



# An *In Vitro* Assessment of the Cytotoxic and Apoptotic Potency of Silymarin and Silymarin Loaded Solid Lipid Nanoparticles on Lung and Breast Cancer Cells

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## ABSTRACT

Cancer disease is a major cause of death worldwide in the last few decades. Recently, the risk of cancer and its mortality rates have increased both in economically less and highly developed countries. Two of the leading causes of the death of cancer are lung and breast cancers among males and females, respectively. A variety of drugs are in use for cancer treatment but their recruiting properties are limited because of the developing resistance and/or finiteness of bioavailability. Current research is aimed at synthesis of a new effective agent of a different type, silymarin-loaded solid lipid nanoparticles and investigation of its effectiveness on treatment of lung and breast cancers. In this respect, these nanoparticles and silymarin were elucidated by using MTT assay for cytotoxicity, annexin-V analyses for cell death mechanism and confocal and transmission electron microscopies for morphological and ultrastructural changes. Based upon our findings, it can be concluded that silymarin-loaded solid lipid nanoparticles significantly reduced the growth of A549 and MCF-7 cells compared to silymarin. Also these nanoparticles induced apoptosis both in A549 and MCF-7 cells in higher percentages than that of silymarin. In microscopic investigations, it was shown clearly that apoptotic cell death hallmarks in silymarin and silymarin-loaded solid lipid nanoparticles treated cells, being more prevalent for the latter. These findings suggested improved bioavailability of silymarin in nanoparticle formulation, consequently silymarin-loaded solid lipid nanoparticles could be considered as a useful drug delivery system for silymarin that has poor water-solubility and an excellent candidate for anticancer drug development.

## Article Information

Received 25 October 2019

Revised 30 October 2019

Accepted 19 November 2020

Available online 23 April 2021

## Key words

Silymarin, Nanoparticles, Cancer, Cytotoxicity, Apoptosis

## INTRODUCTION

Lung cancer is the leading cause of death among males in developed and less developed countries. The disease with maximal mortality among females in less developed countries is reported as breast cancer (Torre *et al.*, 2015). Classical chemotherapy alone became ineffective in cancer treatment as to developing resistance by many of cancer cell types (Liu, 2004; Obradovic *et al.*, 2013). Thus, novel agents offering theranostic potency are required. Eugenol, eucalyptol and camphor promising anticancer potency as natural agents were elucidated in parental and drug-resistant human lung cancer cell lines (Özkan and Erdoğan, 2013). It was proved that these agents are effective in apoptosis promotion but more effective ones are needed to be found (Özkan and Erdoğan, 2013). Apoptosis is defined as a programmed cell death that occurs via cell-derived or extracellular initiators that mainly regulates the tissue

homeostasis of the body. Triggering apoptotic cell death is one of the main research focuses of drug development with anticancer activities. In this respect, research on finding the mode of cell death starts with detection of the physiological and morphological changes that refer to apoptosis such as membrane disintegration, chromatin and nuclear condensation of cells exposed to an anticancer agent (Kerr *et al.*, 1972).

Silymarin a flavonolignan that is a bioactive constituent of *Silybum marianum* possesses chemosensitizing, antioxidant and anti-inflammatory potentials. In addition, as a phytochemical, it has concentration-related anti-apoptotic and cell transporters altering, free radical scavenging, lipid peroxidation inhibitory and cellular membrane stability enhancing and steroid-like activities (Karimi *et al.*, 2011; Prasad *et al.*, 2016; Montgomery *et al.*, 2016). Recently, compounds of natural sources have been reported to inhibit hyperproliferation of the cells, their neoplastic transformation as well as carcinogenesis (Agarwal *et al.*, 2006). Phytochemicals as compounds of natural resources and their usefulness for cancer prevention and/or treatment are on the main focus of cancer research

