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# Effects of tamoxifen-loaded solid lipid nanoparticles on the estrogen receptor-α (ER-α) and vascular endothelial growth factor-A (VEGF-A) genes expression in the endometrial tissue of ovariectomized female Sprague-Dawley rats

Saman Javid, Nasrin Ziamajidi, Shadi Foroughi, Roghayeh Abbasalipourkabir*

*Department of Clinical Biochemistry, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*

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ABSTRACT

*Background:* The effect of tamoxifen on endometrial carcinogenesis stems from its estrogen agonist effect. An *in vivo* study was carried out to compare the effect of tamoxifen-loaded solid lipid nanoparticles and free drug on the ER-α and VEGF-A genes expression.
*Material and methods:* Twenty-four female Sprague-Dawley rats divided into 4 groups of six rats were used for this study. The first and second groups were ovariectomized and given tamoxifen and tamoxifen-loaded SLN respectively for six days continuously. Group 3 served as the untreated ovariectomized control group and group 4 was made up of untreated normal healthy rats. At the end of the study, the rats were sacrificed and study of the genes expression and serum oxidative stress were carried out.
*Results:* The results of this study showed that treatment with tamoxifen-loaded SLN significantly reduced the mRNA levels of ERα and VEGF-A gene and the total oxidant status compared to the ovariectomized control group.
*Conclusions:* The results of this study revealed that encapsulation of tamoxifen in solid lipid nanoparticles may have less adverse effects on the oxidative stress status and incidence of endometrial cancer.


## 1. Introduction

Breast cancer is one of the most important health concerns of contemporary society [16].

Although the mortality rates from breast cancers have decreased in most developed countries owing to more frequent mammographic screening and extensive use of tamoxifen (TMX), it remains the second highest cause of death in women [9].

In hormone-sensitive cancer, patients receive chemotherapy with cytotoxic drugs. The cytotoxic drugs treat cancers by causing cell death or growth arrest. Efficacious cancer chemotherapy has the capacity to shrink a tumor or to help destroy cancer cells [15].

ER-α gene and VEGF-A are involved in the pathogenesis of "tamoxifen dependent" endometrial cancer. In humans and adult mice, during the proliferative phase of the menstrual cycle, estradiol ($E_2$) is the major mitogen in the uterine cells that acts via the transcription factor receptor called ER-α [46]. Estrogen receptor stimulation in uterine cells increases the cell proliferation and risk of developing cancer [23]. In uterine cells, tamoxifen acts as an estrogen agonist [32]. Increased ER-α gene expression in the endometrial tissue during tamoxifen therapy has been reported. The risk of endometrial cancer increases due to estrogenic effect [13].

VEGF-A is a specific mitogen of endothelial cells with potential capability in angiogenesis. Physiological function of VEGF-A gene include reconstruction of the endometrium during monthly periods, but over expression can lead to an increase in cell proliferation and tumor formation in this tissue. It has been shown that the expression of VEGF-A in the uterus increases by two factors; estradiol (E2) and tamoxifen [17]. Considering the fact that the importance of this gene in the development of endometrial carcinoma has been substantiated, it was chosen as a representative of the growth factor genes.

Thus, alternative methods of drug administration like appropriate drug carrier system is required to surmount this problem. Depending on the route of administration, the size of drug carriers

* Corresponding author at: Department of Clinical Biochemistry, School of Medicine, Hamadan University of Medical Sciences, 65178 Hamadan, Iran.
*E-mail addresses:* rpourkabir@hotmail.com, abbasalipourkabir@umsha.ac.ir (R. Abbasalipourkabir).

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may range from a few nanometers (colloidal carriers) to micrometers (microparticles) and to several millimeters (implants). Among these carriers, nanoparticles have shown great promise for parenteral application of chemotherapeutic drugs [33].

In this study, the comparative effect of free tamoxifen (TMX) and tamoxifen-loaded solid lipid nanoparticles (TMX-SLN) on the estrogen receptor-α (ER-α) and vascular endothelial growth factor-A (VEGF-A) genes expression in the uterus of female ovariectomized Sprague-Dawley rats was assessed.

## 2. Materials and methods

### 2.1. Materials

Softisan® 154 (S154) or hydrogenated palm oil was given as a gift by CONDEA (Witten, Germany). Lipoid S100 (soy lecithin) was given as a gift by Lipoid KG (Ludwigshafen, Germany). Thimerosal, sorbitol and tamoxifen were purchased from Sigma.

