Review

Silibinin in cancer therapy: A promising prospect

Prabha Tiwari[1]and Kaushala Prasad Mishra*[2]

[1] Radiological Physics and Advisory Division, Bhabha Atomic Research Centre, Mumbai - 400085, India. Present address: National Institutes of Biomedical Innovation, Health and Nutrition, 7-6-8, Saito Asagi, Ibaraki, Osaka 567-0085, Japan.
[2] Radiation Biology and Health Sciences Division, Bhabha Atomic Research Centre, Mumbai - 400085, India. Present address: President, Foundation for Education and Research, 504 Neelyog Residency, K-1, Mumbai 400075 India.

***Corresponding author**: Prof. Kaushala Prasad Mishra, Radiation Biology and Health Sciences Division, Bhabha Atomic Research Centre, Trombay, Mumbai – 400 085, India. E-mail: mishra_kaushala@rediffmail.com, mishradrlp@gmail.com

Citation: Prabha Tiwari, et al. Silibinin in cancer therapy: A promising prospect. Cancer Research Frontiers. 2015 Sep; 1(3): 303-318. DOI: 10.17980/2015.303

Competing Interests: The authors declare that there are no competing interests.
Received Sept 1, 2015; Revised Dec 17, 2015; Accepted Dec 25, 2015. Published Dec 31, 2015

Abstract
The potential anti-cancer properties of natural plant compounds have elicited widespread interest in recent times since it is believed that they can substantially contribute to the development of newer and more effective anti-cancer therapies. Silibinin, a natural flavonoid, has demonstrated chemopreventive properties against many cancer types. A deeper understanding of the signaling pathways modulated by silibinin is important to realize its potential in developing targeted cancer therapies. This review summarizes the recent advances in understanding the molecular mechanisms of silibinin-induced apoptosis in various cancer types.
Keywords: Silibinin, Natural-plant-compound, anti-cancer-compound, Chemotherapeutic-agent, Apoptosis

1. Introduction

Natural plant compound related research has gained immense importance of late, chiefly because they are derived from millions of years of evolution and natural selection and are primed for bioactivity. This is especially helpful in avoiding the potential side effects of the traditional chemical based therapies. Silibinin is a plant derived compound (flavonone) isolated from milk thistle (*Silybummarianum* L.) (1). It is used for the treatment of liver disease (2) and poisoning (3). In recent years silibinin has been tested in cancer therapy and has shown promising results against various cancer types such as breast, prostate, skin, lung, colon, lung, bladder and ovarian cancers (4, 5).

Natural compounds can induce apoptosis by targeting multiple cellular signaling pathways including transcription factors, growth factors, tumor cell survival factors, inflammatory cytokines, protein kinases, and angiogenesis and therefore they offer immense therapeutic potential for effective and selective killing of cancer cells (6). Studies have suggested that silibinin has multiple targets in the cell (7, 8) (Fig. 1, Table 1). Understanding the detailed mechanisms of action of silibinin in various cancer types will help in development of silibinin as effective anticancer compound.

To minimize the systemic toxicity of chemotherapeutic agents, more efforts are being directed towards the investigation of dietary supplements and other phytotherapeutic agents for their synergistic efficacy in combination with anticancer drugs (9). In this review, we have surveyed key studies

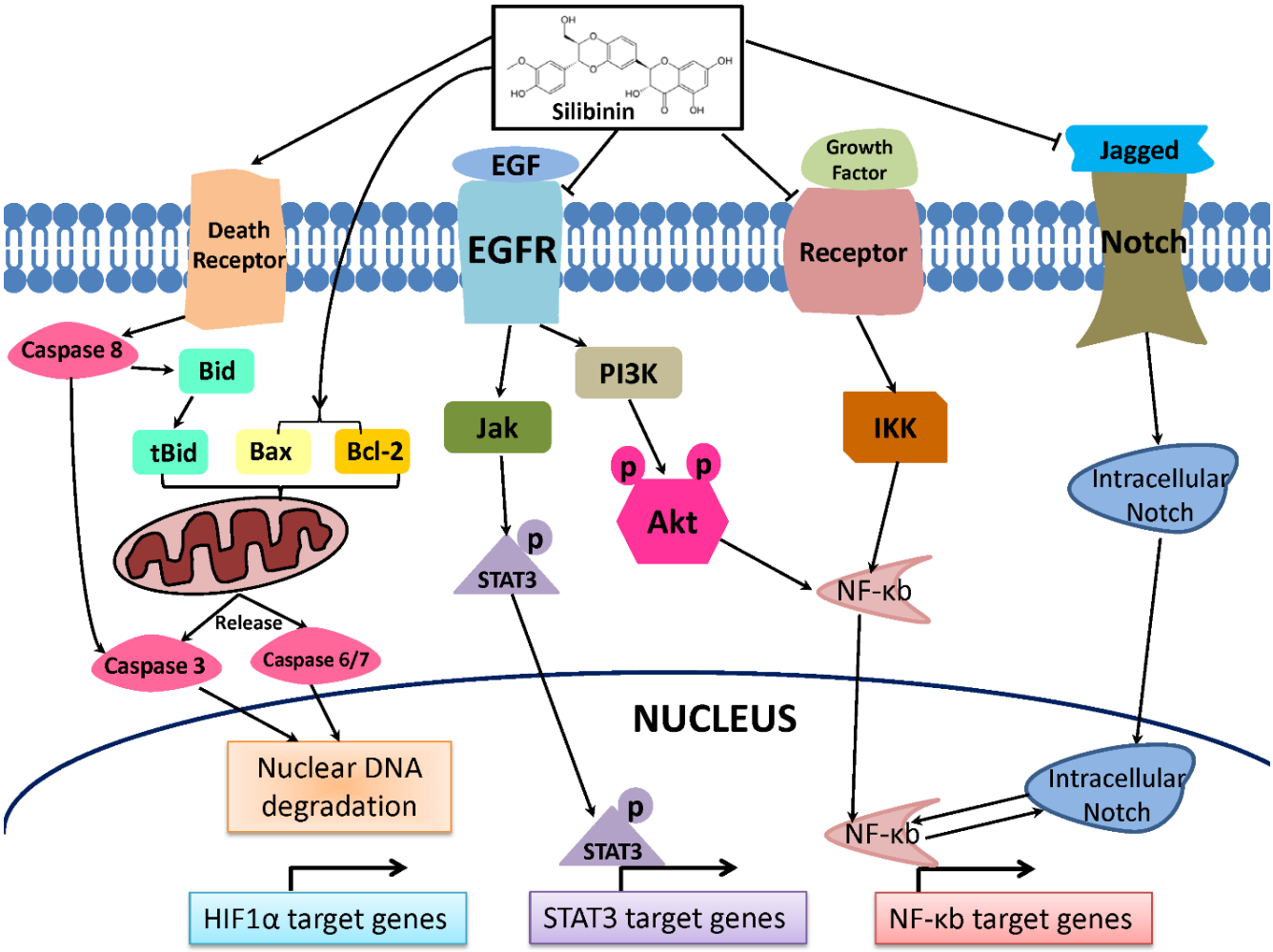

Figure 1: An abridged illustration of the key pathways influenced by silibinin in various cancer types. Silibin-induces cell death by apoptosis via independent mechanisms involving Caspase-8 and mitochondrial changes leading to nuclear DNA fragmentation. Silibinin downregulates Notch and EGFR signaling thereby modulating the decreased expression of the downstream targets of transcription factors STAT3 and NF-κB.

that have investigated the anti-cancer properties of silibinin in various cancer types, together with other chemotherapeutic drugs and compounds that modulate epigenetic states and that offer potential anti-cancer implications and the development of more effective combinatorial anti-cancer therapies.

2.1 Silibinin in Breast cancer Therapy

A large body of research suggests that silibinin has effective anti-breast cancer properties. Within our group, we focused our studies on breast cancers cells MCF-7 and T47D, which differ in the status of tumor suppressor p53 and caspase-3. Silibinin-mediated cytotoxic effects were found to be dose and time-dependent in both cell lines and T47D cells were more sensitive than MCF-7. Our findings suggested that silibinin-induced apoptosis in breast cancer cells MCF-7 and T47D is p53-independent and caspase-dependent mediated by both extrinsic and intrinsic pathways of apoptosis (10). We have demonstrated that silibinin inhibited breast cancer cell growth and induced apoptosis in MCF7 (500-120 μM) and T47D (50-100 μM) cells. Further investigation into the mechanisms of silibinin-induced apoptosis showed an involvement of

Table 1: A summary of and the various cellular pathways influenced by silibinin via molecular targets that have been characterized in various cancer types.