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(Aljuffali *et al.*, 2016). The antioxidant, anti-inflammatory and proapoptotic properties are implying their anticancer efficacy (Iriti and Faoro, 2009). Food like fruits and vegetables has different kind of phytochemicals that have anticancer potency (Montgomery *et al.*, 2016). Currently, silymarin is used as a hepatoprotective drug supplement in both clinical and experimental models due to its above mentioned properties (Karimi *et al.*, 2011; Eminzade *et al.*, 2008). Silymarin has been declared as safe/non-toxic/with low toxicity in human studies (Eminzade *et al.*, 2008). However, the molecular mechanisms underlying the bioactivity of silymarin and its drug/food interactions have still remained unclear (Mayer *et al.*, 2005). In addition, anticancer activities of the compound are limitedly investigated for some cancer types in vivo and in vitro. In the studies carried out by Agarwal *et al.* (2006) and Malewicz *et al.* (2006) the chemo-preventive efficiency of silymarin on breast cancer was elucidated but they considered silymarin as conflicting in mammary carcinogenesis (Agarwal *et al.*, 2006; Malewicz *et al.*, 2006). To unravel the deeper mechanism of the anticancer activities of silymarin and its derivatives in mammary and other type of cancers, deeper investigations are needed to be performed. In the last few decades, investigations on cancer treatment by using natural agents with low toxicity and side effects are in rise. Silymarin has many bioactive properties as to balance the survival and death/apoptosis as well as to be anti-inflammatory, anti-metastatic under the cells. The cytoprotective, cancer protective and anticancer efficacies of silymarin have been investigated under breast, prostate, cervical, liver, colon and lung cancers (Gezgin *et al.*, 2013). Despite the usefulness of silymarin in many cancer research, it has limitations on the bioavailability due to its quite low solubility in water (Provinciali *et al.*, 2007). To diminish this limitation, novel approaches on preparing sophisticated formulations of silymarin for cancer therapy are required. Recent approaches on enhancement of the drug bioavailability have been redirected on preparing nanoscale formulations. Their sizes vary from 1 nm to several hundred nanometers and can be used as carriers to conquer the limitations of drugs (Jabir *et al.*, 2012). Nanoparticles as drug carriers are considered to provide controlled, targeted delivery and high bioavailability of drugs (Almeida *et al.*, 2015). As one of that kind of carriers, solid lipid nanoparticles (SLNs) were involved to eliminate the limitations and lower the side effects of natural products that were used as drugs and their derivatives (Seyfoddin *et al.*, 2015). When used orally, SLNs loaded with drugs in their monolayer phospholipid shell, are beneficial as to their enhanced theranostic opportunity and pharmacokinetics such as lengthening half-life, stability, reduced side effects, clearance, mean

residence time and enhanced bioavailability etc. (Jabir *et al.*, 2012; Li and Huang, 2008; Geszke-Moritz and Moritz, 2016; Chih-Hung *et al.*, 2017). Furthermore, they lower the risk of toxicity with their biodegradable lipid content and offer safe and efficient drug delivery especially for drugs with limited solubility in water (Jaafar-Maalej *et al.*, 2011). SLNs are preferred among other colloid drug carrier systems due to their benefit for being low-priced and easiness for production in big commercial amounts (Harde *et al.*, 2011).

In this study, it is aimed to prepare solid lipid nanoparticles carrying commercial silymarin by using hot homogenization method and to examine their cytotoxicity and apoptosis promoting capability in comparison with silymarin alone on human lung adenocarcinoma and human breast cancer cells.

## MATERIALS AND METHODS

### Materials

MCF-7 (ATCC® HTB-22™) and A549 (ATCC® CCL-185™) purchased from the American Type Culture Collection (Manassas, USA). The silymarin powder (S0292), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl-2H-tetrazolium bromide) (M2003), fetal bovine serum, penicillin-streptomycin, dimethyl sulfoxide (DMSO) were from Sigma-Aldrich (St. Louis, USA), and Roswell Park Memorial Institute medium (RPMI-1640) was obtained from GIBCO (Grand Island, USA). Compritol and polyoxyethylene sorbitan monooleate (Tween 80) were purchased from Merck Schuchardt (Darmstadt, Germany).

### Synthesis of silymarin-loaded solid lipid nanoparticles (SLNs)

Hot homogenization method described before by Muller *et al.* (2000) ([www.sigmaaldrich.com](http://www.sigmaaldrich.com), accessed 24 March 2018) was used for the preparation of silymarin-loaded SLNs. Briefly, used lipid agent was kept in a hot water bath in a glass well until it melted (80 °C). Silymarin (5%) was added in the melted lipid and tween 80 (3%) was included as surfactant. 87% (w/w) of the suspension consists of bidistilled water. This mixture was homogenized via using Ultra-Turrax at 20500 rpm (T25, Janke and Kunkel IKA®, Germany). After it cooled down at room temperature and the filtration was performed, the prepared suspension was stored at +4 °C in dark drug bottles.

