

Recent Advances in Herbal Drug Nanocarriers against Cervical Cancer

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ABSTRACT: Cervical cancer is one of the most common and prevalent cancers affecting women worldwide. Primarily women of reproductive age between 35 and 55 years are the affected population and the disease often leads to mortality if not diagnosed at primary stages. Major etiological factor responsible for development of cervical cancer are high-risk human papilloma virus (HPV) infections. Other causative factors include long term use of hormonal contraceptives, multiple sexual activity, and other co-infections and comorbidities. Present diagnostic practices include unaided visual inspection with acetic acid (VIA), cytology test (pap smear test) followed by colposcopy and subsequent biopsy of suspicious lesions. Effective and accurate diagnostics in timely manner can have huge impact on therapeutics of disease. Choice of treatment modalities adopted in clinical practice is driven by stage of cancer. Surgery, radiation therapy and coupled chemotherapy often lack specificity. Many of the chemotherapeutic agents often develop resistance and the drawbacks associated with chemotherapeutic drugs have led to increased research interests in discovering new molecular entities that are target oriented, with low incidence of resistance and side effects. Drugs of herbal origin or phytoconstituents derived from them are being envisaged either as main therapeutic or adjuvant to existing chemotherapeutic with an intention to mitigate some of these shortcomings. Herbal drugs and derived phytoconstituents exhibit promising activity when delivered in optimum concentration at the cellular target. However potential of herbal drug delivery system is limited in clinical use because of their bioavailability issues. To overcome these disadvantages and formulate a therapeutically effective and target oriented drug delivery systems, nanocarriers are being investigated as carriers for herbal phytoconstituents. This review highlights various phytoconstituents, exhibiting promising effects against cervical cancer and their mechanistic pathways. We also discuss discussed various nanocarriers such as liposomes, micelles, nanoemulsions, nanoparticles, nanogels, various aspects of optimizing drug delivery, and confirming preclinical efficacy.

KEY WORDS: phytoconstituents, liposomes, micelles, nanoparticles, nanoemulsion, hydrogels, solid lipid nanoparticles

ABBREVIATIONS: **2AAECM**, tert-butyl 2-acrylamidoethyl carbamate; **AIF**, apoptosis inducing factor; **AKT**, protein kinase B; **AP-1**, activator protein-1; **CBA**, N,N'-cystamine bisacrylamide; **CDK**, cyclin dependent kinase; **COX**, cyclooxygenase; **DDAB**, didecyldimethylammonium bromide; **DNA**, deoxyribonucleic acid; **EGFR**, epidermal growth factor receptor; **EPR**, enhanced permeability and retention; **ERK**, extracellular signal-regulated kinases; **Fas**, apoptosis antigen 1; **GSH**, glutathione; **GSK**, glycogen synthase kinase; **HEAA**, N-hydroxyethyl acrylamide; **HIV**, human immunodeficiency virus; **HPV**, human papilloma viruses; **HSV**, herpes simplex virus; **Inos**, inducible nitric oxide synthase; **MPP2**, membrane

palmitoylated protein 2 (MPP2); **MTT**, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; **NF-κB**, nuclear factor κB; **NIPA**, poly-N-isopropylacrylamide; **NP**, nanoparticles; **p21/WAF1**, cyclin dependent kinase inhibitor-1; **PAA**, polyacrylic acid; **PARP**, poly ADP ribose polymerase; **PEG/PCL**, poly (ethylene oxide) and poly(ϵ -caprolactone); **PLGA**, poly lactic-co-glycolic acid; **PTX**, paclitaxel; **ROS**, reactive oxygen species; **SCC**, squamous cell carcinoma; **TBP**, TATA binding protein; **TPGS**, using d- α -tocopheryl polyethylene glycol succinate; **VEGF**, vascular endothelial growth factor receptor; **VIA**, visual inspection with acetic acid; **WHO**, World Health Organization

I. INTRODUCTION

The World Health Organization (WHO) proclaims cervical cancer to be the fourth most common type of cancer prevalent amongst women in the year 2018.¹ The GLOBOCAN data of year 2018 also reported that 5,69,847 new cases of cervical cancer were detected with a mortality of 311,365. With 168,411 deaths reported in the year 2018, cervical cancer is the ninth most common cancer in Asia and fourth most prevalent cancer in India after breast cancer, oral cancer, and lung cancer.² Adenocarcinoma and squamous cell carcinoma (SCC) are the principal types of cervical cancer, with SCC accounting for 85% of the reported cases.

Major etiological factor responsible for development of cervical cancer are high-risk human papilloma virus (HPV) infections. There are 15 types of high risk HPVs³ that have been identified to trigger cervical cancer of which HPV 16 type alone contributes to 60% of the cancer cases while HPV 18 is accountable for 5% cases. HPVs are DNA viruses which are approximately 8000 base pairs long and wrapped into a protein shell that is composed of two molecules L1 and L2 recombinant capsid proteins.^{4,5} The virus genome expresses early proteins namely E1, E2, E4, E5, E6, and E7. Out of these, E5, E6, E7 are the oncoproteins which are responsible for progression of cervical cancer. These oncoproteins interfere and affect the normal functioning of tumor suppressor proteins which regulate and control the cell growth and survival. E6 promotes the alteration of p53, to induce tumors while E5 contributes to cervical carcinoma by several mechanisms including activation of epidermal growth factor receptor (EGFR) pathway, modulation of inflammatory cell signaling pathway, induction of angiogenesis through vascular endothelial growth factor receptor (VEGF), and inhibition of apoptosis.⁶ E6 and E7 interacts with several cellular proteins such as activator protein-1 (AP-1), p21, p53, p300, TATA binding protein (TBP), tyrosine kinase 2, membrane palmitoylated protein 2 (MPP2) Epoc 1.⁷