### 2.2. Preparation and characterization of TMX-loaded SLN

SLN was prepared using the high-pressure homogenization (HPH) technique [41]. 70 g of palm oil (S154) and 30 g of soy lecithin (S100) were weighed within a short time, mixed and ground in a ceramic crucible and thereafter heated to 65–70 °C until a clear yellowish solution was obtained. A solution consisting of 1 ml of oleyl alcohol, 0.005 g of thimerosal, 4.75 g of sorbitol and 89.25 ml of bidistilled water was added to each of the lipid matrices. The mixtures were stirred on a magnetic stirrer using a teflon-coated magnet for 30 min at room temperature. The lipophilic drug model, TMX with a concentration of 10 mg was dissolved in 1 ml olive oil and mixed with 50 mg of SLN using an Ultra Turrax® (Ika, Staufen, Germany) at 13,000 rpm for 10 min. The mixture of TMX-SLN was then incubated at 50–60 °C while stirring overnight with a teflon-coated magnet at 500 rpm and then exposed to air until solidification was achieved. TMX-loaded SLN was then characterized by the particle size and polydispersity index (PDI), zeta potential (Zetasizer; Mal 1033452, Malvern Instruments, UK) and fourier transform infrared spectroscopy (FTIR; Perkin Elmer, Spectrum 65, England).

### 2.3. Experimental design and procedure

Twenty-four virgin female Sprague-Dawley rats aged 6–8 weeks, weighing 180–200 g were purchased from Pasteur Institute of Iran. The animals were housed on the basis of two rats per plastic cage and allowed to acclimatize under standard conditions (12 h light/dark cycles) for one week. The rats were given free access to distilled water and commercialized food throughout the experiment. The rats were anaesthetized with a mixture of ketamine/xylazin (100/5 mg/kg B.W.) by intraperitoneal injection and were bilaterally ovariectomized under standard method [22]. After operation, the animals were allowed to recover for 2 weeks before the commencement of the study. The rats were divided into 4 groups of 6 rats each. The first group (T) was given tamoxifen (2 mg/kg B.W.) dissolved in 1 ml olive oil, the second group (TS) was given 2 mg/kg B.W. tamoxifen-loaded SLN (a total of 2 mg/kg B.W. TMX loaded in 10 mg/kg B.W. SLN dispersed in 1 ml olive oil), the third group (C) was made up of untreated ovariectomized rats and served as the ovariectomized control group and group four (H) served as the healthy unovarietomized group. Treatments were given to the animals orally for 6 consecutive days using gastric intubations. At the end of the study, the rats were sacrificed using an overdose of ketamine and their uterus were harvested, washed with ice-cold normal saline and stored at −80° C until the commencement of the gene expression study. The rats' blood samples were also collected by cardiac puncture using 23 G needles and were allowed to clot at room temperature, and centrifuged at 1000*g* for 10 min. Serum were separated and analyzed for total antioxidant capacity (TAC), malondialdehyde (MDA), total oxidant status (TOS) and thiol levels. The experimental procedure was approved at the Hamadan University of Medical Sciences (UMSHA) and the research was conducted according to the guidelines for the care and use of laboratory animals of UMSHA.

**Table 1**
The temperatures and reactions times of Real-time PCR.

| Reaction Phase | Temperature | Period |
|---|---|---|
| Initial activation | 95 °C | 10 min |
| 50 cycles of: | | |
| Denaturation | 95 °C | 15 s |
| Annealing | 60 °C | 60 s |
| Extension | 60 °C | 60 s |

### 2.4. Quantitative real time polymerase chain reaction (qRT-PCR)

Both estrogen receptor-α (ER-α) and vascular endothelial growth factor-A (VEGF-A) mRNA expression were assessed by quantitative reverse transcriptase polymerase chain reaction (RT-PCR) using specific primers for amplification. In this study, by using trizol (Invitrogen), total RNA was extracted from frozen uterine tissue and t the RNA was later removed. The extracted total RNA purity ratio ($A_{260}/A_{280}$) was determined using a spectrophotometer (WPA Bio Wave II). For synthesis of the cDNA by reverse transcription of 1 μg RNA, we used Revert Aid™ First Strand cDNA Synthesis Kit (K1622, Fermentase, USA). Expression of both genes, estrogen receptor-α (ER-α) and vascular endothelial growth factor-A (VEGF-A) was determined by real time polymerase chain reaction (Applied Biosystem Step one plus) using Master Mix SYBR Green method and detection system (AB, Invitrogen). The temperatures and reactions times of real-time PCR are shown in Table 1. The agarose gel electrophoresis (1%) was used to confirm cDNA amplification. The characteristics of the forward and reverse primers of the target genes, ER-α (NM_012689.1), VEGF-A (NM_001110333.2) and house keeping gene, beta actin (NM_031144.3) are shown in Table 2. Cycle threshold (Ct) of both genes was determined and ΔCt and $2^{-\Delta\Delta ct}$ (fold change) of the genes were analyzed [30].