### Analyses of characteristics of the particles

Particle size, polydispersity index, zeta potential and electrical conductivity of the silymarin-loaded SLNs and silymarin were measured in distilled water in triplicate

by using green zeta cells at 25°C under Malvern Zetasizer Nano ZS (Malvern Instruments). For polydispersity index measurement, the Nano Zetasizer (ZS, Malvern, UK) was adjusted to a fixed angle of 90° and 25°C temperature. To measure the potential of the samples, bidistilled water was used with electrical conductivity of 50  $\mu\text{S}/\text{cm}$  (adjusted with NaCl).

#### *Scanning electron microscopy (SEM) for visualization of the morphological characteristics of the particles*

The shape of the particles was imaged by SAM. For preparation of SAM samples, suspensions of silymarin-loaded SLNs and silymarin were dropped onto the carbon stickers on the separate sample holders. Samples were allowed to air for drying. Prepared samples were placed in argon atmosphere and coated with gold for SEM imaging.

#### *Cell culture*

Breast MCF-7 (ATCC® HTB-22™) cancer cells and lung A549 (ATCC® CCL-185™) cancer cells were cultured in phenol red free RPMI 1640 medium (Gibco, USA) containing penicillin-streptomycin (1%) (Gibco, USA), fetal bovine serum (10%, Lonza, Belgium) at 37°C in a humidified atmosphere supplemented with CO<sub>2</sub> (5%). Cells with a confluency of 80% were used in all of the investigations.

#### *Cytotoxicity assay*

Commercial silymarin used in this study was containing (45% w/w) silybin (A and B) isosilybin (A and B), silychristin, silydianin taxifolin finally apigenin 7-glucoside (Muller *et al.*, 2000). The effect of silymarin on the viability of breast and lung cancer cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In this respect, a stock solution of silymarin was prepared (in DMSO with 1% final concentration) (Polyak *et al.*, 2010; Gharagozloo *et al.*, 2013). The prepared silymarin-loaded SLNs were suspended in fresh culture medium. These solutions were applied on different concentrations (2,34-150 $\mu\text{M}$ ) on A549 and MCF-7 cells plated into 96-well plates (1  $\times 10^5$  cells per well). Cells were incubated with the agents for 24 h at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> in air. At the end of incubation period, 20  $\mu\text{L}$  of MTT solution (5mg/mL) was added to each well and incubated for 2 h under the same conditions. After the incubation, the medium from each well was aspired and 200  $\mu\text{L}$  of dimethyl sulphoxide was added to the wells, mixed thoroughly and kept at room temperature for 10 minutes. Plates were read on an ELISA reader at a wavelength of 570 nm (n = 3).

#### *Confocal imaging for apoptosis visualization*

In order to examine the nuclear morphology and

membrane integrity of cells, two fluorescent dyes, phalloidin and acridine orange were used respectively. MCF-7 and A549 cells were seeded in 6-well culture plates at a density of 3 $\times 10^5$  per well and treated with IC<sub>50</sub> inhibition concentrations of silymarin and silymarin loaded solid lipid nanoparticles at 37 °C for 24 h.

At the end of the treatment period, cells were incubated with phalloidin and acridine orange at room temperature for 20 min in the dark. Stained cell was mounted to slides and imaged with a confocal microscope.

#### *Transmission electron microscopy (TEM)*

MCF-7 and A549 cells treated IC<sub>50</sub> value of silymarin and silymarin-loaded solid lipid nanoparticles for 24 h were fixed in glutaraldehyde (2.5%, in 0.1 M phosphate buffer (pH 7.4) and post fixed in osmium tetroxide (2%). After the fixation, cell samples were dehydrated in graded ethyl alcohol (70%, 90%, 96%, and absolute alcohol) then embedded in Epon 812 epoxy and sectioned on ultramicrotome. Thin sections were prepared using a glass knife to a maximum thickness of 100 nm. Thin sections were stained in lead citrate and uranyl acetate and evaluated under TEM.

#### *Annexin-V analysis*

Mode of cell death triggered by silymarin and silymarin-loaded SLNs was elucidated by annexin-V staining in A549 and MCF-7 cells. In this manner, all test cell groups (untreated, silymarin and silymarin-loaded SLNs treated cells prepared in triplicate) were harvested by using trypsin and 100  $\mu\text{L}$  of each group were transferred to separate eppendorf tubes. These cell groups were incubated with 100  $\mu\text{L}$  of annexin-V reagent/sample for 20 min at room temperature in dark as described in the user manual of Muse® Annexin-V and Dead Cell Assay Kit and analyzed by using Muse™ Cell Analyzer (Merck, Millipore, Hayward, California, USA).