Other contributing cofactors such as long term use of hormonal contraceptives, multiple sex partners, high parity, early initiation of sexual activity, tobacco consumption, smoking and comorbidities of human immunodeficiency virus (HIV); *Chlamydia trachomatis*, and herpes simplex virus (HSV) type 2 infections, immunosuppression, poor hygiene, low socioeconomic status and nutritional deficiency of antioxidants in a HPV-infected individual further aggravates the progression of carcinoma.⁸

Cervical cancer predominantly affects sexually active women in their reproductive age that is between 30 to 55 years. However, about 15% cases are also reported in women above 65 years of age. Approximately 90% of deaths resulting from cervical cancer are

found to occur majorly in developing and underdeveloped countries.⁹ Initially, patients show no symptoms with the appearance of symptoms perceived as the disease advances. Abnormal excessive menstrual bleeding, bleeding after intercourse, douching, increased vaginal discharge, pain during sexual intercourse, bleeding after menopause, persistent pelvic and back pain are some of the major signs and symptoms of cervical cancer.¹⁰ WHO guidelines suggest, that the standard practice of diagnosis for cervical cancer is by unaided visual inspection with acetic acid (VIA), cytology test (pap smear test) followed by colposcopy and subsequent biopsy of suspicious lesions.⁵ Routine screening has a huge impact on prevention of cervical cancer. With the advent of regular pap testing, the incidence and mortality associated with cervical cancer have decreased at least 80%.¹¹ Yearly pap testing is recommended by The American College of Obstetricians and Gynecologists after a woman begins having vaginal intercourse. After the age of 30 years, this interval for testing can be reduced to every two to three years.¹²

II. CURRENT TREATMENT MODALITIES IN TREATMENT OF CERVICAL CANCER AND ASSOCIATED LIMITATIONS

The current modalities in the treatment of cervical cancer include chemotherapy, surgery and radiation therapy and/or targeted therapy.¹³ The choice of treatment modalities adopted in clinical practice is driven by stage of cancer (Table 1). It has been observed that as the cancer stages progresses the survival rate of the patients decreases, with about 93% survival chances in women with stage I carcinoma and less than 16% survival in stage IV cancer.

Surgery can be of many forms depending on the location and extent of cancer metastasis. Hysterectomy or any kind of surgical intervention often results in infertility with other complications like bladder emptying problems, bleeding and wound infection. In early stages, surgery can be avoided to preserve the fertility in women; in such cases radiation therapy is recommended. Radiation therapy involves application of high energy radiation to bring about suppression of the growth of tumor cells. But long-term radiation therapy is also associated with several side effects such as changes in skin texture, anemia, weakening of bones in pelvic area and vaginal stenosis. In advanced stages Radiation therapy is often coupled with chemotherapy. Some of the chemotherapeutic agents that are routinely prescribed individually or in combination include Cisplatin, carboplatin, paclitaxel, topotecan, gemcitabine, docetaxel, ifosfamide, 5-fluorouracil, and irinotecan. These agents are ineffective at low doses and if the dosage is higher; they cause severe side effects.¹⁴ Chemotherapeutic agents are designed to destroy the rapidly dividing cells as a result they often lack specificity and differentiating ability between normal and cancerous cells. Therefore, along with cancerous cells these agents are detrimental to the healthy cells of bone marrow, hair follicles, digestive tract. In addition, these chemotherapeutic agents when delivered by conventional routes, limitations like poor solubility, unfavorable, erratic and inconsistent pharmacokinetic profile, rapid deactivation and nonspecific bio-distribution often result into non-specific action, side effects, acquisition of multi-drug resistance and non-tumor selectivity. Chemotherapy

TABLE 1: Current treatment modalities reported for management of cervical cancer based on progression of disease⁸⁰

Stage of cancer	Clinical features	Treatment modalities
0	Carcinoma- <i>in situ</i> , intraepithelial carcinoma	
IA1	Invasion is < 3 mm	Conization of cervix (to preserve fertility) radical hysterectomy
IA2	Invasion is 3–5 mm	Radical hysterectomy with pelvic lymphadenectomy + radiotherapy
IB1	Tumor is < 4 cm	Radical hysterectomy with pelvic lymphadenectomy + chemoradiotherapy
IB2	Tumor is > 4 cm	Radical hysterectomy with pelvic lymphadenectomy + chemoradiotherapy or chemoradiotherapy + adjuvant hysterectomy
IIA	Involvement of upper two-thirds of vagina	Radical hysterectomy with pelvic lymphadenectomy + chemoradiotherapy
IIB	Parametrial extension of tumor	Chemoradiotherapy
IIIA	The cancer has extended to the lower part of the vagina and/or the walls of the pelvis; the cancer may be blocking the ureters	Chemoradiotherapy
IIIB	The tumor has grown into the pelvic wall and/or affects a kidney	Radical radiotherapy (external + brachytherapy) + concurrent weekly cisplatin
IIIC	The tumor involves regional lymph nodes; subsequently, cancer spreads to lymph nodes in the pelvis and to para-aortic lymph nodes	Extended field radiotherapy + concurrent weekly cisplatin
IVA	Local extension of tumor within pelvis; the cancer has spread to the bladder or rectum or it is growing out of the pelvis	Pelvic exenteration + chemoradiotherapy
IVB	The cancer has metastasized to distant organs beyond the pelvic area such as lungs, distant lymph nodes, liver, or bones	Palliative chemotherapy + chemoradiotherapy