### 2.5. Determination of oxidative stress status

**Total Antioxidant Capacity (TAC):** TAC in serum samples was assessed using ferric reducing antioxidant power assay (FRAP) [5].

**Malondialdehyde (MDA)**: MDA as a lipid peroxidation index was determined using fluorometric thiobarbituric acid method [6].

**Total Oxidant Status (TOS):** The oxidation of ferrous ion to ferric ion accompanied with a number of oxidant species in acidic pH was used for the measurement of TOS in serum. The ferric ion was determined using xylenol orange [14].

**Protein Thiol Groups (SH):** Protein SH (P-SH) is a marker of free radical damage to the cells and was determined using DTNB (Ellman's reagent) (2,2-dithiobisnitrobenzoic acid). The absorbance of yellowish complex was assayed at 412 nm [20].

### 2.6. Statistical analysis

The data were expressed as mean ± standard deviation. For statistical analysis, the experimental values were compared with their corresponding control values. One-way analysis of variance (ANOVA) incorporated in SPSS software (version 16.0) was used to show the significant difference between the experimental and

**Table 2**
The characteristics of primers of, ER- α, VEGF-A and Beta actin genes.

| Genes | Primers | Primer lengh | Amount of use (μl) | GC% | Tm (°C) | Product length (bp) |
|---|---|---|---|---|---|---|
| ER-α | Forward: GAGCACATTCCTTCCTTCCGT | 21 | 1 | 52.38 | 60.34 | 147 |
| | Reverse: CGAGGTACAGATTGGCTTCCC | 21 | 1 | 57.14 | 60.47 | |
| VEGF-A | Forward: CAAGGCAGACTATTCAACGG | 20 | 1 | 50.00 | 56.53 | 142 |
| | Reverse: GGCACGATTTAAGAGGGGAA | 20 | 1 | 50.00 | 57.59 | |
| Beta actin | Forward: ATCAGCAAGCAGGAGTACGAT | 21 | 1 | 47.62 | 59.24 | 94 |
| | Reverse: AAAGGGTGTAAAACGCAGCTC | 21 | 1 | 47.62 | 59.39 | |

**Table 3**
Effect of treatment with TMX and TMX-SLN on body weights of female Sprague-Dawley rats.

| Time | Groups | | | | |
|---|---|---|---|---|---|
| | T | TS | C | H | *P* Value |
| Pretreatment (g) | 222.00 ± 6.54 | 223.67 ± 3.50 | 224.33 ± 6.22 | 223.33 ± 6.06 | 0.907 |
| End of the study (g) | 239.00 ± 1.90 [a,b,*] | 241.17 ± 2.79 | 243.00 ± 2.53 | 244.00 ± 0.75 | 0.003 |

All values are expressed as mean ± standard deviation.
[*] $p < 0.05$.
[a] Comparing with Cont.
[b] Comparing with Healthy.

**Table 4**
Effect of treatment with TMX and TMX-SLN on ΔCt values of the genes in uterine tissue of female Sprague-Dawley rats.

| Genes | Groups | | | |
|---|---|---|---|---|
| | T | TS | C | H |
| ER-α | 3.63 ± 0.68 | 5.32 ± 0.41[a,b,c,*] | 2.93 ± 0.77 | 3.58 ± 0.43 |
| VEGF-A | 7.13 ± 0.54 | 7.67 ± 0.99[a,b,*] | 6.87 ± 0.59 | 7.07 ± 0.42 |

[a] comparing with control group.
[b] comparing with healthy group.
[c] comparing with TAM group.
[*] *p* Value < 0.05.

control groups. The significant difference was considered 0.05 or less.

