

Research Article



Insights into Curcumin-Selenium Nanoparticles in Modulating Hormonal Levels and Promoting PTEN Gene Expression in Doxorubicin Treated Rats

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Abstract | This study aimed to investigate the protective impact of synthesized curcumin selenium nanoparticles (CurSeNPs), using a hydroalcoholic extract of *Curcuma longa* rhizomes, in reducing disruption to hormonal profiles, redox homeostasis, inflammatory parameters, and ovarian tissue PTEN gene expressions in rats treated with doxorubicin. CurSeNPs were synthesized using sodium hydrogen selenite and a hydroalcoholic extract derived from *Curcuma longa*. The in vivo study utilized thirty-two mature female rats divided into four experimental groups over a two-week treatment period as follows: Rats in the control group (C) received distilled water orally, Group G1 received intraperitoneal injections of doxorubicin (4.40 mg/kg B.W), Group G2 was administered CurSeNPs (10.47 µg/kg B.W), and Group G3 received both doxorubicin and CurSeNPs at the same doses. Blood samples were collected from anesthetized rats; serum was analyzed to assess levels of FSH, estrogen, TGF-β, and NF-κB. Additionally, ovarian tissue was extracted to measure levels of malondialdehyde (MDA) and superoxide dismutase (SOD) concentrations, and to estimate ovarian gene expression of PTEN. The findings indicated a notable decrease in tissue SOD, serum FSH, and estrogen levels, along with an elevation in tissue MDA, serum TGF-β, NF-κB, and PTEN gene expression levels in the G1 group. However, the results demonstrated that administration of CurSeNPs in the G2 and G3 groups effectively improved oxidative stress-related factors, hormonal profiles, inflammatory criteria, and reduced expression of the PTEN gene. The present investigation has shown that CurSeNPs exhibit preventive actions against doxorubicin toxicity in adult female rats, possibly through their antioxidant, anti-inflammatory, and anticancer properties.

Keywords | Curcumin, Selenium nanoparticles, Doxorubicin, Hormonal profile, PTEN gene

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INTRODUCTION

Nanobiotechnology is a developing field within nanotechnology that has gained global attention. Green nanotechnology is a highly effective strategy for reducing the impacts of nanomaterial production and use, while simultaneously minimizing the potential dangers connected with alternative approaches (Ramalingam et al., 2021; Murthy et al., 2021). The study investigates the

antioxidant activity and renal protective benefits of silver nanoparticles derived from *Salvia officinalis* against the harmful effects of methotrexate in rats (Sood and Khudiar, 2019). Doxorubicin, often known as DOX or adriamycin, is a commonly employed cytotoxic anthracycline antibiotic in treating cancer in premenopausal individuals (Iwamoto et al., 2020). The chemotherapeutic effects of this substance on cancer cells may be attributed to various processes, including its ability to intercalate into deoxyribonucleic

acid (DNA) and inhibit topoisomerase-II-mediated DNA repair. Additionally, it can generate free radicals that cause damage to cellular membranes, DNA, and proteins (Thorn et al., 2011). DOX exhibit deleterious effects on ovarian follicles in rodents, including follicular atresia, overactivation (Wang et al., 2018; Nishi et al., 2018; Wang et al., 2019) and reduced production of 17 β -estradiol (E2) (Xiao et al., 2017). Selenium demonstrates biological properties linked to the defence system, antioxidation, antiviral characteristics, as well as anticancer effects (Zhou et al., 2020). Simultaneously, selenium nanoparticles (SeNPs) also contribute to the reduction and alleviation of heavy metal toxicity, it possess the benefits of a high absorption rate, elevated biological activity, and less toxicity (Zhang et al., 2023; Chen et al., 2023). The stability of these nanoparticles becomes better due to their natural organic molecular coating, which prevents any accumulation over time (Alqayim, 2019; Shareef et al., 2024). The black currant selenium nanoparticle works as an antioxidant and hypolipidemic agent. It exhibits both prophylactic and therapeutic properties in mitigating the harmful impact of D-galactose (Khudair et al., 2020). In rats, *Avena sativa* seed crude extract protects the heart from DOX-induced toxicity (Salim and Hasan, 2022).