is also related to cause other adverse effects like fatigue, loss of hair, loss of appetite, mouth ulcers, leucopenia, diarrhea, and myelosuppression.¹⁵ Multidrug resistance, in

which the tumor cells become insensitive to chemotherapeutic agents by different mechanisms such as expression of multidrug resistance protein (MDR1), low uptake of drugs or over-expression of P-glycoprotein protein, is another factor that may lead to failure of chemotherapeutic drugs.¹⁶

Some newer approaches that are currently being investigated include use of monoclonal antibodies (mAbs) such as VEGFR inhibitors (bevacizumab), EGFR inhibitors (trastuzumab, cetuximab and pertuzumab), folate receptor inhibitors (farletuzumab) individually or in combination to chemotherapeutics. Some of these monoclonal antibody treatments have entered the clinical trial phase (Table 2). Bevacizumab, a vascular endothelial receptor inhibitor, which has been approved by FDA for treatment of advanced stage cervical cancer, is associated with side effects such as rectal and nasal bleeding, hypertension, exfoliative dermatitis, back pain, peripheral sensory neuropathy, and formation of blood clots, myocardial infarction and gastrointestinal perforations. In a study conducted by Tewari et al., it was observed that patient treated with bevacizumab experienced various adverse events such as hypertension of grade 2 (25%), gastrointestinal or genitourinary fistulas of grade 3 or higher (6%), thromboembolic events of grade 3 or higher (8%).¹⁷ EGFR inhibitors have cutaneous side effects like acneiform eruptions, xerosis, fissures, paronychia and decreased WBC count, hypersensitivity. Common side effects observed with folate receptor antagonists are hypersensitivity reactions, pyrexia, chills, headache, fatigue and diarrhea. The side effects associated with mAbs are mild compared with conventional chemotherapeutic agents. However the production cost of mAbs are very high owing to use of very large cultures of cells, which are expensive to maintain, primarily as a consequence of high turnover of disposables, such as media, and the continuous requirement for sophisticated purification steps to ensure clinical quality.^{18,19} Minion et al. reported a study conducted to understand the cost effectiveness of bevacizumab for treatment of advanced cervical cancer, it was observed that the total cost of therapy with bevacizumab was approximately 13.2-fold more than that for chemotherapy alone.²⁰

Considering these aforementioned drawbacks associated with chemotherapeutic drugs and other approaches have led to increased research interests in discovering new molecular entities that are target oriented, with low incidence of resistance and side effects. Drugs of herbal origin or phytoconstituents derived from them are being envisaged either as main therapeutic or adjuvant to existing chemotherapeutic with an intention to mitigate some of these shortcomings.

III. HERBAL DRUGS AND PHYTOCONSTITUENTS AS PROMISING ALTERNATIVES OR ADJUVANTS TO CHEMOTHERAPEUTIC DRUGS

The risk of long-term morbidity and mortality associated with surgeries and side effects of chemotherapeutic and immunotherapeutic system of medicines has escalated the demand of herbs as medicines. Phytomedicines have been shown to benefit patients by providing relief from a plethora of ailments; 65–80% of the population in the developing countries still uses traditional medicine as a source of health care.

TABLE 2: Ongoing clinical trials for use of monoclonal antibodies and synthetic drugs in cervical cancer

Serial no.	Trial #	Title	Phase	Drug	Sponsor
1	NCT03476798	Bevacizumab and rucaparib in treating patients with recurrent cervical or endometrial carcinoma	II	Bevacizumab and rucaparib	University of Oklahoma Health Sciences Center
2	NCT03508570	Nivolumab with or without ipilimumab in treating patients with recurrent or high-grade gynecologic cancer with metastatic peritoneal carcinomatosis	IB	Nivolumab with or without ipilimumab	MD Anderson Cancer Center, United States
3	NCT03367871	Pembrolizumab, bevacizumab, and standard chemotherapy for the treatment of recurrent, persistent, or stage IVB cervical cancer	II	Pembrolizumab and bevacizumab	University of Miami Miller School of Medicine-Sylvester Cancer Center, Miami, Florida
4	CTRI/2009/091/000739	A clinical trial to study the effects of nimotuzumab in cervical cancer patients	II	BIOMAb EGFR nimotuzumab + cisplatin + standard radiotherapy	Bangalore Institute of Oncology, Karnataka, India
5	CTRI/2019/04/018479	Study of durvalumab or placebo given along with chemoradiation therapy in women with locally advanced cervical cancer	III	Durvalumab	Action Cancer hospital, Delhi, India
6	CTRI/2019/02/017477	Trial of nimorazole with chemoradiation as primary treatment for locally advanced cervical cancer compared with chemoradiation alone	II	Nimorazole with cisplatin and radiation	All India Institute of Medical Sciences, Delhi, India