## 3. Results

### 3.1. Preparation and characterization of TMX-loaded SLN

The authors used high pressure homogenization to prepare SLN because the matrix lipid composed of palm oil (i.e., a triglyceride mixture of natural, hydrogenated and unbranched fatty acid chains) was suitable for the incorporation of lipophilic drugs such as tamoxifen [53]. Soy lecithin was the most useful surfactant in SLN dispersions. SLN and TMX-loaded SLN were characterized *in vitro* for particle size, particle size distribution and zeta potential. In this study, the average size of TMX-loaded SLNs (251.65 ± 33.02 nm) was significantly larger than that of the free SLNs (152.87 ± 9.91 nm), and the surfaces of TMX-loaded SLNs carried a positive charge (10.16 ± 0.22 mv). This may be attributed to the fact that drug is either adsorbed to particle surface or entangled in aliphatic chains of triglycerides [34].

The FTIR spectra for drug free SLN, TMX and TMX-loaded SLN are shown in Fig. 1. The specific peaks of SLN were observed at 2849 $cm^{-1}$, 2915 $cm^{-1}$ and 3351 $cm^{-1}$ (related to the stretching of O—H and aliphatic C—H bonds). The peaks at 1725 $cm^{-1}$ and 1736 $cm^{-1}$ (related to the stretching of C=O and C=C bonds) and 1045–1079 $cm^{-1}$ single bonds of C—C and C—O were also observed. As shown in Fig. 1, TMX showed stretching bond of O—H, aromatic and aliphatic C—H bonds at 2700–300 $cm^{-1}$, C=C and C=O bonds peaks at 1400–1700 $cm^{-1}$ and C—C and C—O at 500–1600 $cm^{-1}$. According to the results obtained and presented in Fig. 2, in TMX-loaded SLN spectrum, all the peaks at 2766–3075 $cm^{-1}$ were shifted to less frequency at 2800–2900 $cm^{-1}$. Therefore, it can be concluded that the drug encapsulated in the lipid core is in amorphous state [28].

### 3.2. Effect of TMX and TMX-loaded SLN on the body weight of animals

The results presented in Table 3 showed that before treatment there was no significant difference in body weight among the studied group. The body weight of all animals increased during the study period. Treatment with TMX for 6 days induced significant difference between the TMX group and control/healthy group while no significant changes was observed between TS and C/H groups.

### 3.3. Effect of TMX and TMX-loaded SLN on the gene expression of estrogen receptor-α (ER-α) and vascular endothelial growth factor-A (VEGF-A) in the animals' uterine tissue

Amplification of both genes (ER-α and VEGF-A), which was established by electrophoresis (agarose 1%) is presented in Fig. 2. Expression of the estrogen receptor-α (ER-α) and vascular endothelial growth factor-A (VEGF-A) genes were evaluated by RT-PCR. Tables 4 and 5 shows the results of ΔCT and mRNA fold change