Cancer type	Known target pathways and genes	References
Breast Cancer	Mitochondrial apoptotis pathway [Bcl-2/Bax, BNIP3]; ROS/receptor-mediated apoptosis [AIF, Caspase 3]; Cell growth and survival [Notch-1, ERK, Akt];Transcription factors [NF-κB]; Angiogenesis and metastasis [COX-2, MMP-9, VEGF]	(10, 13, 15, 17, 63)
Bladder Cancer	ROS/receptor-mediated apoptosis [AIF, Caspase 3, PARP], Cell growth and survival [Survivin]; Angiogenesis and metastasis [VIM, MMP-2, cytokeratins (KRT18, KRT19), ZEB1]	(51-53, 64)
Cervical Cancer	Mitochondrial apoptotis pathway [Bax/ Bcl-2, Mtor]; Transcription factors [HIF-1α]; Cell growth and survival [Akt]; ROS/receptor-mediated apoptosis [Caspases (3, 8 and 9)]	(56, 58)
Colon Cancer	Cell growth and survival [ERK1/2, Akt, Cyclins (D1, D3, A and B1), CDKN1A (Cip1/p21), CDKN1B (Kip1/p27)], Transcription factors [HIF-1α]; Angiogenesis and metastasis [COX-1, COX-2, NOS, NOS3, VEGF]	(40, 41)
Lung Cancer	Cell growth and survival [ERK1/2, Akt, Cyclins (D1, D3, A and B1), CDKN1A (Cip1/p21), CDKN1B (Kip1/p27)]; Transcription factors [HIF-1α]; Angiogenesis and metastasis [COX-1, COX-2, NOS, NOS3, VEGF]	(44, 45, 68)
Ovarian Cancer	ROS/receptor-mediated apoptosis [Caspase 3]; Cell growth and survival [Akt, ERK]	(54)
Prostate Cancer	Mitochondrial apoptotis pathway [Bcl-2], Transcription factors [NF-κB]; SLUG, IGFBP-3, Cell growth and survival [CDKN1A (Cip1/p21), CDKN1B (Kip1/p27), ERK 1/2, BIRC5 (survivin), Cyclin B1]; Angiogenesis and metastasis [KRT18 (cytokeratin 18), MMP-2 , VEGF, VIM, ZEB1]	(31-33, 64)
Skin Cancer	Cell growth and survival [MAPK, CDKN1A (Cip1/p21), CDKN1B (Kip1/p27), Cyclin D1, p53, GADD45, ERK1/2, Akt, AP-1];Transcription factors [HIF-1α, NF-κB, STAT3]; Angiogenesis and metastasis [COX2, INOS]	(37, 38, 65, 66)

both casapase-8 activation and mitochondrial changes including loss of mitochondrial membrane potential and Bax/Bcl-2 redistribution. Surprisingly, despite the presence of mutant p53, silibinin was more efficient in T47D cells. Our study also provided further evidence of the existence of another mechanism of DNA fragmentation in the absence of caspase-3.

Subsequently, a number of studies have contributed to a deeper understanding of the molecular mechanisms of silibinin-induced apoptosis in breast cancer. The cytotoxic effects of silibinin on T47D were found to depend on inhibition of estrogen receptor β expression levels and also expression and secretion of leptin hormone (11). Silibinin induced apoptosis in MCF-7 and MDA-MB-231 (5-50μM) was found to be dependent on generation of reactive oxygen species (ROS) (12). Furthermore, silibinin induced cell death through an AIF-dependent mechanism in MCF7 cells and a caspase-3–dependent mechanism in MDA-MB-231 cells, and ROS generation and Notch-1 signaling act upstream of the ERK and Akt pathway (13).The anti-proliferative effect of silibinin (100-300 μM) on

SKBR3, an ErbB2-overexpressed and ER-negative human breast carcinoma cell line is regulated by the high inhibitory effect on NF-κB (11).

It was reported that silibinin treatment increased the expression of chemokine receptors CXCR3, CCR5 and CCR7 in MDA-MB-231 cells (14). *Silibinin*-loaded lipid nanoparticles (SLNs) (20 μg/mL) effectively inhibited the growth of MDA-MB-231 cells. Compared with free silibinin, SLNs exhibited stronger inhibitory effects on the invasion and migration of MDA-MB-231 cells via downregulation of MMP-9 and Snail (15). Silibinin also inhibited Wnt/β-catenin signaling by suppressing Wnt co-receptor LRP6 expression in human breast cancer cells MDA-MB-231 and T-47D (16).

Though it had been established that silibinin has antioxidant and radioprotective properties (17), according to one study silibinin promoted sustained superoxide (O(2)(.-)) production which had protective effects; while the activity of endogenous SOD was not changed by silibinin, exogenous SOD markedly enhanced silibinin-induced apoptosis (18). Another study reported the formation of reactive nitrogen species in MCF-7 cells (19). Recent findings have also revealed that silibinin induced autophagic cell death through ROS-dependent mitochondrial dysfunction and ATP depletion involving Bcl-2 adenovirus E1B 19-kDa-interacting protein 3 (BNIP3), a pro-death Bcl-2 family member, in MCF7 cells (20).

Silibinin (10-50μM) suppressed the epidermal growth factor receptor (EGFR) signaling pathway in SKBR3 and BT474 breast cancer cells and may be used as an effective drug for the inhibition of metastasis of human breast cancer (21).

Silibinin inhibited the expression of Cdc42 and D4-GDI mRNAs but had no effect on the expression of β1-integrin and Raf-1 mRNAs although it indirectly but effectively modulated the β1-integrin signaling pathway and RAF1 function and reduced the rate of metastasis, migration and adhesion of MDA-MB-231 to distant organs (22). The effects of silibinin on IFN-γ-induced FAT10 expression and chromosome instability have also been studied. HCT116 and HepG2 cells were treated with silibinin (50 and 150 μM) before karyotyping was performed. It was observed that silibinin with FAT10 can modulate IFN-γ-induced chromosome instability, apoptosis sensitivity and suppressing TNF-α-induced tumor growth (23).

The effect of silibinin in combination with anti-breast cancer drugs has also been studied in breast cancer cell lines. Effect of silibinin in combination with doxorubicin, cisplatin or carboplatin, the chemotherapeutic drugs, in human breast carcinoma, MCF-7 and MDA-MB468 cells was studied (Table 2). Combination of silibinin (25-100 μM) with doxorubicin (10-75 nM) resulted in much stronger apoptotic death compared to either agent alone in both the cell lines. On the the other hand, silibinin in combination with cisplatin (0.2-2 μg/mL) showed no additional apoptotic effect in either cell line. Silibinin plus carboplatin (2-20 μg/mL) combination induced a stronger apoptotic effect only in MCF-7 cells (24).

Silibinin in combination with curcumin offers a promising therapeutic approach against breast cancer. It was highlighted that silibinin and curcumin combination inhibited the growth of T47D human breast cancer cells. IC50 of silibinin, curcumin and their combination on T47D breast cancer cell line was 110, 30 and 20 μM for 24h (25). Moreover, Curcumin and silibinin together (IC50s for 24, 48 and 72h were 17.5, 15 and 12 μM, respectively) inhibited Telomerase Expression in T47D cells (26). Silibinin was also demonstrated to enhance UVB-induced apoptosis in MCF-7 cells (27).

Subsequent to our investigations into the mechanisms of silibinin-induced apoptosis in MCF-7 and T47 D cells, many other findings have contributed significantly to understanding the molecular mechanism of silibinin-induced apoptosis *in vitro*. It is hoped that all studies would actively contribute to the use of silibinin in breast cancer therapy which is a most challenging task.

2.2 Silibinin in Prostate cancer Therapy

Many studies have reported anti-prostate cancer properties of silibinin. Silibinin inhibited serum- and androgen-stimulated prostate-specific antigen (PSA) protein levels in LNCaP cells concomitant with cell growth inhibition via a G_1 arrest in cell cycle progression (28). Silibinin treatment for 24h at 25- and 75-μg/ml doses led to a 45 and 59% reduction in PSA secretion in the medium, respectively. Silibinin was further reported to upregulate Insulin-like growth-Factor binding protein 3 (IGFBP-3) expression and inhibited proliferation of androgen-independent prostate cancer (PC-3) cells (29). In the medium supplemented with 10% FBS, compared with vehicle or untreated controls, treatment of PC-3 cells with 2 and 20 μm silibinin resulted in 17.3% and 54% growth inhibition, respectively, after 48h.

Table 2: Silibinin in combination with various drugs and their impact on various cancer types.

Cancer type	Silibinin in combination with	Biological outcomes	References
Breast Cancer	Carboplatin	Stronger apoptotic death in MCF-7 cells	(24, 51)
	Cisplatin	No additional apoptotic effect in MCF-7 and MDA-MB468 cells	(24, 51)
	Curcumin	Inhibited growth and Telomerase expression in T47D cells	(24, 51)
	Doxorubicin	Stronger apoptotic death in MCF-7 and MDA-MB468 cells	(24, 51)
Cervical Cancer	Metformin	Synergistic effects in C-33A cells and activated caspase-3 or AIF	(59)
Colon Cancer	HDAC inhibitors TSA and SAHA	Synergistic effects in SW480 and metastatic SW620 cells	(43)
Lung Cancer	DNMT inhibitor Aza	Synergistic effects in NSCLC cells and inhibited invasion and migration	(49)
	Erlotinib	Prevented migration in NSCLC xenografts and cells	(69)
	Indole-3-carbinol	Induced apoptosis in A549 and H460 cells and inhibited ERK, Akt	(47)
	TSA and SAHA	Synergistic effects in NSCLC cells	(48)
Ovarian Cancer	IdB 1016	Inhibited growth and angiogenesis in A2780 xenografts	(71)
	Paclitaxel	Enhanced apoptosis and decreased invasion in A2780/taxol cells	(55)
Prostate Cancer	Carboplatin	Complete S phase arrest and inhibition of cdc2, cyclin B1 and cdc25C in PC-3 cells	(34)
	Cisplatin	Stronger G2-M arrest in PC-3 cells	(34)
	Doxorubicin	Strong G_2-M arrest and inhibition of cdc25C, cdc2/p34, cyclin B1 and cdc2/p34 kinases in DU145P cells	(33)
	Mitoxantrone	Induced apoptosis in PC-3 cells	(35)

Silibinin also inhibited the migration and adhesion of the human prostate adenocarcinoma (PC-3) cell line (30) and the invasion, motility and migration of highly bone metastatic $ARCaP_M$ prostate cancer cells (31) though, they were less sensitive to silibinin. Even at a high concentration of 200 μmol/L, only 18.5% growth inhibition was observed in $ARCaP_M$ cells, but 48.7%, 60.0%, and 73.8% in LNCaP, PC-3, and DU145 cells, respectively, after 48h of treatment. Furthermore, silibinin down-regulated vimentin and MMP-2 and up-regulated cytokeratin-18 and thereby, induced the morphological reversal of epithelial-mesenchymal transition (EMT) phenotype to epithelial phenotype. Silibinin also inhibited the NF-κB p50 translocation via

the up-regulation of I kappaB alpha protein, and down-regulated the expression of two major EMT regulators, ZEB1 and SLUG transcription factors (32).