Curcumin, derived from the rhizomes of *Curcuma longa*, exhibits significant pharmacological effects such as anti-tumor (Joshi et al., 2021), antioxidant (Kamal et al., 2021) and anti-inflammatory (Memarzia et al., 2021) properties. Multiple studies have documented that curcumin possesses the ability to postpone the progression of ovarian cancer, enhance its responsiveness to chemotherapy, and minimize the adverse effects of chemotherapy medications, also evidence has demonstrated the significant potential of this substance in fighting cancer by encouraging apoptosis, limiting the process of cell division, triggering the self-degradation of cells, preventing the spread of tumors, and controlling the activity of enzymes (Liu et al., 2023). These particles have been proven to be nanoparticles, which possess future uses in numerous sectors, medicine, and therapy (Jaffar and Hussein, 2015). One of the many biological functions of curcumin phytosome nanoparticles (CPNPs) is their ability to reduce inflammation and lower cholesterol levels (Jadaan and Khudair, 2023). Previous studies have further confirmed the hypothesis that phytochemicals play a crucial role in regulating the formation of metal nanoparticles (Al-Kurdy and Khudair, 2020). Therefore, this study was designed to explore the risks resulting from exposure to DOX on certain parameters of ovarian tissues in adult female rats.

MATERIALS AND METHODS

The current study was conducted at an animal center affiliated with the College of Veterinary Medicine,

University of Baghdad. Ethical approval (P.G. 2641 dated 29-11-2023) was obtained from the local committee of animal care and use for the study involving animals, conducted in strict compliance with the code of ethics for animal experiments at the University of Baghdad's College of Veterinary Medicine. Thirty-two adults female Wistar rats were used in this experiment and were randomly and evenly divided into four experimental groups after an acclimatization period. The rats received the following treatments for two weeks: Rats in the control group (C) were orally administered distilled water; Group G1 received intraperitoneal injections of doxorubicin (STADA Pharm, Germany) at a dose of 4.40 mg/kg B.W; Group G2 received CurSeNPs at a dose of 10.47 μ g/kg B.W; and Group G3 received both doxorubicin and CurSeNPs at the same doses. Rats were anesthetized and blood samples were collected by heart puncture. Serum levels of FSH, estrogen, NF- κ B, and TGF- β were measured. Additionally, specimen ovarian tissues were discarded to measure serum Malondialdehyde (MDA) and superoxide dismutase (SOD) using enzymatic kits (BTLAB, China), as well as the gene expression of PTEN. Total RNA from the ovarian tissue was extracted using an RNA extraction kit from Genaid Korea. The quantification of gene expression was carried out using forward primer (5'-TGGATTCGACTTAGACTTGACCT-3') and reverse primer (5'-GGTGGGTATGGTCTTCAAAGG-3') for PTEN and forward primer: 5'-CACCCACTCCTCCACCTTTG-3' and reverse primer: 5'-CCACCACCCTGTTGCTGTAG-3' for GAPDH (Chen et al., 2022a) with the AddScript RT-qPCR Syber master kit from AddBio, Korea. The RT-qPCR results were evaluated based on the criteria outlined by Schmittgen and Livak (2008).

STATISTICAL ANALYSIS

The data were analyzed using the statistical software SPSS version 26, and the results are presented as means $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was employed for statistical analysis. The study also utilized the Least Significant Difference (LSD) test for between-group comparisons, with statistical significance set at $P < 0.05$ (Baarda and van Dijkum, 2019).

RESULT AND DISCUSSION

REDOX STATUS

The group treated with doxorubicin revealed a significant rise ($P < 0.05$) in MDA with a significant decline in SOD concentrations in ovarian tissues compared to other groups (Figure 1A, B). Whereas the G2 and G3 groups showed a significant ($P < 0.05$) decrease in MDA associated with a significant ($P < 0.05$) increase in SOD levels in ovarian

tissues as compared to group G1. This study pointed out that doxorubicin-induced oxidative stress in ovarian tissue via changes in redox status through elevating lipid peroxidation (LPO), which was documented by an increase in tissue MDA concentration representing increased cellular membrane tissue injury. This result was agree with (Ren et al., 2017; Al-Okaily and Murad, 2021; Zhang et al., 2023).