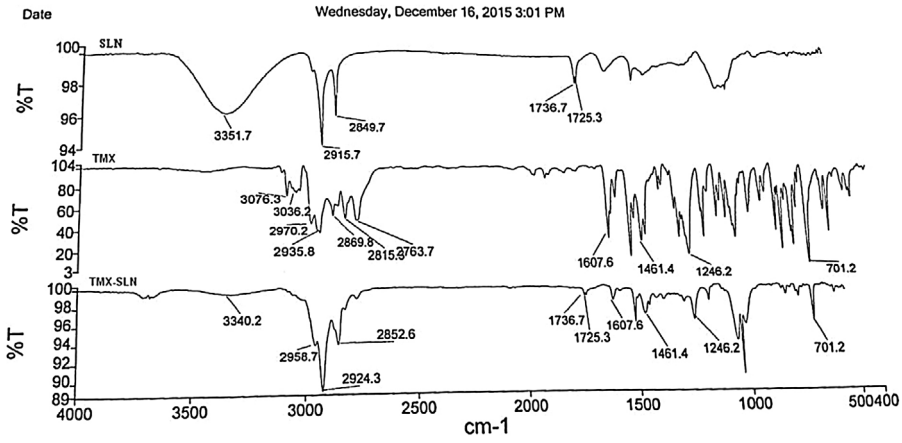


**Fig. 1.** FTIR spectra of SLN, TMX and TMX-SLN.

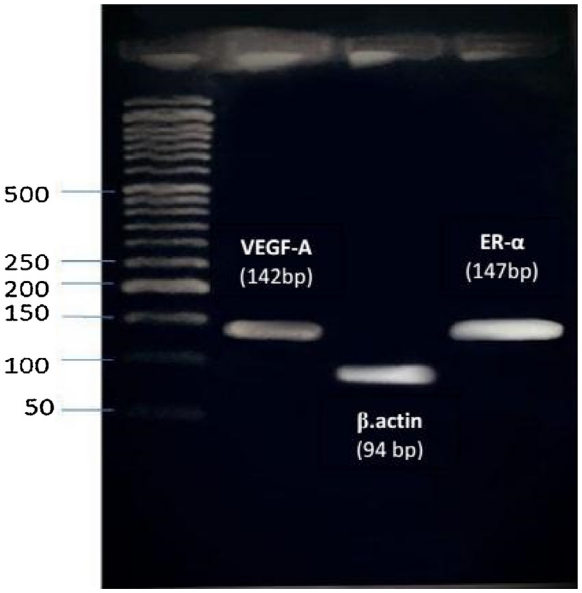


**Fig. 2.** Analysis of PCR product on 1% agarose gel contains: DNA ladder (200 bp), VEGF-A (142 bp), β-actin (94 bp) and ER-α (147 bp).

**Table 5**
Fold change ($2^{-\Delta\Delta ct}$) of ER-α and VEGF-A gene expression in studied groups.

| Groups | ER-α | VEGF-A |
|---|---|---|
| T/C | 0.61 | 0.83 |
| T/H | 0.96 | 0.95 |
| TS/C | 0.19* | 0.57* |
| TS/H | 0.29* | 0.65* |
| TS/T | 0.30* | 0.68 |

* $p$ Value < 0.05.

($2^{-\Delta\Delta CT}$) of both genes in the uterine tissue of the studied groups respectively. The results of ΔΔCt and $2^{-\Delta\Delta Ct}$ showed significant differences between TMX-SLN and other groups. While treatment with free TMX did not produce significant change, the mRNA level of ER-α and VEGF-A in comparison to the control and healthy groups significantly reduced the expression of the ER-α gene compared to the C, H and T groups when the drug was encapsulated in SLN. TMX-SLN also decreased the mRNA level of the VEGF-A when compared to the C group and H group.

### 3.4. *Effect of TMX and TMX-loaded SLN on the rats' Serum oxidative stress status*

In this study, blood samples obtained from the rats on day 6 upon treatment were used to determine the serum oxidative stress status. The effects of TMX and TMX-SLN on the oxidant and antioxidant parameters are presented in Table 6. Although ovariectomy may lead to increased SH group and decreased TOS and MDA compared to the healthy group, those changes were however not significant. While treatment with TMX-SLN insignificantly decreased TAC and SH group in comparison to the control and healthy groups, TMX further decreased TAC and the SH group. According to the results shown in Table 6 regarding the total oxidant status (TOS), TMX insignificantly, reduced TOS compared to the control and healthy group and TMX-SLN only showed significant reduction in TOS compared to the control group. Based on the data in Table 6, treatment with TMX and TMX-SLN led to negligible increase in MDA compared to the control group.

## 4. Discussion

Nano colloidal drug delivery systems can improve drug efficiency by increasing bioavailability of poorly soluble drugs, provide protection for sensitive active compounds [12] and facilitate controlled release of drugs [36]. In the present study, FTIR spectroscopy was used to characterize the bonding status of the TMX-SLN. Two spectrums at 2852 and 2924 $cm^{-1}$ in TMX-SLN spectrum were concurrent with spectrums at 2869 and 2935 $cm^{-1}$ in TMX respectively and wider spectra peak at 3340 $cm^{-1}$ indicating the loading of tamoxifen in solid lipid nanoparticles [50]. These results buttressed previous reports [4] and proved the successful loading of TMX in SLN, and indicated some interactions between SLN and TMX.

The cytotoxic effect of TMX probably involves more than one pathway. One of the pathways may be independent of estrogen receptor. In this pathway, TMX increased the levels of the transforming growth factor-ß1 (TGF-ß1), a pleiotropic cytokine that regulates the multiplication and functional activity of a wide range of cell types [45]. The second pathway is ER dependent, and involves the binding of TMX to the ER to form a TMX-ER complex that competitively inhibits the binding of estrogen to its receptor. The TMX-ER complex binds to an estrogen responsive element (ERE) that contains estrogen sensitive genes. As a result, transcription of estrogen sensitive genes is attenuated. Thus, TMX arrests the cell cycle in the G1 phase, thereby decreasing cell multiplication [10] and promoting apoptosis [8]. The estrogen receptor-mediated antiestrogenic effect of TMX on breast cancers could lead to either cytostatic or cytotoxic effects. This effect of TMX is dependent on