#### *Statistical evaluations*

For statistical analysis of our results, one-way ANOVA was performed for multiple comparisons Tukey post-test of Graphpad Prism 6.0 for Windows and the data was expressed as means  $\pm$  SDs.  $p < 0.05$  and  $p < 0.01$  values were considered as statistically significant.

## RESULTS AND DISCUSSION

Cancer therapy by using the classical chemotherapeutics has too many side effects also exhibit high level of toxicity for patients. Novel approaches on cancer therapy imply the use of phytochemicals and/or other nanoscale sized natural products that offer low

toxicity and fewer side effects. These nano formulations are more effective in low doses than the normal sized agents in cancer treatment (Numanoğlu and Tarımcı, 2006). In this study, silymarin-loaded SLNs were prepared and characterized by measuring the particle size, zeta potential, polydispersity index and conductivity. In addition, the shape of nanoparticles was visualized by using scanning electron microscopy method. The size of the particles refers to the physical stability and activity of SLNs (Numanoğlu and Tarımcı, 2006). The size of the silymarin-loaded SLNs was measured 92.5 nm that is about ten times smaller compared to the size of silymarin particles (997.2 nm) that implies to a nano formulation-derived to reduce of the size of silymarin particles (Table I). Zeta potential was measured to examine the storage stability of SLNs. Zeta potential values of silymarin-loaded SLNs were presented in Table 3. As it can be seen at Table II, silymarin-loaded SLNs were negatively charged and had a potential about  $-11.7$  mV. This value refers to a relatively good dispersion quality and physical stability (Venkateswarlu and Manjunath, 2004). This potential may be affected by storage conditions and aggregation may occur. Aggregation in charged particles does not rarely occur due to the electric repulsion, though (Al-Haj and Rasheed, 2009). Other important values of stability for SLNs dispersion are electrical conductivity and polydispersity index (Al-Haj and Rasheed, 2009). These values were determined in the safe level for a relatively good stability of particles (Tables I and II). Moreover, scanning electron microscopic evaluation results indicated the spherical particles of SLNs loaded with silymarin while a cubic-like shape for silymarin particles (Fig. 1).

The short time treatment with nanoparticles led to the inhibition of the growth of A549 and MCF-7 cells. Growth inhibition of A549 cells exposed to different silymarin concentrations raised when applied concentration increased during 24 h of incubation.  $IC_{50}$  value for these cells was  $33 \mu M$  (Fig. 2). Also, viability of treated A549 cells decreased in accordance with the dose and  $IC_{50}$  value was calculated as  $25 \mu M$  for 24 h of silymarin-loaded SLNs. The half maximal inhibitory concentration ( $IC_{50}$ ) of silymarin under MCF-7 cells for 24 h was not found at the range of applied concentrations (Fig. 3). This might be implying the limited bioavailability of silymarin. However,  $IC_{50}$  concentration of SLNs for MCF-7 cells ( $18 \mu M$ ) was found to be lower than that of SLNs for A549 cells. This might be attributed to the difference between the used cell lines and their sensitivity to applied nanoparticles that are needed to be figured out with further investigations. Similar to our results, Deep *et al.* (2007) have considered that silymarin and its nano formulation have been antiproliferative in human prostate carcinoma LNCap and 22Rv1 cell lines.

Silymarin loaded SLNs showed an antigrowth efficacy on A549 and MCF-7 cells with half-maximal inhibitory concentrations ranging at  $18$ - $25 \mu M$ , respectively. Our results refer to a good anticancer potency in lower doses for silymarin-loaded SLNs for A549 and MCF-7 cells.

