selenium's modulatory role on gene expression in the presence of doxorubicin, suggesting a potential protective mechanism against doxorubicin-induced dysregulation of BCL2 and BAX gene expression.

Table two shows the analysis of oxidative stress markers. Our results revealed distinct responses across different treatment groups. The control group-maintained baseline levels with an MDA concentration of 30 ± 3 and SOD activity at 5641.3 ± 39.9. The Se group slightly increased MDA levels to 37.67 ± 3.51 and significantly elevated SOD activity to 8620.3 ±

48, suggesting an enhanced antioxidative response. In contrast, the DOX group exhibited a marked increase in oxidative stress, evidenced by a substantial rise in MDA concentration to 234.33 ± 2.08 and a decrease in SOD activity to 2797.7 ± 75.6.

The data is described as Mean ± SD. Significant difference a vs. control, b vs. DOX.

Notably, the combined treatment (DOX + Se group) showed a moderated oxidative stress profile, with MDA levels reduced to 82 ± 4 and a partial recovery in SOD activity to 5865.2 ± 91.1. These findings indicate that while DOX significantly induces oxidative stress, co-treatment with Selenium appears to mitigate these effects, suggesting a protective role of Se NPs against DOX-induced oxidative damage.

Table 2: MDA and SOD activity across treatment groups

Group	MDA Conc	SOD activity
Control	30 ± 3	5641.3 ± 39.9
Se	37.67 ± 3.51	8620.3 ± 48
DOX	234.33 ± 2.08 [a]	2797.7 ± 75.6 [a]
DOX + Se	82 ± 4 [ab]	5865.2 ± 91.1 [ab]

The data is described as Mean ± SD.
Significant difference a vs. control, b vs. DOX

Discussion:

Doxorubicin, an anthracycline medication, is a commonly prescribed antineoplastic treatment for a range of malignancies. Hepatotoxicity, which manifests as hepatocyte necrosis, sinusoidal dilatation, and fibrosis, is one of its side effects. Still, there is a lack of information regarding the damage's amount and geographic spread. Doxorubicin is frequently used to treat ovarian, thyroid, breast, and lung cancers as well as leukemias. However, its therapeutic utility is somewhat limited by systemic toxicity at clinically relevant levels **[19]**.

The drug's metabolism in the liver produces ROS, which leads to an imbalanced redoxpoten tial that causes oxidative stress, decreased level s of apoptosis,antioxidant enzymes,inflammatio n, and mitochondrial disfunction.

These are the main molecular mechanisms b y which DOX causes hepatotoxicity. Any type of acute or chronic liver illness can be mimicked by drug-induced liver damage **[20]**.

The detoxification process mostly involves the liver. The liver tissue treated with doxorubicin exhibits changes such as localized necrosis, bile duct hyperplasia, hepatocyte vacuolation, and hepatocyte cord degeneration. The ROS generation, reduced inflammation and oxidative stress, impaired mitochondrial synthesis and function, and increased apoptosis are the primary molecular causes of hepatotoxicity **[21]**.

Due to their potential uses in the rapidly developing fields of nanoscience and technology, nanoparticles are of utmost significance. These nanomaterials' physical, chemical, optical, and electrical capabilities are largely controlled by their size, shape, and surface morphology **[22]**.

Selenium (Se) is a vital trace element needed by both animals and humans. It is a nutrient that is found in food and is significant for many areas of health, particularly for its antioxidant action. It is a crucial component of the catalytic site of over 25 Selenium nanoparticles and enzymes in the human body and protects cells and tissues from oxidative damage. When the body is under oxidative stress, se shortage may upset the delicate balance between oxidants and antioxidants, increasing the hazards linked with oxidation **[23]**.

Due to their higher qualities and advantageous biological activities, selenium nanoparticles (SeNPs), which are elemental, nanosized selenium particles, have obtained more and more attention. Since Se NPs have shown tremendous promise for use in nutritional supplementation, chemoprevention, chemical treatment, and nanomedicine delivery applications, they are regarded as a possible Se supplement. Se NPs' ability to protect animals from toxins or pathogens that cause liver damage suggests that they may have hepatoprotective properties **[24]**.