TABLE 2: (continued)

7	CTR1/2017/12/010726	A prospective randomized open label study to evaluate the response rate and toxicity of gefitinib in the treatment of carcinoma cervix patients on chemoradiotherapy	III	Gefitinib	Mysore Medical College and Research Institute, Karnataka, India
8	CTR1/2017/08/009265	Role of nelfinavir during chemoradiation for cervical cancer	III	Nelfinavir	ACTREC, Tata Memorial Hospital, Maharashtra, India
9	NCT04221945	Study of chemoradiotherapy with or without pembrolizumab (MK-3475) for the treatment of locally advanced cervical cancer (MK-3475-A18/ KEYNOTE-A18/ENGOT-cx11)	III	Pembrolizumab	Merck, United States

Traditional herbal drugs have been considered as important therapeutic regimens in countries such as India, China, and Egypt mainly because they are economical, indigenous, easily accessible and ancient and traditional cultural beliefs are associated with them. Herbal plants exhibit anticancer activity because of their versatile immune modulatory and antioxidant properties. These drugs act prominently by stimulating both specific and non-specific immunity. Also, they are not as toxic as chemotherapeutic drugs. They help to sensitize the cancer cells against chemotherapeutic drugs and radiation therapy when used in conjunction with them. Studies indicate that these drugs often exhibit synergism with chemotherapeutic agents; thereby there is significant reduction of dose of the chemotherapeutics.^{21,22} Herbal drug at times are more target oriented as they can interact with target at cellular level.^{16,17} For instance, plant alkaloid berberine has effective anti-inflammatory, anticancer, anti-proliferative and proapoptotic activity with 90 identified targets. Berberine causes increased expression of BCL2-associated X protein (Bax), decreased G0/G1 phase-associated cyclins (D1, D2, E, Cdk2, Cdk4, and Cdk6) acts on Bcell CLL/lymphoma 2 (BCL2), pro-caspase-3 and -9, and poly (ADP-ribose) polymerase (PARP), acts on 72 kDa type IV collagenase (MMP2), Cdc42 effector protein 1 (CDC42EP1), and rasrelated C3 botulinum toxin substrate 1 (RAC1), transforming protein RhoA (RHOA) and urokinase-plasminogen activator A (PLAU) in various cell lines showing anti-metastatic activity.^{23,24} Several novel therapeutic agents derived from medicinal plants are recently being investigated for their mechanistic pathway and activity against cervical cancer (Table 3, Fig. 1).

IV. ONGOING CLINICAL TRIALS OF HERBAL DRUGS FOR CERVICAL CANCER THERAPY

From the above discussed drugs in Table 3, a few drugs such as curcumin and paclitaxel managed to enter the clinical trial to demonstrate its efficacy as anticancer agent against cervical neoplasia. The clinical trials on-going for herbal drugs for treatment and management of cervical cancer have been discussed in Table 4.

V. NECESSITY FOR NANOCARRIERS OF HERBAL DRUGS

Herbal drugs and derived phytoconstituents exhibit promising activity when delivered in optimum concentration at the cellular target. They surmount many drawbacks associated with delivery of synthetic anti-cancer molecules. However potential of herbal drug delivery system is limited in clinical use because of their bioavailability issues. The factors accountable for low bioavailability of herbal moieties are instability in gastric pH, enzymatic degradation, low solubility, rapid metabolism, rapid excretion, high molecular size and inability to cross biological membranes. These obstacles decrease the therapeutic efficacy of many herbs. To overcome these disadvantages and formulate a therapeutically effective and target oriented drug delivery systems, nanocarriers are being investigated as carriers for herbal phytoconstituents. It has been widely proposed

TABLE 3: Herbal drugs and phytoconstituents with reported mechanistic pathway and activity against cervical cancer

Serial no.	Herbal drugs/phytoconstituent	Source	Molecular targets and mechanism of action	Refs.
Alkaloids				
1	Lycopodine	Alkaloids obtained from whole plant of <i>Lycopodium clavatum</i> (Lycopodiaceae)	Initiates chromatin condensation and inter nucleosomal DNA fragmentation, increases ROS generation and mitochondrial membrane potential depolarization, causes release of cytochrome c and activation of caspase-3. Induction of mitochondrial dependent apoptosis.	81
2	(-)-Anonaine	Alkaloids obtained from leaf of <i>Michelia alba</i> (Magnoliaceae)	Induces apoptosis by upregulation of Bax and p53 proteins expression, increases intracellular NO, induces ROS generation, glutathione depletion and by disrupting mitochondrial transmembrane potential.	82
3	Berberine	Alkaloids obtained from rhizome of <i>Coptis chinensis</i> (Ranunculaceae)	Induction of DNA topoisomerase poisoning, downregulation of Bcl-2 and Bcl-XL, upregulation of Bax and increase of ROS generation.	24
4	Vincristine	Alkaloid obtained from <i>Catharanthus roseus</i> (Rosaceae)	Arrest proliferation of cancer cells by binding tubulin filaments in mitotic spindle; induce apoptosis.	83
5	Paclitaxel	Alkaloid obtained from <i>Taxus brevifolia</i> (Taxaceae)	Induces mitotic arrest by activating spindle assembly check point.	84
Flavonoids: Flavonols, flavones, isoflavones, flavonone				
6	Isoliquiritigenin (ISL)	Flavonoids obtained from roots and rhizome of <i>Glycyrrhiza glabra</i> (Fabaceae)	Arrests cell cycle in both the G2 and M phases via inhibition of topoisomerase II activity and regulation of DSB-mediated ATM/Chk2 signalling pathway.	85
7	Hesperetin	Flavanoid obtained from citrus fruits like <i>Citrus limonis</i> , <i>Citrus aurantium</i> (Rutaceae)	Induces apoptosis by increased expression of caspase-3, caspase-8, caspase-9, p53, Bax, and Fas death receptor due to the dwindling of the mitochondrial membrane.	86