**Table I. The particle size and polydispersity index of silymarin and silymarin loaded solid lipid nanoparticles.**

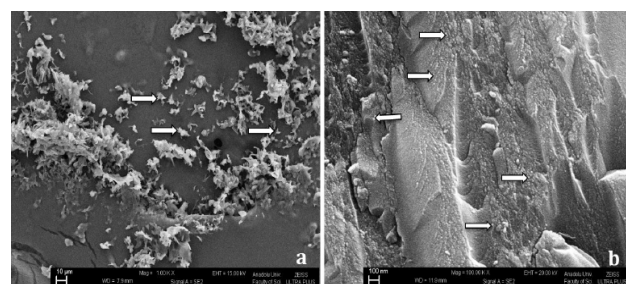
| Formulations         | Particle size (nm) |       | Polydispersity index (PI) |        |
|----------------------|--------------------|-------|---------------------------|--------|
|                      | Medium (n = 3)     | S.D.  | Medium (n = 3)            | S.D.   |
| Silymarin            | 997.2              | 53.73 | 0.752                     | 0.0001 |
| Silymarin loaded SLN | 92.54              | 1.03  | 1.000                     | 1.063  |

Data are presented as mean values and showing standard deviation (n= 3).

**Table II. The zeta potential and electrical conductivity of silymarin and silymarin-loaded solid lipid nanoparticles.**

| Formulations         | Zeta potential (mV) |      | Electrical conductivity ( $mS \cdot cm^{-1}$ ) |        |
|----------------------|---------------------|------|------------------------------------------------|--------|
|                      | Medium (n = 3)      | S.D. | Medium (n = 3)                                 | S.D.   |
| Silymarin            | -19.2               | 6.59 | 0.01                                           | 0.0005 |
| Silymarin loaded SLN | -11.7               | 1.09 | 0.01                                           | 0.002  |

Data is shown as mean values and standard deviation (n= 3).



**Fig. 1. The shape and morphology of silymarin (a, arrows-silymarin particles) and silymarin loaded SLN (b, arrows-silymarin-loaded SLN particles).**

On the confocal images of A549 cells exposed to  $IC_{50}$  value of silymarin alone for 24 h, holes on cytoskeleton, nuclei with condensed chromatin were detected as morphological changes. On A549 cells treated with silymarin-loaded SLNs were seen morphological alterations as fragmented cytoskeleton, pyknotic nucleus, chromatin condensation as well as holes on cytoskeleton

(Fig. 4). Similarly, in MCF-7 cells exposed to silymarin alone, condensed chromatin, fragmented nuclei and diminished number of cells in a typical cell cluster were found. Moreover, in silymarin-loaded SLN treated MCF-7 cells were detected to be shrunken and excessive chromatin condensation and highly perforated cytoskeleton were seen alterations in these cells (Fig. 5). Our confocal finding implies apoptosis in both cell lines treated with silymarin alone and silymarin loaded SLNs, latter was more effective in promoting apoptotic cell death with apparent hallmarks of apoptosis especially for A549 cells.

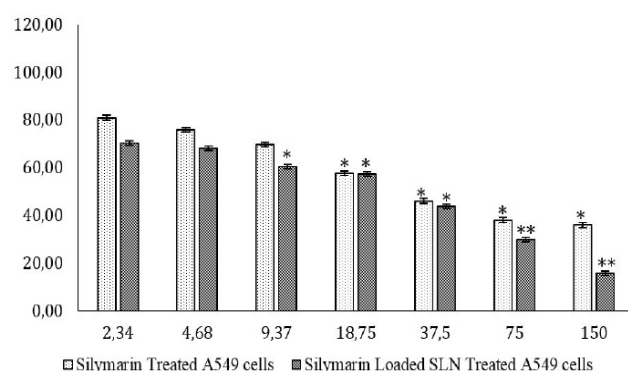


Fig. 2. Viability percentages of silymarin and silymarin-loaded SLN treated A549 cells for 24 h.  $IC_{50}$  value was detected to be 33  $\mu$ M (for silymarin) and 25  $\mu$ M (for silymarin-loaded SLN). \* $p < 0.05$ , \*\* $p < 0.01$

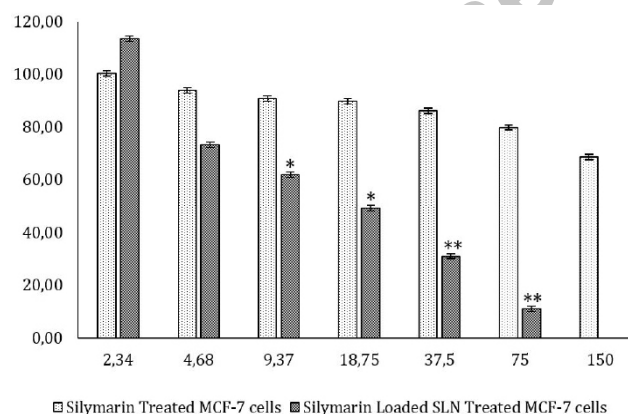


Fig. 3. Viability percentages of silymarin and silymarin-loaded SLN treated MCF-7 cells for 24 h.  $IC_{50}$  value for silymarin treated MCF-7 cells was not detected in this concentration range of the agent for 24 h.  $IC_{50}$  value for silymarin-loaded SLN treated MCF-7 cells was detected to be 18  $\mu$ M for 24 h. \* $p < 0.05$ , \*\* $p < 0.01$