The anti-prostate cancer effects of silibinin have also been examined in combination therapy. In a particular study, prostate carcinoma DU145 cells were treated with silibinin and doxorubicin in combination. The cells were treated with either DMSO (control), 100 μm silibinin, 25 nm doxorubicin alone, or 100 μm silibinin, and 24h later, 25 nm doxorubicin or *vice versa*, or a combination of both agents simultaneously for a total of 48h with 74-87% growth inhibition. Silibinin strongly synergized the growth-inhibitory effect of doxorubicin that was associated with a strong G_2-M arrest in cell cycle progression with a strong inhibitory effect of combination on cdc25C, cdc2/p34, and cyclin B1 protein expression and cdc2/p34 kinase activity. Silibinin and doxorubicin in combination were also effective in inhibiting the growth of androgen-dependent prostate carcinoma LNCaP cells (33). The cells were treated with either DMSO (*Control*), 25 μm silibinin, 15 nm doxorubicin alone, or 25 μm silibinin and 24h later 15 nm doxorubicin or *vice versa*, or a combination of both agents simultaneously for a total of of 48h. The silibinin-doxorubicin combination induced a 62–69% growth inhibition in the treated cells.

Combination of cisplatin or carboplatin with silibinin inhibited the growth of cells with a stronger G2-M arrest in cisplatin and silibinin, and a complete S phase arrest with carboplatin by a substantial decrease in the levels of cdc2, cyclin B1 and cdc25C (Table 2). Apoptosis induction was further confirmed by PARP and caspases 3, 9 and 7 whose cleaved levels were also enhanced by combination treatment with a significant increase in cytochrome *c* release in the cytosol with these combinations (34). Cisplatin alone at 2 μg/ml dose produced a 48% cell growth inhibition, whereas a combination with 50-100 μM silibinin resulted in 63-80% growth inhibition. Similarly, compared to 68% growth inhibition at 20 microg/ml carboplatin, addition of 50-100 μM doses of silibinin caused 80-90% inhibition after 48h. Silibinin in combination with mitoxantrone also inhibited cell growth and induced apoptosis in human prostate cancer cells (35). Combination of silibinin (10, 20 and 40 μM) with 50 nM of mitoxantrone after 72h showed decreased viability in DU145 and LNCaP cells while minimum changes in PC-3 cells.

Silibinin also attenuated the ionizing radiation-induced pro-angiogenic response and EMT in prostate cancer cells (36).

These research findings have significant implications in developing silibinin as an effective anti-prostate cancer therapeutic agent.

2.3 Silibinin in Skin cancer Therapy

Silibinin has also been well studied against skin cancer. Silibinin treatment showed preventive effects in human epidermoid carcinoma A431 cells and showed a marked inhibition of mitogen-activated protein kinase-extracellular signal-regulated kinase-1 and -2 activation. Silibinin treatment also induced Cip1/p21 and Kip1/p27 together with a significant decrease in cyclin-dependent kinase (CDK)-4, CDK2, and cyclin D1 (37). Silibinin prevented UVB-induced skin carcinogenesis and modulated mitogenic and survival signaling, p53, Cip1/p21 and other cell cycle regulatory molecules and increased repair of UV-induced DNA damage in mouse skin (38).

Silibinin (100 μM) treatment in JB6 mouse epithelial cell model before or immediately after UVB exposure, or both, resulted in a strong decrease in UVB-induced phosphorylation of ERK1/2 and Akt (39). Furthermore, silibinin suppressed UVB-induced activator protein-1 (AP-1) and NF-κB activation; silibinin also prevented EGF-induced ERK1/2, JNK1/2, and p38K as well as Akt phosphorylation, and also suppressed EGF-induced AP-1 and NF-κB activation (38).

Moreover, silibinin was also reported to enhance UVB-induced apoptosis in mouse epithelial JB6 cells when treated with silibinin (100 μm) for 24h via the up-regulation of DNA-Protein Kinase-dependent p53 activation (39).

The above studies suggest that silibinin offers an attractive possibility to develop a safe and natural cure against skin cancer.

2.4 Silibinin in Colon cancer Therapy

Silibinin has exhibited anti-cancer properties against colon cancer. Silibinin mediated apoptosis in human colorectal carcinoma LoVo cells in culture was associated with increased levels of cleaved caspases (3 and 9) and cleaved PARP. Silibinin (50–200 μM) treatment for 24h reduced the growth of LoVo cells by 30–49%. Also, silibinin caused a strong cell cycle arrest at G1 phase, and a slight but significant G2/M phase arrest at highest concentration. silibinin further decreased the levels of cyclins (D1, D3, A and B1), CDK1, CDK2, CDK4 and CDK6 and phosphorylation of Rb protein and increased the level of CDKIs (p21 and p27) (40).

The anti-angiogenic effect of silibinin was coupled with a strong decrease in inducible NOS and NOS3, COX-1 and COX-2, and HIF-1α and VEGF (Table 1) (41). In another study, silibinin together with TNF-related apoptosis-inducing ligand (TRAIL), synergistically induced cell death in primary colon tumor cells (SW480) and their derived TRAIL-resistant metastatic cells (SW620). Silibinin upregulated death receptor 4 (DR4) and DR5 and synergistically activated caspase-3, -8, and -9 by silibinin together with TRAIL. Moreover, silibinin and TRAIL potentiated the activation of the mitochondrial apoptotic pathway and down-regulated the anti-apoptotic proteins Mcl-1 and XIAP (42).

Silibinin has been investigated in combination with epigenetically active compounds in colon cancer. SW480 and SW620 cells were treated with vehicle or silibinin (300 μM) for 24, 48 or 72h. Silibinin in combination with HDAC (Histone Deacetylase) inhibitors TSA (Trichostatin A)) and SAHA (Suberoylanilidehydroxamic acid) exerted synergistic effects in colorectal SW480 and metastatic SW620 cells (43).

2.5 Silibinin in Lung cancer Therapy

Silibinin is also effective against lung cancer. Silibinin inhibited the invasion of human highly metastatic A549 lung cancer cells at various concentrations, up to 100 μM, via decreased productions of urokinase-plasminogen activator and matrix metalloproteinase-2 (44).

Silibinin inhibited human non-small-cell lung cancer cells growth namely large cell carcinoma cells (H1299 and H460) and a bronchioalveolar carcinoma cell line (H322). Silibinin treatment (10–75 μM) targeted cell-cycle progressing causing a prominent G(1) arrest and modulated the protein levels of cyclin-dependent kinases (CDKs) (4, 6, and 2), cyclins (D1, D3, and E), CDKIs (p18/INK4C, p21/Cip1, and p27/Kip1) in a differential manner in these three cell lines (Table 1) (45).

Silibinin (60 μM) in combination with doxorubicin (25 nM) also inhibited A549 cell culture *in vitro*. *Silibinin* also inhibited doxorubicin-caused increased translocation of p65 and p50 from cytosol to nucleus and cyclooxygenase-2, an NFκB target, in doxorubicin combination (46).

Silibinin (75 μM) showed inhibitory effects in both the human lung adenocarcinoma A549 cell line and the human large-cell lung cancer cell line. Silibinin in combination with indole-3-carbinol caused stronger antiproliferative effects than the individual agents. Treatment of A549 cells with 100 μM of indole-3-carbinol plus 75 μM of silibinin or 200 μM of indole-3-carbinol plus 75 μM for 24h, reduced the proliferation of the cells by 40 and 62%, respectively. The corresponding effects in H460 cells were 31% and 69%, respectively. At the molecular level, silibinin in combination with indole-3-carbinol inhibited ERK and Akt activation and induced apoptosis (47).

Silibinin (75 μM) in combination with HDAC inhibitors—trichostatin A (TSA) (0.5 μM) and suberoylanilide hydroxamic acid (SAHA)- (5 μM) synergistically inhibited the growth of NSCLC cells (Table 2). At molecular level silibinin inhibited HDAC activity and decreased HDAC1–3 levels in NSCLC cells, leading to an overall increase in p21 (*Cdkn1a*) level and degradation of cyclin B1 and induced apoptosis. Similar results were observed *in vivo* (48).

Silibinin also synergizes with DNMT (DNA Methyltransferase) inhibitors. Silibinin (3.75 μM) combined with HDAC inhibitor TSA (0.5 μM) or DNMT inhibitor Aza (5'-Aza-deoxycytidine) (5 μM) modulated EMT events in NSCLC cells, leading to a significant inhibition in their migratory and invasive potentials (Table 2) (49).

Silibinin has also shown therapeutic potential against cancer stem cells in lung cancer. A pre-clinical model of acquired erlotinib resistance was established by growing NSCLC cells containing a TKI-sensitizing *EGFR* exon 19 deletion in the continuous presence of high doses of Erlotinib knon as Erlotinib-refractory PC-9/Erl-R cells. Silibinin (50-100 μg/mL) treatment decreased the number of lung cancer spheres in Erlotinib-refractory PC-9/Erl-R cells. These results have suggested the benefit of administration of silibinin in combination with EGFR tyrosine kinase inhibitor erlotinib to target CSC in EGFR-mutant NSCLC patients (50).