TABLE 3: (continued)

Serial no.	Herbal drugs/phytocomponent	Source	Molecular targets and mechanism of action	Refs.
Flavonoids: Flavonols, flavones, isoflavones, flavonone				
8	Quercetin	Quercetin is a plant pigment flavonoid found in many plants such as onions, Gingko biloba, apples, buckwheat, tea etc.	Decreases O-GlcNAcylation in cervical cancer cells thus activating AMPK pathway and reducing cancer progression and proliferation of cancer cells. Induces G2/M phase cell cycle arrest, causes upregulation of proapoptotic Bcl-2 family proteins, cytochrome c, Apaf-1 and caspases and downregulation of anti-apoptotic Bcl-2 proteins ultimately leading to apoptosis.	87
9	Apigenin	Apigenin is a flavone found in many fruits and vegetables, but parsley, celery, celeriac, and chamomile tea are the most common sources. Apigenin is particularly abundant in the flowers of chamomile plants, constituting 68% of total flavonoids.	Triggers apoptotic pathway by induction of G1 arrest, DNA fragmentation, increased expression of p21/WAF1, caspase-3 and decreased protein expression of anti-apoptotic factor Bcl-2 protein.	88,89
10	Genistein	Isoflavone found in soya beans <i>Glycine max</i> (Fabaceae)	Inhibits Cell Growth by modulating mitogen-activated protein kinase (MAPK) pathway and PK-B/AKT pathway in cervical cancer cells.	90,91
11	Kaempferol	Flavanol derived from rhizome of <i>Kaempferia galanga</i> L. (Zingiberaceae)	Initiates cellular apoptosis by regulating the p13k/AKT and hTERT pathways.	92,93
12	Kaempferol-7-O- β -D-glucoside	Flavonoid obtained from rhizome of <i>Smilax china</i> (Liliaceae)	Antiproliferative activity. Induces arrest of G2/M phase, decrease of cyclin B1 and CDK1, inhibition of NF- κ B nuclear translocation, upregulation of Bax and downregulation of Bcl-2.	94
13	Liquiritigenin	Flavonoids obtained from roots and rhizome of <i>Glycyrrhiza</i> (Papilionaceae)	Antiproliferative in action and induces apoptosis by upregulation of p53, release of cytochrome c and elevation of caspase-9 and -3 activities.	95

TABLE 3: (continued)

14	Eupafolin	Flavonoids from leaf of <i>Artemisia princeps</i> (Compositae)	Eupafolin activates caspases -3, -6, -7, -8 and -9 and increases cleavage of their substrate and induces apoptosis via mitochondrial death pathway	96
15	Oroxylin A	Flavonoids obtained from roots of <i>Scutellaria baicalensis</i> (Labiatae)	Induces apoptosis by down regulation of Bcl-2 protein expression and degradation of PARP.	97
16	Fisetin	Flavonoids obtained from trees and shrubs in the family Fabaceae, such as the acacias <i>Acacia greggii</i> and <i>Acacia berlandieri</i> , the parrot tree (<i>Butea frondosa</i>), the honey locust (<i>Gleditsia triacanthos</i>)	Activation of the phosphorylation ERK1/2, inhibition of ERK1/2 by PD98059, activation of caspase-8/-3 pathway leading to apoptosis and significantly reduced tumor growth.	98,99
17	Protoapigenone	Flavonoid isolated from <i>Thehpteris toressiana</i>	Causes inhibition of PIK3 signaling, inhibition of AKT1/mTOR activity and activation of caspases-9, -8 and -3, PARP cleavage and promotes apoptosis.	100
18	Naringin	Flavanone-7-O-glycoside between the flavanone naringenin and the disaccharide neohesperidose. The flavonoid naringin occurs naturally in citrus fruits, especially in grapefruit, where naringin is responsible for the fruit's bitter taste.	Induction of apoptosis through both death-receptor and mitochondrial pathways.	101,102
19	Eupatorin	Eupatorin is a natural flavone isolated from several plants including <i>Tanacetum vulgare</i> , <i>Lantana montevidensis</i> , and <i>Orthosiphon stamineus</i>	Cell cycle arrest at the G2/M phase and induction of apoptosis.	103
20	Luteolin	Flavone obtained from flowering plant <i>Saltia tomentosa</i> (Lamiaceae). Dietary sources include celery, broccoli, green pepper, parsley, thyme, chamomile etc.	Luteolin sensitized HeLa cells to TRAIL-induced apoptosis by both extrinsic and intrinsic apoptotic pathways.	104