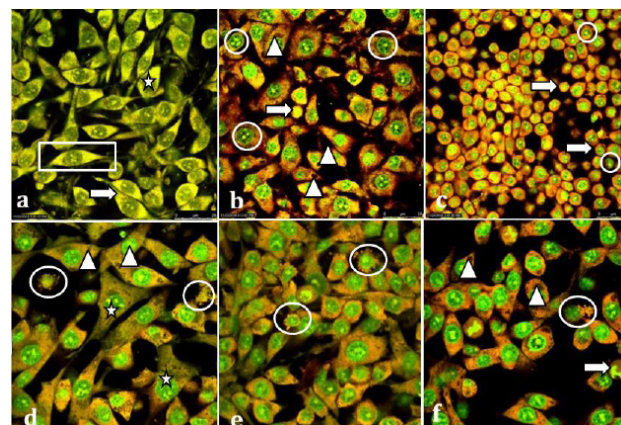


Fig. 4. Untreated A549 cells (a), asterisk-nucleus, arrow-cytoskeleton, rectangle-fusiform cell shape. A549 cells treated with  $IC_{50}$  value of silymarin (b and c), arrow-shrunken (circular shaped) cells, arrow head-holes on cytoskeleton, circle-nuclei with condensed chromatin and A549 cells treated with  $IC_{50}$  value of silymarin-loaded SLN (d, e and f) for 24 h; circle-cells with fragmented cytoskeleton, arrow-pyknotic nucleus, asterisk-chromatin condensation, arrow head-holes on cytoskeleton.

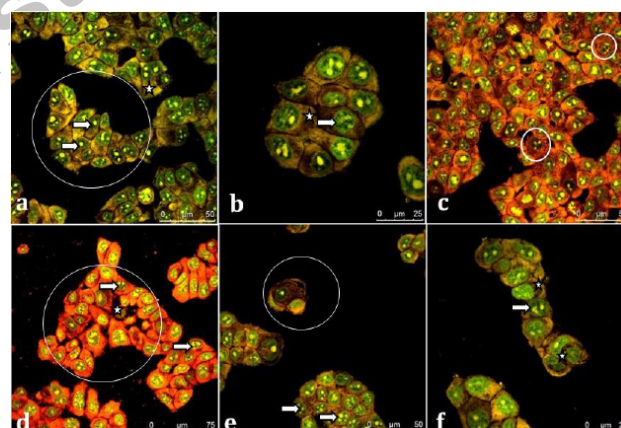


Fig. 5. Untreated MCF-7 cells (a and b); asterisk-cytoskeleton, arrow-nuclei, circle-typical cell cluster. MCF-7 cells treated with  $IC_{50}$  value of silymarin (c and d); arrow-condensed chromatin, asterisk-condensed cell, circle-lacerated cell cluster, small circle-fragmented nuclei and MCF-7 cells treated with  $IC_{50}$  value of silymarin-loaded SLN (e and f) for 24 h; circle-shrunken cell cluster with decreased number of cells, arrow- excessive chromatin condensation, asterisk-highly perforated cytoskeleton.

Cytotoxicity assays involve detection of cell death mode. The ultrastructural and physiological changes that occur during the death of cells can be visualized by transmission electron microscopic imaging that remains “gold standard” in this kind of examining. Some of these

changes that can be taken into consideration for apoptotic cell death are reported as degradation of specific cellular proteins, condensation and fragmentation of nuclear chromatin as well as loss of membrane integrity of cellular membrane and organelles such as mitochondria (Kerr *et al.*, 1972). In our study, in silymarin treated A549 and MCF-7 cells (Fig. 7), swollen mitochondria, lacerated cisterna of endoplasmic reticulum, disintegration of cytoskeleton with holes on it, granular cytoplasm, condensed chromatin (Fig. 6), secondary lysosomes, loss of cristae, granulated cytoplasm, cleavage of nuclear membrane (pyknotic nucleus) were detected TEM findings, respectively. However, in A549 and MCF-7 cells exposed to silymarin-loaded SLNs for the same incubation time pyknotic nucleus, disintegrated mitochondrion with excessive loss of cristae, highly swollen cisterna of endoplasmic reticulum fragmentation of nucleus (Fig. 6), and chromatin condensation, disintegrated cell membrane and fragmented cytoskeleton containing huge holes (Fig. 7) were determined ultrastructural changes. From our TEM results it can be seen that SLNs treatment increased apoptosis when compared to silymarin alone application. Additionally, extensive cytoplasmic vacuolization was seen both in lung and breast cancer cells treated with SLNs implying an incompleting autophagy.