Due to its extensive roles in biologic systems, including antiviral, immune adjustment, cancer inhibition, and antioxidant, selenium (Se) is a trace element that is vital for preserving the health of mammal animals. It also plays a critical role in regulating immune function, preventing antioxidant damage, and endocrine homeostasis **[25]**. Nonetheless, there is a very small window of time between the lowest level of selenium compounds that can be

consumed and their deadly concentration [26] and the chemical form determines the toxicity. In the environment, this metalloid element is typically found in the oxidation states selenide (Se^{-2}), selenite (Se^{+4}), and selenite (Se^{+6}) **[27]**. The liver, the primary metabolic organ, is selenium-sensitive **[28]** and the liver may exhibit a preferred reflection of a selenium deficit **[29].**

As developing nanomedicine, SeNPs have had considerable interests due to their superior antioxidant effects and lower toxicity associated to the other selenocompounds **[30]**. According to earlier studies, SeNPs enhanced testicular antioxidant activity and enhanced sperm concentration, motility, and viability in rats as compared to SeNPs **[31]**. Furthermore, numerous studies have shown that SeNPs are actual in ameliorating testicular damage produced by the anticancer drug cisplatin **[32]** and other environmental agents **[31]**. A critical aspect of their biological activity is their dose-dependent antioxidant effects, closely associated with selenoenzymes such as glutathione peroxidases (GPXs) and thioredoxin reductase (TR). Notably, SeNPs have shown efficacy in mitigating oxidative stress induced by chromium, marked by the restoration of crucial antioxidant markers including superoxide dismutase (SOD), catalase (CAT), and glutathione levels [33]

Additionally, SeNPs have demonstrated potential in reducing the neurotoxic and nephrotoxic effects of cadmium chloride, primarily by diminishing oxidative stress and apoptosis [34]. This reduction is further evidenced by the decrease in malondialdehyde (MDA) levels, a marker of oxidative damage, alongside an increase in glutathione (GSH) levels. Furthermore, SeNPs have been effective in enhancing liver function parameters and reducing the levels of inflammatory cytokines and MDA, while concurrently elevating GSH levels, thus providing a protective effect against various oxidative and inflammatory challenges [35].

In a specific study utilizing a mouse liver model, SeNPs, with sizes ranging from 48 to 67 nm, were applied in vivo to counteract the hepatotoxic effects of acrylamide. This hepatotoxicity, characterized by reduced GSH and elevated MDA, ALT, and AST levels, was significantly ameliorated upon concurrent administration of SeNPs. The study highlighted an increase in antioxidant enzymes such as glutathione reductase (GR), CAT, SOD, and glutathione-s-transferase (GST) following SeNPs treatment, demonstrating their potent antioxidative capability [36]

Furthermore, research led by de Freitas et al. underscored the physiological role of SeNPs as a component of GSH-Px, a key Selenium nanoparticle. Interestingly, the beneficial effects of selenium supplementation in reducing doxorubicin (DOX)-induced hepatic injury were primarily attributed to the enhancement of SOD recovery rather than GSH-Px. This finding aligns with observations where diphenyldiselenide, a simple organ selenium compound, effectively mitigated hepatic oxidative stress caused by methylmercury, although it did not significantly alter hepatic GSH-Px, CAT, or SOD activities [37]

In summary, this paper has explored the multifaceted roles of SeNPs in mediating oxidative stress and their potential therapeutic applications, particularly in the context of combating the hepatotoxic side effects of DOX. Our findings underscore the significance of SeNPs' antioxidant properties, especially in modulating key enzymes like SOD and mitigating MDA levels, thereby offering a protective mechanism against oxidative damage.

Concurrently, the study highlights the challenges posed by DOX, a widely used anthracycline medication, whose efficacy in treating various malignancies is often marred by its hepatotoxic side effects. The potential of SeNPs to not only enhance antioxidant activity but also to provide a safeguard against the hepatotoxicity induced by anticancer treatments. These insights pave the way for integrating SeNPs into oncological therapy, proposing a balanced approach that maximizes treatment efficacy while minimizing adverse effects. Overall, the paper contributes to the growing body of research on nanomedicine and its application in cancer therapy, suggesting a promising future where SeNPs play a critical role in enhancing patient outcomes and advancing cancer treatment modalities.

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