TABLE 3: (continued)

Serial no.	Herbal drugs/phytocomponent	Source	Molecular targets and mechanism of action	Refs.
Flavanoids: Flavonols, flavones, isoflavones, flavonone				
21	Baicalein	Flavone isolated from the roots of <i>Scutellaria baicalensis</i> and <i>Scutellaria lateriflora</i> (Lamiaceae)	Baicalein induces apoptosis in cancer cells through multiple mechanisms, such as increasing the ROS level, inactivating 12-lipoxygenase (12-LOX), inhibiting PI3K/Akt and its downstream proteins, as well as upregulating tumor suppressors p38 and p53.	105
Terpenes-diterpenes, triterpenes, sesquiterpenes and cyclic terpenes				
22	Carnosic acid	Major bioactive compound benzenediol abietane diterpene of <i>Rosamarius officinalis</i> L. (Lamiaceae)	Upregulation of apoptosis and ROS production in cervical cancer cells. Acceleration of ROS production causes phosphorylation of c-Jun N-terminal kinase and activation of endoplasmic reticulum stress thus promoting apoptosis through stimulating caspase-3 expression.	106
23	23-hydroxyursolic acid	Pentacyclic triterpene obtained from stems and bark of <i>Cussonia paniculata</i> (Araliaceae)	Causes decrease of Bcl-XL and Bcl-2 expression and NF-κB p65 protein level.	107
24	3a,23-isopropylidenedioxyolean-12-en-27-oic acid	Triterpenes from leaflets and stem of <i>Aceriphyllum rossii</i> (Saxifragaceae)	Induces apoptosis by release of cytochrome c, activation of caspase-9, increase of ER stress, Ca ²⁺ release and activation of calpain	108
25	Saikosaponin-a and d	Triterpene saponins obtained from roots of <i>Bupleurum falcatum</i> (Umbelliferae)	Induction of cellular ROS accumulation mediates synergistic cytotoxicity in saikogenins and cisplatin co-treated cancer cells.	109
26	AMDT ((Z)-7-acetoxy-methyl-11-methyl-3-methylene-dodeca-1,6,10-triene)	Sesquiterpenes obtained from hairy root of <i>Artemisia annua</i> (Compositae)	Induces apoptosis through the mitochondria-dependent pathway and activation of caspase cascade.	110
27	Celastrol	Triterpenes from whole plant of <i>Trypterygium wilfordii</i> Hook (Celastraceae)	Antiproliferative in activity. Induces apoptosis by depolarization of Δψm and by increasing caspase activity.	—

TABLE 3: (continued)

28	Cucurbitacin D	Cucurbitacin is a bitter-tasting principle that can be isolated from members of the family Cucurbitaceae, such as cucumber (<i>Cucumis sativus</i>) and melon (<i>Cucumis melo L.</i>)	Arrests the cell cycle in G1/S phase, inhibits constitutive expression of E6, Cyclin D1, CDK4, pRb, and Rb and induces the protein levels of p21 and p27. Enhances the expression of tumor suppressor microRNAs (miR-145, miRNA-143, and miRNA34a) in cervical cancer cells.	111
Terpenoids				
29	Crocetin	Apocarotenoid dicarboxylic acid found in <i>Crocus sativus</i> (Iridaceae)	Causes increased DNA fragmentation, activates caspase dependent apoptosis and p53 mediated cell cycle arrest.	112,113
30	Astilbotriterpenic acid	Triterpenoids from rhizome of <i>Astilbe chinensis</i> (Saxifragaceae)	Induction of caspase activation, release of ROS, downregulation of Bcl-2 and upregulation of Bax.	114
31	ATA (93b-hydroxy-12-oleanen-27-oic acid)	Triterpenoids obtained from rhizome of <i>Astilbe chinensis</i> (Saxifragaceae)	Causes downregulation of Bcl-2 expression, upregulation of Bax expression, decrease of $\Delta\mu m$ and activation of the caspase-3 pathway leads to apoptosis.	115
32	Parviflorene F (1)	Sesquiterpenoids obtained from whole plant of <i>Cucurma parviflora</i> (Zingiberaceae)	Enhancement of mRNA and protein expression of TRAIL-R2, and activation of caspase-8, -9 and -3 thus inducing apoptosis.	116
33	Oridonin	Diterpenoids obtained from aerial parts of <i>Rabdosia rubescens</i> (Labiatae)	Anti-proliferative action by downregulation of the protein kinase B (Akt) activation, expression of FOXO transcription factor and GSK3.	117
34	Amooranin	Triterpenoidal compound isolated from <i>Amoora rohituka</i> (Meliaceae)	Arrests G2/M phase of cell cycle and causes induction of apoptosis.	118
35	Corosolic acid	Triterpenoids obtained from roots of <i>Actinidia valhava</i> (Actinidiaceae)	Induction of apoptosis by activation of caspase-dependent mitochondrial pathway.	119
36	Ursolic acid	Pentacyclic triterpenoids isolated from plants such as <i>Mirabilis jalapa</i> and fruits and herbs such as apples, cranberries, peppermint, rosemary, basil, oregano and prunes	Antiproliferative activity, induces apoptosis through activating caspases, p53 and suppressing apoptosis-related signals.	66

TABLE 3: (continued)