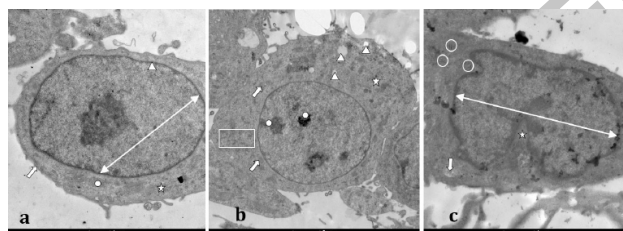


Fig. 6. Untreated A549 cell (a), arrow-cell membrane, two-headed arrow-nucleus, circle-mitochondrion with compact cristae, asterisk-cytoskeleton. A549 cell treated with  $IC_{50}$  value of silymarin (b), Arrow-swollen mitochondria, rectangle-lacerated cisterna of endoplasmic reticulum, arrow head- holes on cytoskeletal disintegration, asterisk-granular cytoplasm, circle-condensed chromatin. A549 cell exposed to  $IC_{50}$  value of silymarin-loaded SLN for 24 h (c), two-headed arrow-pyknotic nucleus with extremely condensed chromatin, arrow-disintegrated mitochondrion with excessive loss of cristae, circle-highly swollen cisterna of endoplasmic reticulum and asterisk-fragmentation of nucleus.

In a study by Deep *et al.* (2007) silymarin was examined for its antiproliferative and anticancer activities in human breast, skin, prostate, cervical, and bladder cancers. They reported that silymarin triggers apoptotic cell death in colonic mucosa by reducing beta-glucuronidase activity

and PGE2 level. Externalization of phosphatidylserine to the cell surface implies membrane disintegration and has been clear hallmark of early apoptosis (Kerr *et al.*, 1972). These changes are determined by annexin-V staining in this study. Our results showed that silymarin caused total apoptotic cell death about 26% in A549 cells while about 36% of silymarin-loaded SLN treated A549 cells underwent apoptosis (Fig. 8). The percentage of total apoptotic cells in MCF-7 cells exposed to silymarin (Fig. 9) was found as 23%. This percentage was detected to be about 34% in silymarin-loaded SLN treated MCF-7 cells. These results refer to apparent high levels of apoptotic cell death derived from SLNs application. Similarly, in a study by Deep *et al.* (2007) silymarin and its nano formulations were reported to trigger apoptosis by arresting the cell cycle in G1 phase in LNCap and 22Rv1 cell lines (Deep *et al.*, 2007).

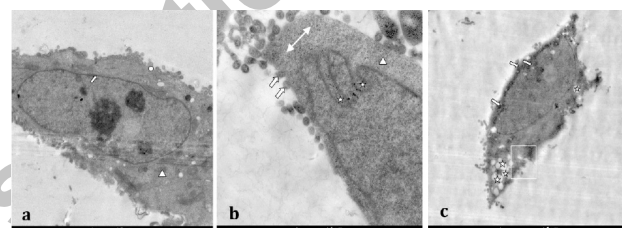


Fig. 7. Untreated MCF-7 cell (a), arrow-nuclear membrane, circle-cell membrane, arrow head-compact mitochondrion. MCF-7 cell treated with  $IC_{50}$  value of silymarin (b), Arrow-secondary lysosomes, Arrow head-loss of cristae, two headed arrow-granulated cytoplasm, asterisk-cleavage of nuclear membrane (formation of pyknotic nucleus). Silymarin-loaded SLN treated MCF-7 cell (c), arrow-extreme chromatin condensation, rectangle-disintegrated cell membrane and asterisk-fragmentation of the cytoskeleton (huge holes on cytoskeleton).