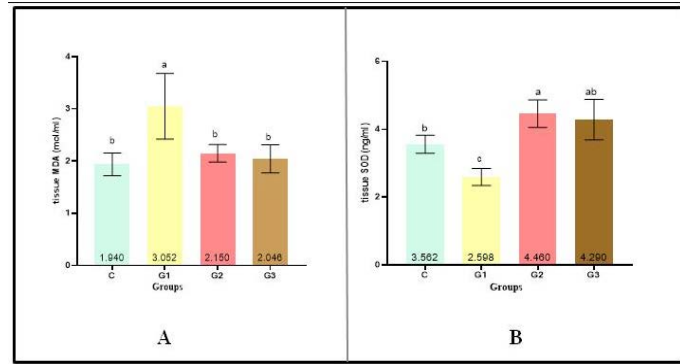


Figure 1: Effect of CurSeNPs, doxorubicin and both on (A) tissue MDA and (B) tissue SOD in adult female rats after 14 days.

Values are expressed as mean $\pm$ SD, n=5. C= Control group. G1 = Injected with i/p doxorubicin 4.40 mg /kg. B.W. G2 = Administrated with CurSeNPs 10.47 μ g / kg B.W orally. G3 = Administrated with CurSeNPs 10.47 μ g / kg B.W orally and with doxorubicin 4.40 mg/ kg B.W i/p. Different small letters present on bars indicate significant differences between groups at P<0.05.

Numerous studies confirm that curcumin treatments have been demonstrated to significantly increase the concentrations of antioxidant enzymes, such as CAT, SOD and GSH-Px, as observed in different investigations (Chen et al., 2018, 2020). Curcumin is believed to have a role in alleviating the oxidative stress and cardiotoxicity caused by DOX (Wu et al., 2019; Yarmohammadi et al., 2020a). Particularly it can improve the antioxidant defense mechanisms by activating the Nrf2 pathway and its associated signaling molecules (Yadav et al., 2019; Mohammed et al., 2020; Yarmohammadi et al., 2021b) via four separate mechanisms, including inhibiting Keap1, altering the upstream regulators of Nrf2, modulating the expression of Nrf2 and its target genes, and enhancing the nuclear translocation of Nrf2 (Bellezza et al., 2018; Wafi et al., 2019; Ashrafizadeh et al., 2020). The modulation of gene and protein expressions of Nrf2, Keap1, HO-1, and NQO1, confirmed the antioxidant modulation mechanism of curcumin on mice subjected to intense exercise (Chen et al., 2022b). Another findings reported that curcumin can reduce oxidative stress via activating the Keap1-Nrf2-ARE signaling pathway (Xie et al., 2018), as well as administering hexa-hydrocurcumin to rats revealed a considerable reduction in oxidative stress

levels accompanied with an increase in the expression of Nrf2 and HO-1, antioxidative enzyme activity, and SOD activity (Wicha et al. 2017).

FSH AND ESTROGEN

The findings demonstrated a significant decline in serum FSH and estrogen concentrations in group G1 compared to the control group and group G2. Rats administered CurSeNPs (G2) demonstrated a significant parallel increase ($P > 0.05$) in serum FSH and estrogen levels compared to groups C, G1, and G3. The FSH and estrogen hormones in group G3 showed a non-significant difference ($P > 0.05$) in FSH and estrogen concentrations compared to the control group (Figure 2A, B). These results agree with (Samare-Najaf et al., 2020; Rad et al., 2021; Rabah et al., 2023; Fareed et al., 2023). Doxorubicin disturbs the hormonal balance of the hypothalamic-pituitary-gonadal (HPG) axis, affecting essential hormones causing impairment in the process of folliculogenesis and oogenesis, resulting in the loss of ovulation and the uterine cycle (Mohan et al., 2021) which is associated with reduction in the duration of the pro-estrous and estrous phases in rodent models. Additionally, doxorubicin induces mitochondrial failure in oocytes that have compromised calcium signaling and endoplasmic reticulum stress (Wang et al., 2019; Mohan et al., 2021) and caused a significant reduction in the average number of primary, secondary, and Graafian follicles in treated rats (Bhardwaj et al., 2023).