Serial no.	Herbal drugs/phytocomponent	Source	Molecular targets and mechanism of action	Refs.
Polyphenols and phenolic compounds				
37	Curcumin	Natural polyphenolic compound extracted from rhizome of <i>Cucurma longa</i> (Zingiberaceae)	Induces apoptosis by causing downregulation of the TGF-β signaling pathway, Bcl-2, Bcl-XL, COX-2, iNOS and cyclin D1 and upregulation of Bax, AIF, and release of cytochrome c.	120,121
38	Manchartin C	Phenolics obtained from whole plant of <i>Dumortiera angust</i> (Marchantiaceae)	Arrests cell cycle at G2/M phase, decreases expression of Bcl-2, and increases expression of cyclin B1, Bax and Caspase-3.	122
39	Gallic acid	Polyhydroxy phenolic compound widely distributed in gall nuts, sumac, grape, green tea, strawberry, lemon, witch hazel, oak bark	Increases reactive oxidative species level and causes depleted level of glutathione in cervical cancer cells thus inducing apoptosis and/or necrosis.	123
40	Resveratrol	Polyphenol obtained from seeds and skin of grapes, red wine, mulberries and peanuts	Inhibits proliferation and induces autophagy and apoptotic death in cervical cancer cells. Resveratrol inhibits NF-κB and AP-1 transactivation suppressing the transcription of MMP- leading to suppression of migration and invasion of cervical cancer cells.	124
41	Pterostilbene	Polyphenols naturally found in various dietary sources, such as grapes, blueberries, red wine, peanuts	p53 and p21 expression and subsequent downregulation of cyclin E1 and cyclin B1 leading to cell cycle arrest at S and G2/M phases.	125
Quinones and Benzoquinones				
42	2-acetylifuro-1,4-naphthoquinone	Quinones from root of <i>Newbouldia laevis</i> (Bignoniaceae)	Induction of cell cycle arrest in S-phase.	126
43	Thymoquinone	Benzoquinones obtained from seeds of <i>Nigella sativa</i> (Ranunculaceae)	Induction of apoptosis by elevation of p53 and downregulation of the Bcl-2 protein.	127
44	Tan II-A	Quinones obtained from roots and rhizome of <i>Salvia miltiorrhiza</i> (Labiatae)	Induction of mitotic arrest and apoptosis through the JNK-mediated mitochondrial pathway.	128

TABLE 3: (continued)

Coumarins			
45	Osthole (7methoxy8isopentenoxycoumarin)	Coumarin derivative found in mature fruit of <i>Cnidium monnierii</i> (Fructus Cnidii)	Induces apoptosis by increasing Bcl-2 associated X protein (Bax)/Bcl2 ratio, and the levels of cleaved caspase 3 and cleaved caspase9 thus activating caspase dependent pathway.
46	Coumarin A	Coumarins obtained from fruit of <i>Mammea americana</i> (Mammea)	Activation of an apoptosis-like cell death program, release of the pro-apoptotic protein AIF, but without disturbance of cell cycle.
47	Decursin and decursinol angelate	Coumarins from roots of <i>Angelica gigas</i> (Umbelliferae)	Activates caspases, causes cleavage of PARP, increases TRAIL and TRAIL receptors expression, regulation of the Bcl-2, Bcl-XL, surviving, clap-1, -2 and XIAP expression.
Catechins			
48	(-)Epigallocatechin-3-Gallate (EGCG)	Ester of Epigallocatechin and Gallic acid found in green tea <i>Camellia sinensis</i> (Theaceae)	Arrests cell cycle at the G1 phase and causes induction of apoptosis.
Lectins			
49	Frutalin	A binding lectin isolated from breadfruit seeds obtained from two different sources: native frutalin, purified from its natural origin and recombinant frutalin which was produced and purified from <i>Pichia pastoris</i>	Induces cell apoptosis and inhibits cell proliferation.
Proteins			
50	Trichosanthin	Proteins obtained from roots and tubers of <i>Trichosanthes kirilowii</i> (Cucurbitaceae)	Causes downregulation of α - and β -tubulin mRNAs decreases the amount of γ -actin mRNA.
51	Nebrodelysins	A monomeric protein obtained from fruiting body of <i>Pleurotus nebrodensis</i> (Graminae)	Exhibits hemolytic activity towards the rabbit erythrocytes, and causes efflux of potassium ions from erythrocytes and induces apoptosis in HeLa cells.

TABLE 3: (continued)