2.6 Silibinin in Bladder cancer Therapy

Silibinin efficacy against bladder cancer has also been explored. Silibinin in various dosages (50, 100 and 200 μM) induced cell cycle arrest and apoptosis in human bladder transitional cell carcinoma cells by regulating CDKI-CDK-cyclin cascade, and caspase 3 and PARP cleavages (Table 1) (51).

Silibinin in various dosages (50, 100, and 200 μM) induced apoptosis in human bladder carcinoma 5637 cells in caspase-dependent and -independent manner, which was associated with disruption of mitochondrial membrane potential and selective release of

cytochrome c, Omi/HtrA2 and AIF from mitochondria and the downregulation of survivin and nuclear translocation of AIF. (52).

Silibinin (50 or 100 μM) also suppressed the migration and invasion of highly metastatic T24-L cell model *in vitro* (53).

These findings have demonstrated the anti-tumor efficacy of silibinin against bladder cancer suggesting attractive possibilities for the development of newer and more effective chemopreventive therapies.

2.7 Silibinin in Ovarian cancer Therapy

In a specific study, silibinin (50 μM) demonstrably inhibited the growth of human ovarian cancer cells, A2780 and SKOV3 and induced apoptosis via the activation of caspase-3 and inhibition of p-ERK and p-Akt (Table 1) (54).

In another study, A2780/taxol cells were treated with different concentrations of silibinin (25-200 μM), paclitaxel alone (1-10000 nM), or in combination for 72h. Silibinin enhanced the sensitivity of A2780/taxol cells to paclitaxel and in combination with paclitaxel (Table 2), enhanced apoptosis and accumulated cells in the G2/M phase likely via lowering the expression levels of survivin and P-gp. This combination also decreased invasiveness of cells and suppressed MMP-2 and MMP-9 expression (55).

The above studies that silibinin may be employed as a part of combinatorial therapies against ovarian cancer.

2.8 Silibinin in Cervical cancer Therapy

Silibinin (500 μM) inhibited the proliferation of human cervical (HeLa) cells. Silibinin-inhibited HIF-1α accumulation correlated with strong dephosphorylation of mammalian target of rapamycin (mTOR) and its effectors ribosomal protein S6 kinase (p70S6K) and eukaryotic initiation factor 4E-binding protein-1 (4E-BP1) and activated Akt (56).

In another set of studies, silibinin (40-100 μM) inhibited the growth of HeLa cells and induced autophagic and apoptotic cell death with an increase ROS and RNS (Reactive nitrogen species) (57). Further investigations have demonstrated that silibinin downregulated the expression of CDK1 and CDK2 and induced G2/M arrest and activated caspase 3, caspase 8 and caspase 9; silibinin also downregulated the ratio of Bcl-2/Bax (Table 1). Overall, silibinin activated both the mitochondrial-mediated pathway and the death receptor-mediated pathways in HeLa cells (58). Furthermore, silibinin in combination with metformin synergized the inhibition of cervical cancer cells (C-33A) and induced apoptosis (Table 2) via increased the expression of activated caspase-3 or AIF (59).

Thus, silibin has proved to an effective chemopreventive agent against cervical cancer and offers attractive possibilities for the development of better therapeutic strategies against cervical cancer.

3. *In vivo* studies

There are a few studies that have investigated silibinin-induced anti-tumor effects in various cancer models *in vivo*. For instance, the effect of silibinin on Myeloid-derived suppressor cells (MDSCs) in tumor-bearing mice and antitumor activity of silibinin in a mouse model of breast cancer has been studied. In this study, mouse xenograft models were prepared by injecting 4T1 luciferase-transfected mammary carcinoma cells into the mammary fat pad female BALB/c mice, and female CB17-Prkdc Scid/J mice. Treatment groups received silibinin at 150 mg/kg of body weight by gavage for 4–5 weeks starting 4 or 14 days after tumor inoculation. Treatment with silibinin increased the overall survival in mice harboring tumors derived from the 4T1-luciferase breast cancer cell line, and reduced the tumor volumes and numbers of CD11b+Gr-1+MDSCs in the blood and tumor, and increased the content of T cells in the tumor microenvironment. However, silibinin failed to inhibit tumor growth in immune compromised severe combined immunodeficiency mice, suggesting that the anticancer effects of silibinin are immune-mediated (60).

Anti-tumor effects of silibinin in 2D and 3D models of MDA-MB-468 have been highlighted in recent research. A recent study (61) showed that silibinin-induced inhibition of cell growth and apoptosis is mediated by alteration of the cell cycle, reduction of stemness properties and function, and induction of tumoral differentiation. The mechanisms of silibinin action and the response of tumor cells differed when the cells were cultured in a 3D model compared with a 2D model. Moreover, tumor-initiating cells were more sensitive to silibinin in a 3D culture than in a 2D culture.

Combination of silibinin and epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKI) Erlotinib effectively suppressed tumor growth in erlotinib resistance-bearing PC-9 xenografts. Mice xenograft models were prepared by implanting PC-9 cells into the mouse flanks in female severe combined immunodeficiency (SCID) mice. Both drugs were orally administered for 24 days, erlotinib (100 mg/kg, 5 days a week), silibinin (200 mg/kg, 5 days a week), or erlotinib plus silibinin (62).

A recent study established the xenograft model by injecting the MDA-MB-468 cells into female Balb/c-nude mice (63) and reported that silibinin (200 mg/kg for 45 days), had a pronounced anti-cancer effect on xenograft model of MDA-MB-468 cells by preventing the phosphorylation of EGFR and suppressed COX-2, VEGF and MMP-9 expression. Moreover, the tumor volume of the xenograft models was decreased after administration of silibinin.

Silibinin was also effective in *in vivo* studies against prostate cancer. Athymic male mice were orthotopically implanted with PC-3 cells in prostate and 1 week later after surgical recovery were gavaged daily with silibinin (100 mg/kg body weight) for 7 weeks. Silibinin inhibited *in vivo*, the growth of human prostate carcinoma PC-3 tumor xenografts by 40% via increased IGFBP-3, Cip1/p21, Kip1/p27 levels and ERK1/2 activation and decreased Bcl-2, survivin and VEGF levels in tumors (64).

Silibinin has also been tested against skin cancer in *in vivo* models. Silibinin (9 mg silibinin in 200 μL acetone/mouse) topically prevented ultraviolet B radiation-induced epidermal damages in mouse SKH1 hairless mouse skin, in a p53-GADD45-dependent manner (65).

Silibinin (9 mg silibinin in 200 μL acetone/mouse) was applied topically, showed a protective effect against photocarcinogenesis via down-regulation of inflammatory and angiogenic responses, involving HIF-1α, STAT3, and NF-κB transcription factors, as well as COX2 and iNOS in SKH-1 Hairless Mice (66).

Silibinin inhibited human colorectal carcinoma (CRC) HT29 xenograft growth by upto 48% in xenografted mice dosed with 200 mg/kg/d dose of silibinin or 100 and 200 mg/kg/d doses of silybin-phytosome (5 days per week) for 32 days. The antitumor effects of silibinin were correlated with down-regulated ERK1/2 expression, Akt phosphorylation and decreased cyclin D1 levels (41).

In animal studies, oral administration of silibinin for 6 weeks (at 100 and 200 mg/kg/day, 5 days per week) significantly inhibited the growth of LoVo xenograft (40).

Silibinin has been also tested against colon cancer stem cells (colon CSCs). Silibinin treatment reduced number of spheres in the colorectal cancer spheroid culture system (PP2Ac). Furthermore in a xenograft tumor model, silibinin inhibited tumor formation rate and tumor growth by suppressing the PP2Ac/AKT Ser473/mTOR pathway (67). Effect of silibinin on colon CSCs has been reported recently. Silibinin strongly inhibited the growth kinetics of colon CSC-enriched spheroids by modulating interleukin 4/6-mediated survival signals (67), thereby suggesting a promising application of silibinin in colon CSC therapy.

Oral silibinin (742 mg/kg body weight, 5 days/wk for 10 wks) had a pronounced negative effect on the growth and progression of established lung adenocarcinomas in A/J mice. Silibinin also inhibited lung adenocarcinomas in A/J mice by decreasing tumor-associated macrophages and cytokines, inhibition of HIF-1α, NF-κB, and STAT-3 activation, and up-regulation of the angiogenic inhibitors, Ang-2 (Angiopoietin-2) and Tie-2 (Ang-receptor tyrosine kinase) (68).

In a specific study, oral silibinin (200 mg/kg body weight, 5 d/wk) was observed to suppress human non–small-cell lung carcinoma A549 xenograft growth and enhance the therapeutic response of intraperitoneally administered doxorubicin in athymic BALB/c *nu/nu* mice. Silibinin also inhibited tumor angiogenesis and enhanced antiangiogenic effects of doxorubicin (46).

Silibinin enhanced the inhibition of lung adenocarcinoma by combinatorial treatment with indole-3-carbinol in A/J mice. In mice pretreated with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and given indole-3-carbinol (10 μmol/g diet) plus silibinin (7 μmol/g diet), multiplicities of adenoma were reduced by 84% and by 68% and 92% in mice given indole-3-carbinol, silibinin or indole-3-carbinol plus silibinin, respectively. Similarly the multiplicities of adenocarcinoma were reduced by 83%, 50% and 95% in mice given indole-3-carbinol, silibinin or indole-3-carbinol plus silibinin, respectively. The observed effects of silibinin in combination with indole-3-carbinol on lung adenocarcinoma was mediated by the decreased levels of phospho-Akt, phospho-ERK and cyclin D1 and poly (ADP-ribose) polymerase (47).