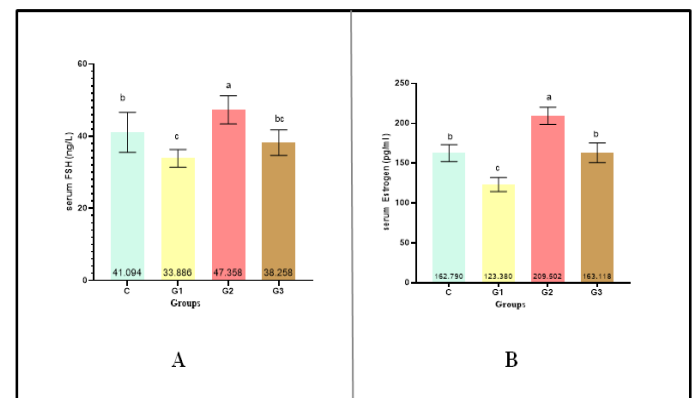


Figure 2: Effect of CurSeNPs, doxorubicin and both on (A) serum FSH and (B) serum estrogen in adult female rats after 14 days.

Values are expressed as mean $\pm$ SD, n=5. C= Control group. G1 = Injected with i/p doxorubicin 4.40 mg /kg. B.W. G2 = Administrated with CurSeNPs 10.47 μ g / kg B.W orally. G3 = Administrated with CurSeNPs 10.47 μ g / kg B.W orally and with doxorubicin 4.40 mg/ kg B.W i/p. Different small letters present on bars indicate significant differences between groups at P<0.05.

DOX prevent the synthesis of topoisomerase II and the generation of reactive oxygen species, which results

in impairment of the cell membrane, DNA damage, hyperactivation of primordial follicles and apoptosis (Chen et al., 2018; Lee et al., 2023), consequently exhaust the supply of ovarian follicles and disruption of the female reproductive capacity (Wang et al., 2018; Niringiyumukiza et al., 2019; Spears et al., 2019; van der Zanden et al., 2021). The synergistic properties of CurSeNPs, which result from the combination of selenium and the active substances found in *Curcuma longa*, have been found to reduce the impact of free radicals (Ishaq et al., 2021) and maintain the levels of FSH and estrogen within the normal range (Azami et al., 2020). Curcumin shows a beneficial impact on ovarian disorders due to its anti-inflammatory (Memarzia et al., 2021), anti-apoptotic (Xia et al., 2020; Lu et al., 2023) and antioxidant characteristics (Kamal et al., 2021). Moreover, curcumin effectively decreases hyperglycemia, hypogonadism and impaired insulin resistance in diverse conditions, such as polycystic ovary syndrome (PCOS) (Akter et al., 2023).

NF-KB AND TGF- β

The results presented in Figure 3A, B, exhibited that there is a remarkable ($P < 0.05$) increase in the serum NF-KB and TGF- β concentrations in the doxorubicin treated group. These results are in line with (Kabel, 2018; Ismail et al., 2021). Doxorubicin treatment has been found to increase levels of proinflammatory mediators such as TNF- α , intercellular adhesion molecule-1 (ICAM-1), cyclooxygenase-2 (COX-2), TGF- β , NF- κ B, MPO, IL-1 β , and IL-6 in cardiac cells (Alanazi et al., 2020; Al-Aziz et al., 2020). TGF- β acting as a cytokine, can enhance proliferation and differentiation and promote fibrosis by stimulating fibroblast proliferation and conversion to myofibroblasts (Kim et al., 2019; Gencer et al., 2017; Liu et al., 2017). Findings showed that DOX enhanced the mRNA expression of TGF- β 1, TNF- α , IL-6, and IL-10, initiating strong inflammatory responses that cause severe damage to cardiomyocytes (Subburaman et al., 2014). Also, oxidative stress can directly stimulate the activation of NF- κ B, which in turn leads to the activation of NF- κ B, causing an increase in the expression of NLRP3 (Liu et al., 2017). A recent study demonstrated that lowered extracts have significant antioxidative and anti-inflammatory effects due to the combined action of selenium and phytochemicals (Al-Brakati et al., 2021; Mohamed et al., 2023). Curcumin effectively minimises or inhibits the inflammatory process by decreasing the generation of inflammatory factors and possesses the capacity to regulate cellular signaling pathways, so it could be preventing the development of inflammation by modulation of cell division and programmed cell death signaling pathways, or by suppressing the expression of genes associated with tumor growth (Li et al., 2024).