**Table 6**
Effect of treatment with TMX and TMX-SLN on serum stress oxidative parameters in female Sprague-Dawley rats.

| Parameters | Groups | | | | |
|---|---|---|---|---|---|
| | T | TS | C | H | *P* Value |
| TAC(mmol/ml) | 0.44 ± 0.05 | 0.48 ± 0.10 | 0.58 ± 0.30 | 0.58 ± 0.70 | 0.126 |
| SH (mmol/ml) | 0.26 ± 0.09 | 0.34 ± 0.24 | 0.40 ± 0.03 | 0.30 ± 0.05 | 0.64 |
| TOS(mmol/ml) | 1.24 ± 0.33 | 1.06 ± 0.28[a,*] | 1.77 ± 0.12 | 2.06 ± 0.57 | 0.009 |
| MDA (μm/l) | 0.79 ± 0.06 | 0.83 ± 0.22 | 0.75 ± 0.12 | 0.90 ± 0.14 | 0.827 |

All values are expressed as mean ± standard deviation. TAC = total antioxidant capacity; MDA = maolndialdehyde; TOS = total oxidant status.
[*] $p < 0.05$.
[a] Comparing with Healthy.

concentration. Nanomole concentrations of TMX only cause growth arrest whereas at micromole concentrations, the drug induces cell death [7,31,38].

By regulating estrogen receptors, tamoxifen forestalls tumor growth in mammary tissue. However, tamoxifen has some negative side effects such as endometrial cancer. In the current study, the effects of free tamoxifen and tamoxifen encapsulated in solid lipid nanoparticles (SLNs) on the expression of estrogen receptor alpha (ER-α) and vascular endothelial growth factor A (VEGF-A) genes were investigated. It was found that ER-α and VEGF-A are involved in endometrial complication. To remove the variation of estrogenic effect in the sexual cycles, the animals were ovariectomized before treatment.

The results obtained from the study showed that ovariectomy increased the expression of ER-α ($p = 0.04$) and VEGF-A ($p = 1.000$) compared to the healthy group. It is possible that due to decreased level of estrogen, ovarietomy led to activation of a compensatory mechanism to increase the expression of the ER-α gene [39]. Increased expression of VEGF-A gene maybe due to localization of its mRNA in endometrial stroma following ovariectomy [27]. Based on the result presented in Table 5 treatment with tamoxifen at a dose of 2 mg/kg bw led to reduced expression of ER-α gene compared to the ovariectomized control group, although it was not significant ($p = 0.225$). Regarding estrogenic effect of tamoxifen on the endometrial tissue [24], it can be concluded that administration of tamoxifen at the dose of used in the current study, reduced the compensatory mechanism in activating the ER-α gene expression when comparing to the control group.

There is less investigation on the effects of tamoxifen on ER-α expression. Jordan et al., found that tamoxifen is an agonist of estrogen receptor in the endometrial tissue but it has an estrogen receptor antagonist role in the mammary gland of humans and animals [25]. The estrogen receptor has two activator transcriptional domains in endometrial and mammary gland tissue. Tamoxifen respectively activates and inhibits mammary gland and endometrial domain [44].

Tsai et al., reported that tamoxifen stimulates migration of the tumor cells by increasing ER-α expression [47]. In the other study, it was found that in women with benign mammary tumor who were treated with tamoxifen, ER-α expression was higher than those who did not receive it [35]. It can be adduced to the fact that there are different variants of ER-α and tamoxifen may increase one of the variants. In agreement with this result, Lin et al., reported that in cells with low ER-α gene expression, tamoxifen may increase ER-α 36, a variant of ER-α [29].

The results of the present study also showed that the group treated with encapsulated tamoxifen in SLN significantly reduced the endometrial level of ER-α gene expression in comparison with the ovariectomized control, healthy and tamoxifen treated groups ($p$ value < 0.001). Therefore, tamoxifen encapsulated in SLNs showed less estrogenic effects on the endometrium compared to free tamoxifen. It means that encapsulation of the drug in SLNs may decrease its side effect. However, administration of tamoxifen-loaded SLNs after menopause may regulate the carcinogenic effects of the drug on the endometrial tissue.