Recently, promoting apoptotic cell death by using natural agents in cancer treatment has been main research topic (Ghobrial *et al.*, 2005). In this study, the investigated agents A549 and MCF-7 agents have triggered apoptosis but that of silymarin-loaded solid lipid particles was detected significantly higher than the silymarin alone. Silymarin and its newly synthesized Nano formulation showed cytotoxic, antiproliferative and apoptosis promoting activities on breast and lung cancer cells. Moreover, SLNs caused cytoplasmic vacuolization that imply an incompleting autophagy. Taken all together, these results are good fundamentals for understanding the anticancer efficacy of silymarin and silymarin-loaded solid lipid nano formulation to conquer the limitations of silymarin in anticancer research. Furthermore, the number of researches for preparing solid lipid nanoparticles as

to use them as successful drug carriers with improved bioavailability have been in rise, recently. Thus, our results indicate the usage of drug delivery systems of colloidal characters to improve the pharmaceutical properties. It seems that silymarin-loaded SLNs exhibit a quite good efficacy for treatment of lung and breast cancers offering strong theranostic potency.

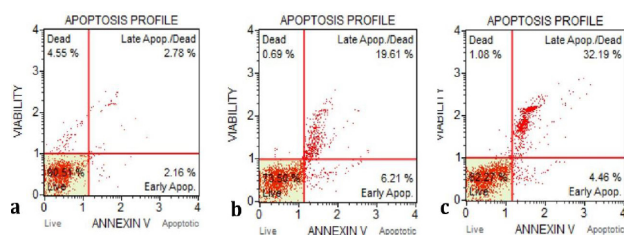


Fig. 8. Apoptosis profiles of untreated A549 cells (a) and A549 cells treated with IC<sub>50</sub> values of silymarin (b) and silymarin-loaded SLN for 24 h (c). Apoptosis profiles of A549 control cells (a) showed 90.51% live, 2.16% early and 2.78% late apoptotic cells. In this group of cells percentage of dead cells was detected to be 4.55%. A549 cells treated with IC<sub>50</sub> concentration of silymarin (b) percentage of live cells was detected 73.50. Early apoptotic and late apoptotic cells of this group were 6.21% and 19.61%, respectively. A negligible percentage (0.69%) of the same group of A549 cells were dead. Total percentage of cells that underwent apoptosis was 36.75 of that 32.19% were in late apoptosis in silymarin-loaded SLN treated A549 cells (c). The percentage of live cells in the same group was detected to 62.27 for 24 h.

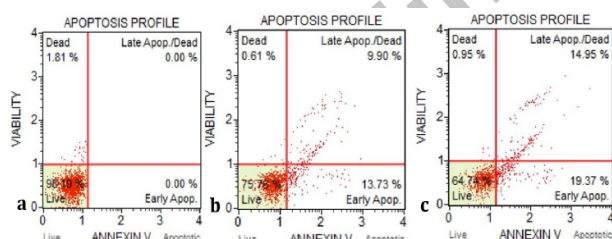


Fig. 9. Apoptosis profiles of untreated MCF-7 cells (a) and MCF-7 cells treated with IC<sub>50</sub> values of silymarin (b) and silymarin-loaded SLN (c) for 24 h. On the apoptosis profiles of untreated MCF-7 cells (a) were detected 98.19% live and 1.81% dead cells. In silymarin treated MCF-7 cells (b) the percentage of live cells was 75.76 and 13.73% and 9.90% of these cells were in early and late apoptotic stage, respectively. 0.61% of dead cells were detected in this group of cells. Early apoptotic and late apoptotic cells of this group were 6.21% and 19.61%, respectively. Live cells percentage in silymarin-loaded SLN treated MCF-7 cells (c) was detected as 64.74%. Early apoptotic and late apoptotic cell percentages were 19.37 and 14.95 for this cell group.

## CONCLUSIONS

As a conclusion, our findings suggest that both silymarin and silymarin-loaded SLN formulation have been cytotoxic under lung and breast cancer cells in dose dependent manner, latter being more effective. Due to the apoptosis initiating efficacy and growth inhibition potency of silymarin-loaded SLNs at lower doses than silymarin alone both in two cell lines SLNs was considered as a solution for eliminating the limitations of silymarin. After the required deeper sequential investigations to figure out clearly its total molecular mechanism in a whole organism, silymarin-loaded SLNs might be a strong candidate for a sophisticated chemotherapeutic agent offering potentials for theranostic cancer treatment.

### Statement of conflicts of Interest

The authors have declared no conflict of interest.

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