Silibinin in combination with erlotinib abrogated tumor growth in NSCLC xenografts. Five weeks of treatment with silibinin (5 days/week at 100 mg/kg body weight by oral gavage) led to a substantial time-dependent reduction in tumor growth in animals xenografted with PC-9/Erl-R cells. Daily oral gavage of the mice with erlotinib-treated xenografts with silibinin resulted in a drastic decrease in the mean tumor volume by 85%. Silibinin fully reversed the EMT-related high *miR-21*/low *miR-200c* microRNA signature and repressed the mesenchymal markers *SNAIL*, *ZEB*, and *N-cadherin* observed in erlotinib-refractory tumors. Silibinin fully activated a reciprocal mesenchymal-to-epithelial transition (MET) in

Table 3: List of clinical trials involving silibinin-based anti-cancer therapies.

Condition	Intervention	Phase	Reference
Prostate cancer	Silibinin-Phytosome	II	ClinicalTrials.gov Identifier: NCT00487721 (72)
Radiodermatitis	Difinsa53; Aquaphor	II	ClinicalTrials.gov Identifier: NCT02534129
Advanced Hepatocellular Carcinoma	Silibinin-Phytosome	I	ClinicalTrials.gov Identifier: NCT01129570 (75)
EGFR Mutant Lung Adenocarcinoma	Silibinin-Phytosome With Erlotinib (Tarceva)	II	ClinicalTrials.gov Identifier: NCT02146118

erlotinib-refractory cells and prevented the highly migratogenic phenotype of erlotinib-resistant NSCLC cells and suggested silibinin as a suitable candidate for upcoming clinical trials following erlotinib treatment (69).

Silibinin inhibited human bladder transitional cell papilloma RT4 tumor xenograft growth. RT4 tumor xenograft was implanted s.c. in athymic nude mice, and then animals were given 100 and 200 mg/kg/day doses of silibinin 5 days/week by oral gavage for 12 weeks. Furthermore, silibinin decreased survivin protein expression, but increased p53 and cleaved caspase-3 levels in tumors. However, the silibinin mediated decrease in survivin was independent of p53 (70).

The cultured human bladder carcinoma 5637 cells were injected subcutaneously into the right flank of athymic BALB/c nu/nu mice to initiate tumor growth and gavaged with 100, 200 and 300 mg/kg/day of silibinin for 32 days. Oral silibinin suppressed the growth of 5637 xenografts with the activation of caspase-3, downregulation of survivin, and increased translocation of AIF. Silibinin also inhibited the carcinogenesis and progression of bladder cancer in rats initiated by N-methyl-N-nitrosourea by reducing the incidence of superficial and invasive bladder lesions (52).

Silibinin decreased bladder cancer lung metastasis and prolonged animal survival in vivo. To establish xenograft animal models T24-L cells were instilled in to the bladder of female athymic BALB/c/ nu/mu mice. Mice were gavaged daily with silibinin (200 mg/kg body weight) for 6 weeks. Silibinin inhibited glycogen synthase kinase-3β (GSK3β) phosphorylation, β-catenin nuclear translocation and transactivation, ZEB1 gene transcription and the expression of cytokeratins, vimentin and MMP2 and reversed EMT. Silibinin also inhibited ZEB1 expression and suppressed the properties of CSCs (53).

Oral administration of silibinin (50 and 100 mg/kg) in female Balb/c nude mice injected with subcutaneous human ovarian cancer cells A2780 cells reduced tumor volume. Silibinin reduced tumor growth through inhibition of ERK and Akt in human ovarian cancer cells (54).

Silibinin in combination therapy showed promising results in ovarian cancer. IdB 1016 (Silipide, a complex of silybin/phosphatidylcholine). 450 mg/kg/day IdB 1016 daily was given by oral gavage inhibited tumor growth in female nude mice bearing human ovarian cancer xenografts (A2780). dB 1016 was significantly active in inhibiting ovarian tumour growth. VEGR receptor 3 was downregulated and angiopoietin-2 was upregulated as potential mechanisms for the antiangiogenic activity (71).

4. Clinical trials

Silibinin has been taken up for clinical trials involving patients with prostate cancer, breast cancer, hepatocellular carcinoma and lymphoblastic leukemia (Table 3). A clinical trial was performed in patients

with localized prostate cancer using high dose oral silibinin-phytosome followed by prostatectomy (72). This study revealed high silibinin peak blood levels but low levels of silibinin in prostate tissues after 2 weeks of therapy. Oral silibinin-phosphatidylcholine (2g per day) was administered for 12 weeks to patients with advanced hepatocellular carcinoma (HCC), who were not eligible for other therapies. In the three patients tested, only one patient showed some improvement in liver function abnormalities and inflammatory biomarkers; however, none of the patients survived (73).

Silymarin whole extract has been also used in clinical trials for the treatment of hepatotoxicity in childhood acute lymphoblastic leukemia (ALL). Silymarin (The target dose of silibinin was 5.1 mg/kg/day) was administered orally for 28 days and it significantally reduced liver toxicity in children with ALL (74). Recently a new silibinin drug formulation Legasil® administration improved hepatic failure due to extensive liver infiltration in a breast cancer patient (75).

A Phase II Study to Assess Efficacy of Combined Treatment with Erlotinib (Tarceva) and Silybin-phytosome (Siliphos) in Patients with EGFR mutant lung adenocarcinoma is going on (ClinicalTrials.gov Identifier: NCT02146118). A Phase II trial of the silibin containing cream, Difinsa53 to determine efficacy in delaying, ameliorating, or preventing radiation dermatitis in patients with breast cancer undergoing whole breast radiation has also been proposed (ClinicalTrials.gov Identifier: NCT02534129).

5. Enhancement of bioavailability of Silibinin

Silibinin is poorly water-soluble (solubility<50 µg/mL), because of its highly hydrophobic and nonionizable structure) (76). Thus, there have been extensive attempts to enhance the bioavailability of silibinin such as synthesis of the silibinin-phospholipid complex. Nanomedicine is an innovative field with immense potential and promises more effective cancer treatment outcomes and it has ushered in several established drug delivery platforms (77, 78). Controlled drug delivery improves bioavailability by preventing premature degradation and enhancing drug uptake, maintining drug concentration within the therapeutic threshold by controlling the drug release rate, and reduces the side effects by effective and more precise targeting to the disease site and target cells (79). Nanostructured lipid carriers are delivery systems in which partial-crystallized lipid particles with a mean radii ≤ 100 nm are dispersed in an aqueous phase containing emulsifier(s); they are useful nutraceutical delivery systems since they enable high drug loading, encapsulation efficiency and stability (80).

In a specific study aimed at enhancing silibinin bioavailability, the researchers described a new protocol to prepare a silibinin-phospholipid complex. Solubility studies confirmed the higher solubility of silibinin-phospholipid complex in water (78.25±2.68 µg/mL) and n-octanol (62.67±1.86 mg/mL). Following an oral administratin of 9.1 mg/kg of of silibinin-phospholipid complex and silibinin-N-methylglucamine to rats, there was a significant increase in the bioavailability of silibinin after the oral administration of silibinin-phospholipid complex compared to silibinin-N-methylglucamine (81).

In another series of approaches, stealth SLNs as colloidal carriers for silibinin have been developed; these stealth SLNs were composed of stearic acid and surfactant Brij 78 (polyoxyethylene 20 stearyl ether) and incorporated high amounts of silibinin up to 7.55%. (82). Recently, sodium cholate/phospholipid-mixed micelles containing silibinin were obtained and were found to be readily soluble; the maximum solubility being 10.0±1.1mg/mL. When silibinin–mixed micelles and silibinin-N-methylglucamine were orally administered to dogs (90 mg, expressed as silibinin equivalents), it increased the bioavailability of silibinin-mixed micelles *versus* silibinin-*N*-methylglucamine by up to 252.0% and led to a marked reduction in effective silibinin doses (76). *Silibin loaded nanoparticles have displayed promising results against various cancer types. For instance, Silibinin*-loaded lipid nanoparticles effectively inhibited growth of MDA-MB-231 cells. To increase the effect of silibinin, silibinin-loaded PLGA-PEG-Fe_3O_4 was prepared to determine the inhibitory effect of this nanodrug on Telomerase gene expression. 1 mg of silibinin-loaded PLGA-PEG-Fe_3O_4 included 760 µg silibinin. Silibinin-loaded PLGA-PEG-Fe_3O_4 had upto 98% greater cytotoxic effect on T47D cell line than free silibinin. The level of telomerase gene expression (70%) was markedly more decreased with silibinin-loaded PLGA-PEG-Fe_3O_4 than with free silibinin alone (83). A specific study prepared chitosan-tripolyphosphate based silibinin nanoparticles which showed high encapsulation efficiencies (82.94±1.82%). The nanoparticles-based delivery of silibinin induced a much higher dissolution and improved cytotoxicity against human prostate cancer cells (DU145) than

silibinin alone (84). The non-biodegradable polymer Eudragit RL 100 nanoparticles loaded with silibinin and coated by Eudragit FS30D (75/mg/kg/day) were administered orally to rats for 5 days and demonstrated significantly improved results in treatment of ulcerative colitis (85).

Taken together, these studies have demonstrated that addressing the bioavailability issues plaguing silibin-based therapes can be an extremely effective and potent approach to ehance the anti-cancer potential of silibinin.

6. Conclusion

Silibinin is an effective anticancer plant derived natural compound. Cellular signaling pathways such as those that control cell division and differentiation are altered significantly in cancer cells. A deeper understanding of how various drugs and plant compounds regulate these pathways is key for effective cancer therapy. In this review, we have aimed to summarise the key signaling pathways modulated by silibinin in various cancer types, a better understanding of which is very important to develop silibinin as effective anticancer drug. Silibinin is water insoluble and demonstrates low bioavailability. Thus, recent studies have been directed to increase the bioavailability of silibinin. Consequently, silibinin is now being pursued as a potential anti-cancer therapeutic agent in clinical studies involving different cancer types; for instance silibinin is currently being evaluated in clinical trials against prostate cancer.