We previously reported that IC50 of TAM-loaded SLN on the breast cancer cell lines were generally lower than that of free TAM [1]. This indicates that TAM cytotoxicity may be the result of improved drug internalization through encapsulation in SLN matrix and endocytosis [43].

It was reported that encapsulation of tamoxifen in nano-sized colloidal system will improve drug efficiency and viability, thus its toxic side effects can be reduced [1,52].

The results of the current study showed insignificantly increased expression of VEGF-A in ovariectomized rats compared to the healthy group. Karuri et al. reported similar result, that ovariectomy may increase VEGF-A mRNA level in endometrial stroma [27].

According to Table 5 tamoxifen negligibly decreased ($p = 1.000$) the expression of VEGF-A gene compared to the ovariectomized control and healthy groups while treatment with TAM-loaded SLN significantly decreased ($p = 1.00$) it when compared to those groups, suggesting that loading of TMX in SLN may decrease the negative side effects of drug on the endothelium due to sustained drug release.

VEGF-A is an important angiogenesis factor in menstrual cycle. According to the study by (Cullinan-Bove and Koos) [11] estrogen regulates VEGF-A gene expression in ruminant uterus. Some anti-estrogen molecules such as tamoxifen may increase VEGF-A expression in human and animal uterus [21,48]. Some studies proposed that women taking tamoxifen develop abnormal vaginal bleeding due to an increase in the effect of tamoxifen expression of VEGF-A gene followed by endothelial blood vessel growth [42]. Based on the study by (Adams et al.) [2] tamoxifen induces VEGF-A expression and leads to endothelial hyperplasia. (Yamamoto et al.) [51] reported similar result, that the plasma level of VEGF-A was significantly higher in women with endothelial tumor compared to healthy women. According to (Helmestam et al.) [19] tamoxifen stimulates the expression of VEGF-A while it has inhibitory effects on VEGF-R1 gene expression.

This study showed that ovariectomy led to higher serum antioxidant parameters (TAC and SH) and lower oxidant parameters (TOS and MDA) compared to the healthy group. This result is comparable to (Kankofer et al.) [26], who found that ovariectomy first increased TAC and glutathione peroxidase (GSH-Px) in the 2nd, 4th, and 5th week and then significantly increase them in the 3rd, 6th and 7th week of the study. However, there are some reports that have shown high oxidative stress in ovariectomized rats [18,37]. According to the data obtained, which is presented in Table 6, although the antioxidant status (TAC and SH) was reduced in the group treated with TMX-SLN compared to the control and healthy groups, treatment with TMX further reduced them. The rats that received TMX showed no significant reduction in TOS and MDA compared to the control and healthy groups while TMX-SLN significantly reduced TOS compared to the ovariectomized control rats. When tamoxifen was encapsulated in the SLN formulation, its impact on the oxidative stress improved because its release was sustained. In

spite of the fact, that some research has introduced tamoxifen as an effective antioxidant and membrane protectant against oxidative damage [49], other reports have shown that oxidative stress could occur as a result of prolonged tamoxifen therapy on the MCF-7 cell lines [40]. (Atakisi et al.) [3] reported that plasma TAC levels were not affected by 6 and 24 h tamoxifen therapy. The current study showed that when tamoxifen was encapsulated in SLN, its properties improved and its side effect decreased.

## 5. Conclusion

Breast cancer is the most prevalent cancer and the second leading cause of cancer death among women in the world. Multiple drug resistance (MDR) is a major obstacle to the success of cancer chemotherapy. The main challenge in cancer chemotherapy is toxic side effects induced by chemotherapeutic drugs. Single dose or short time application (1–2 weeks) will probably induce serious health problems, but the use of biodegradable nano-sized particles for long-term or lifetime therapy may produce other serious side effects. Increasing the encapsulation efficiency of poorly water-soluble molecules will lead to the development of improved SLN formulations. In the near future, it is expected that more studies will focus on improving SLN and drug-loaded SLN formulations to increase the efficacy, and reduce the side effects of chemotherapeutic drugs for anticancer treatment. These studies should include preparation of formulations with different particle size and distributions, different matrix lipids and additional ingredients. Thus, if nanoparticulate drug delivery systems are to be used effectively and routinely, the issue of toxicity of the components of the nanoparticles must be addressed. Indeed, SLN requires further development before it can be utilized as a new drug delivery system for chemotherapy drugs in the treatment of human cancers. Further studies are needed to develop and optimize this drug delivery system in the treatment of cancers.

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