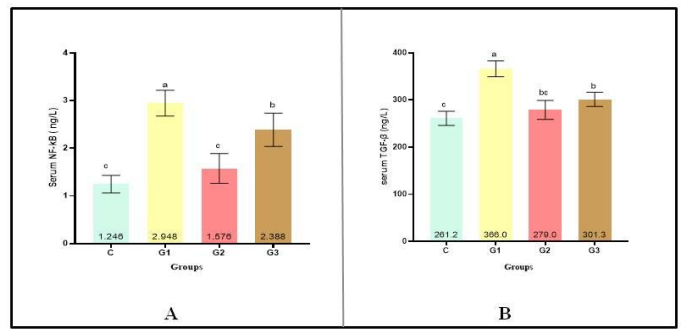


Figure 3: Effect of CurSeNPs, doxorubicin and both on (A) serum NF- κ B and (B) serum TGF- β in adult female rats after 14 days.

Values are expressed as mean $\pm$ SD, n=5. C= Control group. G1 = Injected with i/p doxorubicin 4.40 mg /kg. B.W. G2 = Administrated with CurSeNPs 10.47 μ g / kg B.W orally. G3 = Administrated with CurSeNPs 10.47 μ g / kg B.W orally and with doxorubicin 4.40 mg/ kg B.W i/p. Different small letters present on bars indicate significant differences between groups at $P < 0.05$.

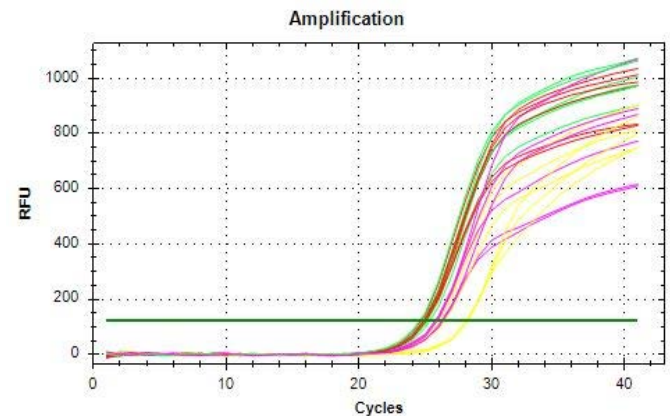


Figure 4: The real-time amplification plots of PTEN gene expression of ovarian tissue in rats experimental samples. The purple plots (control group), green plots (G1 group), red plots (G2 group), yellow plots (G3 group).

PTEN

The mRNA protein from rats' ovarian tissues showed a significant increase ($P < 0.05$) in PTEN gene expression observed in doxorubicin-treated rats (Figures 4, 5, and 6), whereas groups G2 and G3 exhibited a significant reduction in this gene expression. Doxorubicin has the potential to increase PTEN gene expression by modulating the PI3K and mTOR signaling pathways (Wang et al., 2017). In contrast, CurSeNPs may mitigate doxorubicin-induced ovarian damage in rats by targeting these pathways (dos Santos Silva et al., 2023; Rabah et al., 2023). Major signaling pathways controlling the recruitment and growth of primordial follicles include phosphatase and tensin homolog on chromosome 10 (PTEN), phosphatidylinositol 3-kinase (PI3K), and protein kinase B (PKB, Akt). Additionally, impaired DNA damage response (DDR)

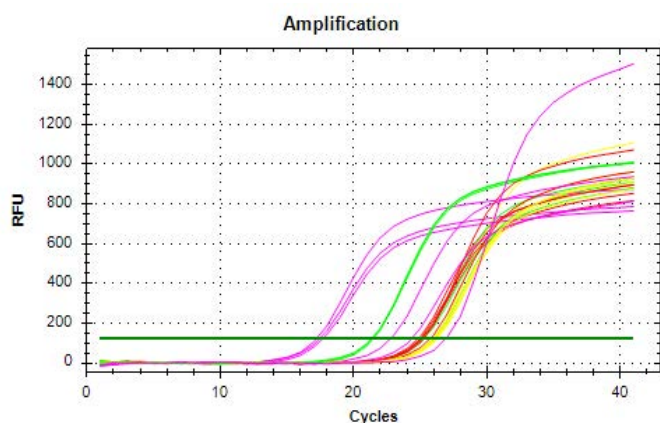


Figure 5: The real-time amplification plots of housekeeping (GAPDH) gene expression of ovarian tissue in rats experimental samples. The purple plots (control group), green plots (G1 group), red plots (G2 group), yellow plots (G3 group).