Silibinin has been demonstrated to induce apoptosis in various cancer types *in vitro* and *in vivo* by targeting multiple factors and influencing multiple signaling pathways such as β-Catenin, Bcl2, Cip1/p21, COX-2/PGE2, Kip1/p27, MAPK/ERK, STAT3, PI3K/AKT and VEGF, which are frequently deregulated in cancers. This broad spectrum of action is especially useful since targeting multiple signaling pathways in cancer may sidestep the problem of drug resistance which has become a major stumbling block in several anti-cancer therapies that involve anticancer drugs that are designed to specifically block a particular signaling pathway. Thus, silibinin offers an attractive possibility to be developed into a potential lead compound for anticancer therapy.

Recent studies have suggested that silibinin is highly effective in combination with other drugs or compounds and epigenomic modifiers and can be used in future treatment of cancer. Silibinin has also shown promising results against cancer stem cells, supporting further development of anti-cancer therapeutics that target tumor stem cells.

Taken together, the wealth of accumulated research that has been surveyed here, suggests an immense potential of silibin as anti-cancer therapeutic compound. These are likely to contribute to improved clinical outcomes of anti-cancer therapies involving silibinin.

Acknowledgements

PT was supported by the senior research fellowship from the Council of Scientific and Industrial Research (CSIR), Govt. of India for her research cited in this article. No explicit funding was required for preparation of this manuscript.

Abbreviations:

ALL, Acute lymphoblastic leukemia;
CDK, Cyclin-dependent kinase;
CSCs, Cancer stem cells;
EMT, Epithelial-mesenchymal transition;
MET, Mesenchymal-to-epithelial transition;
EGFR, Epidermal growth factor receptor;
HCC, Hepatocellular carcinoma;
HDAC, Histone deacetylase;
ROS, Reactive oxygen species;
RNS, Reactive nitrogen species;
SLNs; Silibinin-loaded lipid nanoparticles;
TRAIL, TNF-related apoptosis-inducing ligand.

References

1. Zhao J, Agarwal R. Tissue distribution of silibinin, the major active constituent of silymarin, in mice and its association with enhancement of phase II enzymes: implications in cancer chemoprevention. Carcinogenesis. 1999 Nov;20(11):2101-8.
2. Hackett ES, Twedt DC, Gustafson DL. Milk thistle and its derivative compounds: a review of opportunities for treatment of liver disease. J Vet Intern Med. 2013 Jan-Feb;27(1):10-6. DOI: 10.1111/jvim.12002.
3. Carducci R, Armellino MF, Volpe C, Basile G, Caso N, Apicella A, et al. [Silibinin and acute poisoning with Amanita phalloides]. Minerva Anestesiol. 1996 May;62(5):187-93.
4. Cheung CW, Gibbons N, Johnson DW, Nicol DL. Silibinin--a promising new treatment for cancer. Anticancer Agents Med Chem. 2010 Mar;10(3):186-95.

5. Chhabra N, Buzarbaruah S, Singh R, Kaur J. Silibinin: A promising anti-neoplastic agent for the future? A critical reappraisal2013 July 1, 2013. 206-18 p.
6. Millimouno FM, Dong J, Yang L, Li J, Li X. Targeting apoptosis pathways in cancer and perspectives with natural compounds from mother nature. Cancer Prev Res (Phila). 2014 Nov;7(11):1081-107. DOI: 10.1158/1940-6207.CAPR-14-0136.
7. Li L, Zeng J, Gao Y, He D. Targeting silibinin in the antiproliferative pathway. Expert Opin Investig Drugs. 2010 Feb;19(2):243-55. DOI: 10.1517/13543780903533631.
8. Singh RP, Dhanalakshmi S, Agarwal C, Agarwal R. Silibinin strongly inhibits growth and survival of human endothelial cells via cell cycle arrest and downregulation of survivin, Akt and NF-kappaB: implications for angioprevention and antiangiogenic therapy. Oncogene. 2005 Feb 10;24(7):1188-202. DOI: 10.1038/sj.onc.1208276.
9. Raina K, Agarwal R. Combinatorial strategies for cancer eradication by silibinin and cytotoxic agents: efficacy and mechanisms. Acta Pharmacol Sin. 2007 Sep;28(9):1466-75. DOI: 10.1111/j.1745-7254.2007.00691.x.
10. Tiwari P, Kumar A, Balakrishnan S, Kushwaha HS, Mishra KP. Silibinin-induced apoptosis in MCF7 and T47D human breast carcinoma cells involves caspase-8 activation and mitochondrial pathway. Cancer Invest. 2011 Jan;29(1):12-20. DOI: 10.3109/07357907.2010.535053.
11. Nejati-Koshki K, Zarghami N, Pourhassan-Moghaddam M, Rahmati-Yamchi M, Mollazade M, Nasiri M, et al. Inhibition of leptin gene expression and secretion by silibinin: possible role of estrogen receptors. Cytotechnology. 2012 Dec;64(6):719-26. DOI: 10.1007/s10616-012-9452-3.
12. Kim TH, Woo JS, Kim YK, Kim KH. Silibinin induces cell death through reactive oxygen species-dependent downregulation of notch-1/ERK/Akt signaling in human breast cancer cells. J Pharmacol Exp Ther. 2014 May;349(2):268-78. DOI: 10.1124/jpet.113.207563.
13. Yousefi M, Ghaffari SH, Zekri A, Hassani S, Alimoghaddam K, Ghavamzadeh A. Silibinin induces apoptosis and inhibits proliferation of estrogen receptor (ER)-negative breast carcinoma cells through suppression of nuclear factor kappa B activation. Arch Iran Med. 2014 May;17(5):366-71. DOI: 0141705/AIM.0011.
14. Khannazer N, Paylakhi SH, Mirshafiey A, Azizi G, Motamed N. Silibinin, up-regulates chemokine receptor expression in MDA-MB-231 breast cancer cell line. Bangladesh Journal of Medical Science. 2015 Apr;14(2):190. DOI: 10.3329/bjms.v14i2.16740.
15. Xu P, Yin Q, Shen J, Chen L, Yu H, Zhang Z, et al. Synergistic inhibition of breast cancer metastasis by silibinin-loaded lipid nanoparticles containing TPGS. Int J Pharm. 2013 Sep 15;454(1):21-30. DOI: 10.1016/j.ijpharm.2013.06.053.
16. Lu W, Lin C, King TD, Chen H, Reynolds RC, Li Y. Silibinin inhibits Wnt/beta-catenin signaling by suppressing Wnt co-receptor LRP6 expression in human prostate and breast cancer cells. Cell Signal. 2012 Dec;24(12):2291-6. DOI: 10.1016/j.cellsig.2012.07.009.
17. Tiwari P, Kumar A, Ali M, Mishra KP. Radioprotection of plasmid and cellular DNA and Swiss mice by silibinin. Mutat Res. 2010 Jan;695(1-2):55-60. DOI: 10.1016/j.mrgentox.2009.11.007.
18. Wang HJ, Jiang YY, Wei XF, Huang H, Tashiro S, Onodera S, et al. Silibinin induces protective superoxide generation in human breast cancer MCF-7 cells. Free Radic Res. 2010 Jan;44(1):90-100. DOI: 10.3109/10715760903300717.
19. Wang HJ, Wei XF, Jiang YY, Huang H, Yang Y, Fan SM, et al. Silibinin induces the generation of nitric oxide in human breast cancer MCF-7 cells. Free Radic Res. 2010 May;44(5):577-84. DOI: 10.3109/10715761003692495.
20. Jiang K, Wang W, Jin X, Wang Z, Ji Z, Meng G. Silibinin, a natural flavonoid, induces autophagy via ROS-dependent mitochondrial dysfunction and loss of ATP involving BNIP3 in human MCF7 breast cancer cells. Oncol Rep. 2015 Jun;33(6):2711-8. DOI: 10.3892/or.2015.3915.
21. Kim S, Han J, Kim JS, Kim JH, Choe JH, Yang JH, et al. Silibinin suppresses EGFR ligand-induced CD44 expression through inhibition of EGFR activity in breast cancer cells. Anticancer Res. 2011 Nov;31(11):3767-73.
22. Dastpeyman M, Motamed N, Azadmanesh K, Mostafavi E, Kia V, Jahanian-Najafabadi A, et al. Inhibition of silibinin on migration and adhesion capacity of human highly metastatic breast cancer cell line, MDA-MB-231, by evaluation of beta1-integrin and downstream molecules, Cdc42, Raf-1 and D4GDI. Med Oncol. 2012 Dec;29(4):2512-8. DOI: 10.1007/s12032-011-0113-8.
23. Gao Y, Theng SS, Mah WC, Lee CG. Silibinin down-regulates FAT10 and modulate TNF-alpha/IFN-gamma-induced chromosomal instability and apoptosis sensitivity. Biol Open. 2015;4(8):961-9. DOI: 10.1242/bio.011189.
24. Tyagi AK, Agarwal C, Chan DC, Agarwal R. Synergistic anti-cancer effects of silibinin with conventional cytotoxic agents doxorubicin, cisplatin and carboplatin against human breast carcinoma MCF-7 and MDA-MB468 cells. Oncol Rep. 2004 Feb;11(2):493-9.
25. Nejati-Koshki K, Akbarzadeh A, Pourhasan-Moghaddam M, Abhari A, Dariushnejad H. Inhibition of leptin and leptin receptor gene expression by silibinin-curcumin combination. Asian Pac J Cancer Prev. 2014 Jan;14(11):6595-9.