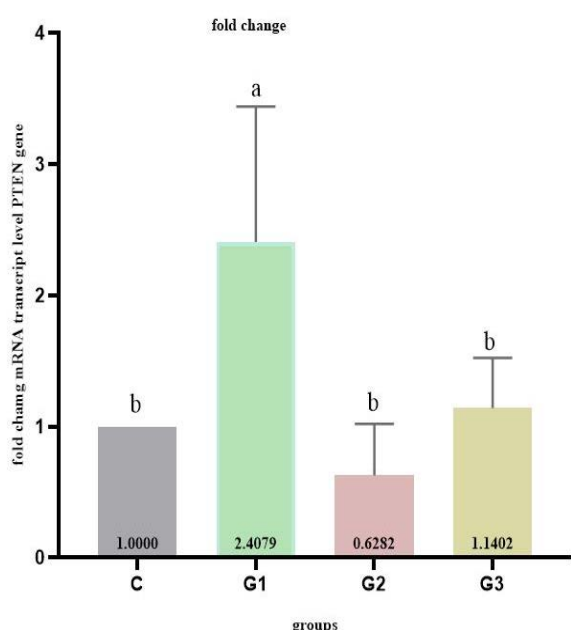


Figure 6: Assessment of PTEN gene expression in ovarian tissues of rats treated with CurSeNPs, doxorubicin and both after 14 days.

Values are expressed as mean $\pm$ SD, n=5. C= Control group. G1 = Injected with i/p doxorubicin 4.40 mg /kg. B.W. G2 = Administrated with CurSeNPs 10.47 μ g / kg B.W orally. G3 = Administrated with CurSeNPs 10.47 μ g / kg B.W orally and with doxorubicin 4.40 mg/ kg B.W i/p. Different small letters present on bars indicate significant differences between groups at P<0.05.

may occur due to accelerated primordial follicle activation through this mechanism (Maidarti et al., 2020). The PI3K/ Akt signaling pathway disruption significantly impacts ovarian follicular granulosa cells which involved in the development of insulin resistance, mitochondrial mutations and polycystic ovary syndrome (PCOS) (Qiu et al., 2020;

Dabravolski et al., 2021; Liu et al., 2021). Curcumin, a major active polyphenolic compound, impacts the female reproductive system by exerting anti-inflammatory effects and regulating endocrine function (Lv et al., 2021). Many investigations have demonstrated that curcumin plays a role in preserving the ovarian reserve's steadiness is achieved via regulating the PTEN-AKT-FOXO3a pathway. Collectively, the findings indicate that curcumin has the potential to serve as a preventive measure against chemotherapy-induced ovarian damage and as a viable pharmaceutical option for treating patients (Akbari et al., 2020; Pistollato et al., 2017).

CONCLUSIONS AND RECOMMENDATIONS

CurSeNPs utilize *Curcuma longa* extract to naturally produce nanoparticles that demonstrate preventive and therapeutic actions against ovarian toxicity induced by doxorubicin in mature female rats. CurSeNPs represent a therapeutic approach with antioxidant, anti-inflammatory, and anticancer properties.

ACKNOWLEDGEMENT

This experiment was carried out with the aid of the University of Baghdad's College of Veterinary Medicine.

NOVELTY STATEMENT

The novelty of this manuscript lies in illustrating how curcumin selenium nanoparticles increase PTEN gene expression in rat ovarian tissues by mitigating the adverse effects of anticancer drugs through inhibition of the PI3K and mTOR pathways.

AUTHOR'S CONTRIBUTION

Baraa Najim was responsible for designing the experiment. Rawya Shakir conducted data analysis, interpretation, and experimental procedures. Baraa Najim provided technical support, wrote the article, and also provided conceptual advice.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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