26. Nasiri M, Zarghami N, Koshki KN, Mollazadeh M, Moghaddam MP, Yamchi MR, et al. Curcumin and silibinin inhibit telomerase expression in T47D human breast cancer cells. Asian Pac J Cancer Prev. 2013;14(6):3449-53.
27. Noh EM, Yi MS, Youn HJ, Lee BK, Lee YR, Han JH, et al. Silibinin enhances ultraviolet B-induced apoptosis in mcf-7 human breast cancer cells. J Breast Cancer. 2011 Mar;14(1):8-13. DOI: 10.4048/jbc.2011.14.1.8.
28. Zi X, Agarwal R. Silibinin decreases prostate-specific antigen with cell growth inhibition via G1 arrest, leading to differentiation of prostate carcinoma cells: implications for prostate cancer intervention. Proc Natl Acad Sci U S A. 1999 Jun 22;96(13):7490-5.
29. Zi X, Zhang J, Agarwal R, Pollak M. Silibinin up-regulates insulin-like growth factor-binding protein 3 expression and inhibits proliferation of androgen-independent prostate cancer cells. Cancer Res. 2000 Oct 15;60(20):5617-20.
30. Mokhtari MJ, Motamed N, Shokrgozar MA. Evaluation of silibinin on the viability, migration and adhesion of the human prostate adenocarcinoma (PC-3) cell line. Cell Biol Int. 2008 Aug;32(8):888-92. DOI: 10.1016/j.cellbi.2008.03.019.
31. Wu KJ, Zeng J, Zhu GD, Zhang LL, Zhang D, Li L, et al. Silibinin inhibits prostate cancer invasion, motility and migration by suppressing vimentin and MMP-2 expression. Acta Pharmacol Sin. 2009 Aug;30(8):1162-8. DOI: 10.1038/aps.2009.94.
32. Wu K, Zeng J, Li L, Fan J, Zhang D, Xue Y, et al. Silibinin reverses epithelial-to-mesenchymal transition in metastatic prostate cancer cells by targeting transcription factors. Oncol Rep. 2010 Jun;23(6):1545-52.
33. Tyagi AK, Singh RP, Agarwal C, Chan DC, Agarwal R. Silibinin strongly synergizes human prostate carcinoma DU145 cells to doxorubicin-induced growth Inhibition, G2-M arrest, and apoptosis. Clin Cancer Res. 2002 Nov;8(11):3512-9.
34. Dhanalakshmi S, Agarwal P, Glode LM, Agarwal R. Silibinin sensitizes human prostate carcinoma DU145 cells to cisplatin- and carboplatin-induced growth inhibition and apoptotic death. Int J Cancer. 2003 Sep 20;106(5):699-705. DOI: 10.1002/ijc.11299.
35. Flaig TW, Su LJ, Harrison G, Agarwal R, Glode LM. Silibinin synergizes with mitoxantrone to inhibit cell growth and induce apoptosis in human prostate cancer cells. Int J Cancer. 2007 May 1;120(9):2028-33. DOI: 10.1002/ijc.22465.
36. Nambiar DK, Rajamani P, Singh RP. Silibinin attenuates ionizing radiation-induced pro-angiogenic response and EMT in prostate cancer cells. Biochem Biophys Res Commun. 2015 Jan 2;456(1):262-8. DOI: 10.1016/j.bbrc.2014.11.069.
37. Bhatia N, Agarwal C, Agarwal R. Differential responses of skin cancer-chemopreventive agents silibinin, quercetin, and epigallocatechin 3-gallate on mitogenic signaling and cell cycle regulators in human epidermoid carcinoma A431 cells. Nutr Cancer. 2001;39(2):292-9. DOI: 10.1207/S15327914nc392_20.
38. Singh RP, Agarwal R. Mechanisms and preclinical efficacy of silibinin in preventing skin cancer. Eur J Cancer. 2005 Sep;41(13):1969-79. DOI: 10.1016/j.ejca.2005.03.033.
39. Dhanalakshmi S, Agarwal C, Singh RP, Agarwal R. Silibinin up-regulates DNA-protein kinase-dependent p53 activation to enhance UVB-induced apoptosis in mouse epithelial JB6 cells. J Biol Chem. 2005 May 27;280(21):20375-83. DOI: 10.1074/jbc.M414640200.
40. Kaur M, Velmurugan B, Tyagi A, Deep G, Katiyar S, Agarwal C, et al. Silibinin suppresses growth and induces apoptotic death of human colorectal carcinoma LoVo cells in culture and tumor xenograft. Mol Cancer Ther. 2009 Aug;8(8):2366-74. DOI: 10.1158/1535-7163.MCT-09-0304.
41. Singh RP, Gu M, Agarwal R. Silibinin inhibits colorectal cancer growth by inhibiting tumor cell proliferation and angiogenesis. Cancer Res. 2008 Mar 15;68(6):2043-50. DOI: 10.1158/0008-5472.CAN-07-6247.
42. Kauntz H, Bousserouel S, Gosse F, Raul F. The flavonolignan silibinin potentiates TRAIL-induced apoptosis in human colon adenocarcinoma and in derived TRAIL-resistant metastatic cells. Apoptosis. 2012 Aug;17(8):797-809. DOI: 10.1007/s10495-012-0731-4.
43. Kauntz H, Bousserouel S, Gosse F, Raul F. Epigenetic effects of the natural flavonolignan silibinin on colon adenocarcinoma cells and their derived metastatic cells. Oncol Lett. 2013 Apr;5(4):1273-7. DOI: 10.3892/ol.2013.1190.
44. Chu SC, Chiou HL, Chen PN, Yang SF, Hsieh YS. Silibinin inhibits the invasion of human lung cancer cells via decreased productions of urokinase-plasminogen activator and matrix metalloproteinase-2. Mol Carcinog. 2004 Jul;40(3):143-9. DOI: 10.1002/mc.20018.
45. Mateen S, Tyagi A, Agarwal C, Singh RP, Agarwal R. Silibinin inhibits human nonsmall cell lung cancer cell growth through cell-cycle arrest by modulating expression and function of key cell-cycle regulators. Mol Carcinog. 2010 Mar;49(3):247-58. DOI: 10.1002/mc.20595.
46. Singh RP, Mallikarjuna GU, Sharma G, Dhanalakshmi S, Tyagi AK, Chan DC, et al. Oral silibinin inhibits lung tumor growth in athymic nude mice and forms a novel chemocombination with doxorubicin targeting nuclear factor kappaB-mediated inducible chemoresistance. Clin Cancer Res. 2004 Dec 15;10(24):8641-7. DOI: 10.1158/1078-0432.CCR-04-1435.

47. Dagne A, Melkamu T, Schutten MM, Qian X, Upadhyaya P, Luo X, et al. Enhanced inhibition of lung adenocarcinoma by combinatorial treatment with indole-3-carbinol and silibinin in A/J mice. Carcinogenesis. 2011 Apr;32(4):561-7. DOI: 10.1093/carcin/bgr010.
48. Mateen S, Raina K, Jain AK, Agarwal C, Chan D, Agarwal R. Epigenetic modifications and p21-cyclin B1 nexus in anticancer effect of histone deacetylase inhibitors in combination with silibinin on non-small cell lung cancer cells. Epigenetics. 2012 Oct;7(10):1161-72. DOI: 10.4161/epi.22070.
49. Mateen S, Raina K, Agarwal C, Chan D, Agarwal R. Silibinin synergizes with histone deacetylase and DNA methyltransferase inhibitors in upregulating E-cadherin expression together with inhibition of migration and invasion of human non-small cell lung cancer cells. J Pharmacol Exp Ther. 2013 May;345(2):206-14. DOI: 10.1124/jpet.113.203471.
50. Corominas-Faja B, Oliveras-Ferraros C, Cuyas E, Segura-Carretero A, Joven J, Martin-Castillo B, et al. Stem cell-like ALDH(bright) cellular states in EGFR-mutant non-small cell lung cancer: a novel mechanism of acquired resistance to erlotinib targetable with the natural polyphenol silibinin. Cell Cycle. 2013 Nov 1;12(21):3390-404. DOI: 10.4161/cc.26417.
51. Tyagi A, Agarwal C, Harrison G, Glode LM, Agarwal R. Silibinin causes cell cycle arrest and apoptosis in human bladder transitional cell carcinoma cells by regulating CDKI-CDK-cyclin cascade, and caspase 3 and PARP cleavages. Carcinogenesis. 2004 Sep;25(9):1711-20. DOI: 10.1093/carcin/bgh180.
52. Zeng J, Sun Y, Wu K, Li L, Zhang G, Yang Z, et al. Chemopreventive and chemotherapeutic effects of intravesical silibinin against bladder cancer by acting on mitochondria. Mol Cancer Ther. 2011 Jan;10(1):104-16. DOI: 10.1158/1535-7163.MCT-10-0577.
53. Wu K, Ning Z, Zeng J, Fan J, Zhou J, Zhang T, et al. Silibinin inhibits beta-catenin/ZEB1 signaling and suppresses bladder cancer metastasis via dual-blocking epithelial-mesenchymal transition and stemness. Cell Signal. 2013 Dec;25(12):2625-33. DOI: 10.1016/j.cellsig.2013.08.028.
54. Cho HJ, Suh DS, Moon SH, Song YJ, Yoon MS, Park do Y, et al. Silibinin inhibits tumor growth through downregulation of extracellular signal-regulated kinase and Akt in vitro and in vivo in human ovarian cancer cells. J Agric Food Chem. 2013 May 1;61(17):4089-96. DOI: 10.1021/jf400192v.
55. Zhou L, Liu P, Chen B, Wang Y, Wang X, Chiriva Internati M, et al. Silibinin restores paclitaxel sensitivity to paclitaxel-resistant human ovarian carcinoma cells. Anticancer Res. 2008 Mar-Apr;28(2A):1119-27.
56. Garcia-Maceira P, Mateo J. Silibinin inhibits hypoxia-inducible factor-1alpha and mTOR/p70S6K/4E-BP1 signalling pathway in human cervical and hepatoma cancer cells: implications for anticancer therapy. Oncogene. 2009 Jan 22;28(3):313-24. DOI: 10.1038/onc.2008.398.
57. Fan S, Li L, Chen S, Yu Y, Qi M, Tashiro S, et al. Silibinin induced-autophagic and apoptotic death is associated with an increase in reactive oxygen and nitrogen species in HeLa cells. Free Radic Res. 2011 Nov;45(11-12):1307-24. DOI: 10.3109/10715762.2011.618186.
58. Zhang Y, Ge Y, Chen Y, Li Q, Chen J, Dong Y, et al. Cellular and molecular mechanisms of silibinin induces cell-cycle arrest and apoptosis on HeLa cells. Cell Biochem Funct. 2012 Apr;30(3):243-8. DOI: 10.1002/cbf.1842.
59. Liou W-S, Chen L-J, Niu H-S, Yang T-T, Cheng J-T, Lin K-C. Herbal product silibinin-induced programmed cell death is enhanced by metformin in cervical cancer cells at the dose without influence on nonmalignant cells. Journal of Applied Biomedicine. 2015 5/1/;13(2):113-21. DOI: 10.1016/j.jab.2014.11.001.
60. Forghani P, Khorramizadeh MR, Waller EK. Silibinin inhibits accumulation of myeloid-derived suppressor cells and tumor growth of murine breast cancer. Cancer Med. 2014 Apr;3(2):215-24. DOI: 10.1002/cam4.186.
61. Abdollahi P, Ebrahimi M, Motamed N, Samani FS. Silibinin affects tumor cell growth because of reduction of stemness properties and induction of apoptosis in 2D and 3D models of MDA-MB-468. Anticancer Drugs. 2015 Jun;26(5):487-97. DOI: 10.1097/CAD.0000000000000205.
62. Rho JK, Choi YJ, Jeon BS, Choi SJ, Cheon GJ, Woo SK, et al. Combined treatment with silibinin and epidermal growth factor receptor tyrosine kinase inhibitors overcomes drug resistance caused by T790M mutation. Mol Cancer Ther. 2010 Dec;9(12):3233-43. DOI: 10.1158/1535-7163.MCT-10-0625.
63. Kil WH, Kim SM, Lee JE, Park KS, Nam SJ. Anticancer effect of silibinin on the xenograft model using MDA-MB-468 breast cancer cells. Ann Surg Treat Res. 2014 Oct;87(4):167-73. DOI: 10.4174/astr.2014.87.4.167.
64. Singh RP, Raina K, Deep G, Chan D, Agarwal R. Silibinin suppresses growth of human prostate carcinoma PC-3 orthotopic xenograft via activation of extracellular signal-regulated kinase 1/2 and inhibition of signal transducers and activators of transcription signaling. Clin Cancer Res. 2009 Jan 15;15(2):613-21. DOI: 10.1158/1078-0432.CCR-08-1846.
65. Roy S, Deep G, Agarwal C, Agarwal R. Silibinin prevents ultraviolet B radiation-induced epidermal damages in JB6 cells and mouse skin in a p53-GADD45alpha-dependent manner. Carcinogenesis. 2012 Mar;33(3):629-36. DOI: 10.1093/carcin/bgr299.

66. Gu M, Singh RP, Dhanalakshmi S, Agarwal C, Agarwal R. Silibinin inhibits inflammatory and angiogenic attributes in photocarcinogenesis in SKH-1 hairless mice. Cancer Res. 2007 Apr 1;67(7):3483-91. DOI: 10.1158/0008-5472.CAN-06-3955.
67. Wang JY, Chang CC, Chiang CC, Chen WM, Hung SC. Silibinin suppresses the maintenance of colorectal cancer stem-like cells by inhibiting PP2A/AKT/mTOR pathways. J Cell Biochem. 2012 May;113(5):1733-43. DOI: 10.1002/jcb.24043.
68. Tyagi A, Singh RP, Ramasamy K, Raina K, Redente EF, Dwyer-Nield LD, et al. Growth inhibition and regression of lung tumors by silibinin: modulation of angiogenesis by macrophage-associated cytokines and nuclear factor-kappaB and signal transducers and activators of transcription 3. Cancer Prev Res (Phila). 2009 Jan;2(1):74-83. DOI: 10.1158/1940-6207.CAPR-08-0095.
69. Cufi S, Bonavia R, Vazquez-Martin A, Oliveras-Ferraros C, Corominas-Faja B, Cuyas E, et al. Silibinin suppresses EMT-driven erlotinib resistance by reversing the high miR-21/low miR-200c signature in vivo. Sci Rep. 2013;3:2459. DOI: 10.1038/srep02459.
70. Singh RP, Tyagi A, Sharma G, Mohan S, Agarwal R. Oral silibinin inhibits in vivo human bladder tumor xenograft growth involving down-regulation of survivin. Clin Cancer Res. 2008 Jan 1;14(1):300-8. DOI: 10.1158/1078-0432.CCR-07-1565.
71. Gallo D, Giacomelli S, Ferlini C, Raspaglio G, Apollonio P, Prislei S, et al. Antitumour activity of the silybin-phosphatidylcholine complex, IdB 1016, against human ovarian cancer. Eur J Cancer. 2003 Nov;39(16):2403-10.
72. Flaig TW, Glode M, Gustafson D, van Bokhoven A, Tao Y, Wilson S, et al. A study of high-dose oral silybin-phytosome followed by prostatectomy in patients with localized prostate cancer. Prostate. 2010 Jun 1;70(8):848-55. DOI: 10.1002/pros.21118.
73. Siegel AB, Narayan R, Rodriguez R, Goyal A, Jacobson JS, Kelly K, et al. A phase I dose-finding study of silybin phosphatidylcholine (milk thistle) in patients with advanced hepatocellular carcinoma. Integr Cancer Ther. 2014 Jan;13(1):46-53. DOI: 10.1177/1534735413490798.
74. Ladas EJ, Kroll DJ, Oberlies NH, Cheng B, Ndao DH, Rheingold SR, et al. A randomized, controlled, double-blind, pilot study of milk thistle for the treatment of hepatotoxicity in childhood acute lymphoblastic leukemia (ALL). Cancer. 2010 Jan 15;116(2):506-13. DOI: 10.1002/cncr.24723.
75. Bosch-Barrera J, Corominas-Faja B, Cuyas E, Martin-Castillo B, Brunet J, Menendez JA. Silibinin administration improves hepatic failure due to extensive liver infiltration in a breast cancer patient. Anticancer Res. 2014 Aug;34(8):4323-7.
76. Yu JN, Zhu Y, Wang L, Peng M, Tong SS, Cao X, et al. Enhancement of oral bioavailability of the poorly water-soluble drug silybin by sodium cholate/phospholipid-mixed micelles. Acta Pharmacol Sin. 2010 Jun;31(6):759-64. DOI: 10.1038/aps.2010.55.
77. Blanco E, Hsiao A, Mann AP, Landry MG, Meric-Bernstam F, Ferrari M. Nanomedicine in cancer therapy: innovative trends and prospects. Cancer Sci. 2011 Jul;102(7):1247-52. DOI: 10.1111/j.1349-7006.2011.01941.x.
78. Wicki A, Witzigmann D, Balasubramanian V, Huwyler J. Nanomedicine in cancer therapy: challenges, opportunities, and clinical applications. J Control Release. 2015 Feb 28;200:138-57. DOI: 10.1016/j.jconrel.2014.12.030.
79. Zhang Y, Chan HF, Leong KW. Advanced materials and processing for drug delivery: the past and the future. Adv Drug Deliv Rev. 2013 Jan;65(1):104-20. DOI: 10.1016/j.addr.2012.10.003.
80. Tamjidi F, Shahedi M, Varshosaz J, Nasirpour A. Nanostructured lipid carriers (NLC): A potential delivery system for bioactive food molecules. Innovative Food Science & Emerging Technologies. 2013 7//;19:29-43. DOI: 10.1016/j.ifset.2013.03.002.
81. Yanyu X, Yunmei S, Zhipeng C, Qineng P. The preparation of silybin-phospholipid complex and the study on its pharmacokinetics in rats. Int J Pharm. 2006 Jan 3;307(1):77-82. DOI: 10.1016/j.ijpharm.2005.10.001.
82. Zhang JQ, Liu J, Li XL, Jasti BR. Preparation and characterization of solid lipid nanoparticles containing silibinin. Drug Deliv. 2007 Aug;14(6):381-7. DOI: 10.1080/10717540701203034.
83. Ebrahimnezhad Z, Zarghami N, Keyhani M, Amirsaadat S, Akbarzadeh A, Rahmati M, et al. Inhibition of hTERT Gene Expression by Silibinin-Loaded PLGA-PEG-Fe3O4 in T47D Breast Cancer Cell Line. Bioimpacts. 2013;3(2):67-74. DOI: 10.5681/bi.2013.005.
84. Pooja D, Babu Bikkina DJ, Kulhari H, Nikhila N, Chinde S, Raghavendra YM, et al. Fabrication, characterization and bioevaluation of silibinin loaded chitosan nanoparticles. Int J Biol Macromol. 2014 Aug;69:267-73. DOI: 10.1016/j.ijbiomac.2014.05.035.
85. Varshosaz J, Minaiyan M, Khaleghi N. Eudragit nanoparticles loaded with silybin: a detailed study of preparation, freeze-drying condition and in vitro/in vivo evaluation. J Microencapsul. 2015;32(3):211-23. DOI: 10.3109/02652048.2014.995728.