Serial no.	Herbal drugs/phytoconstituent	Source	Molecular targets and mechanism of action	Refs.
Lignans and flavolignans				
52	Oblongifolin C	Lignan obtained from whole plant of <i>Garcinia yunnanensis</i> (Guttiferae)	Induction of apoptosis by inducing Bax translocation, cytochrome C release, mitochondrial fission and swelling and reduction of mitochondrial membrane potential.	136
Lignans and flavolignans				
53	Silibinin	Flavolignans from <i>Silybum marianum</i> (Compositae)	Induction of G2 arrest and the decrease in cyclin dependent kinase (CDKs) involved in both G1 and G2 progression, elicitation of apoptosis in HeLa cells via both the mitochondrial and death receptor-mediated pathways.	137
Iridoids				
54	Iridomyrmecin	Plant iridoid compound found in variety of plants including <i>Actinidia polygama</i>	Induces early and late apoptosis, loss of mitochondrial membrane potential, sub-G1 cell cycle arrest, downregulation of PI3K/Akt protein expressions, and upregulation of lncRNA CCAT2 expression.	138
Nucleosides				
55	Clitocine	Nucleosides obtained from fruiting body of <i>Leucopaxillus gigantus</i> (Tricholomataceae)	Causes downregulation of Bcl-2, upregulation of Bax, activation of caspase-3 and release of cytochrome c.	139
Actinoporins				
56	Actinoporin RTX-A	Actinoporins obtained from <i>Heteractis crispa</i> (Actiniidae)	Induction of p53-independent apoptosis and inhibition of activation of the oncogenic AP-1 and NF-κB nuclear transcriptional factors.	140
Xanthones				
57	Xanthone V1	Xanthones from root and leaf of <i>Cratoxylum formosum</i> , <i>Vismia laurentii</i> (Guttiferae)	Induction of cell cycle arrest in S-phase and activation of caspase -3 and -7.	141
58	Paucinervins A-D	Benzophenones and xanthones from leaf of <i>Garcinia paucinervis</i> (Guttiferae)	Causes induction of apoptosis by activating caspase-3 with paucinervin B showing strongest inhibitory activity.	142

Cannabinoids			
59	Cannabidiol	Cannabinoid obtained from <i>Cannabis sativa</i> (Cannabinaceae)	Anti-invasion activity. The decrease of invasion is by upregulation of TIMP-1. Knockdown of cannabidiol induced TIMP-1 expression by siRNA led to a reversal of the cannabidiol-elicted decrease in tumor cell invasiveness.
Volatile organic compounds			
60	Methyl jasmonate	Volatile organic compound derived from jasmonic acid produced by plants in response to biotic and abiotic stresses	Upregulation of Bax level, reduction of p53 and p21 levels. Antiproliferative in action.
143			
144			

TABLE 3: (continued)

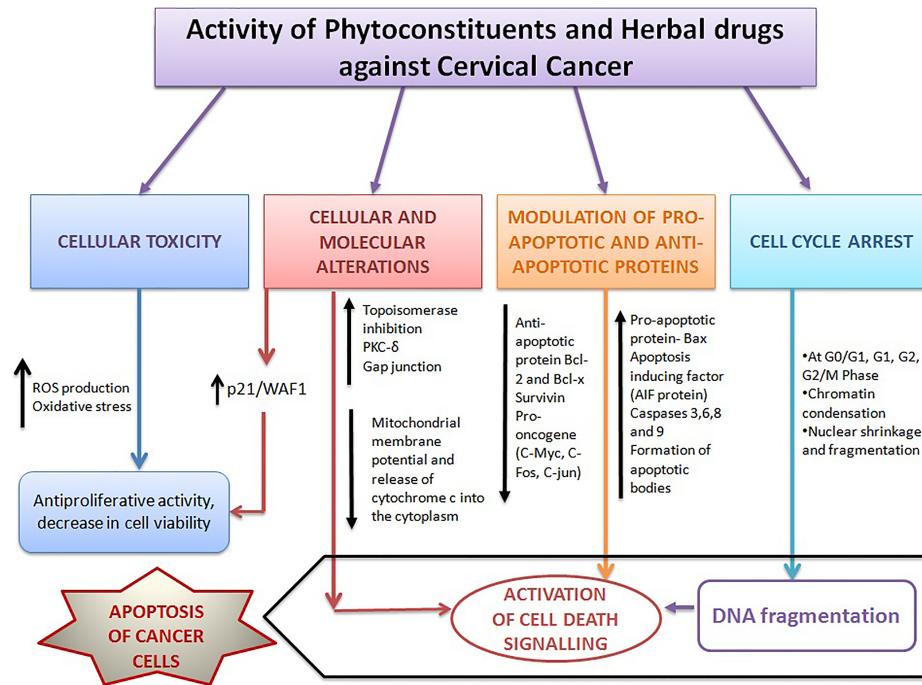


FIG. 1: Pictorial depiction of herbal drugs and phytoconstituents with reported mechanistic pathway and activity against cervical cancer. The figure summarizes all reported pathways of anti-cancer activity exhibited by phytoconstituents (see Table 1). The black upward arrow indicates increase and black downward arrow indicates decrease in factors associated in the pathway.

that combining nanotechnology with plant medicines will help reduce the required dose and side effects, help potentiate the activity of plant extract and improve the activity.²⁵

Nanocarriers are submicron sized colloidal delivery systems (diameter 1–1000 nm) that can alter the basic properties and bioavailability due to their high surface area to volume ratio. Nanocarrier based delivery system have enabled effective delivery of anticancer molecules into the tumor tissues by exploiting the pathophysiology of tumor microenvironment. Nanocarriers provide an excellent platform for treatment of cancer by increasing the retention of drugs at the site of action and reducing non-specific bi-distribution and toxicity. They can maneuver the physicochemical characteristics thereby enhancing the solubility and stability of the herbal moieties. Nanocarriers are being extensively investigated for cervical cancer management. They serve as a suitable, efficient delivery system for various routes of administration, including oral, injectable and topical applications. Additionally, these nanoparticles can be further processed into capsules, tablets, fast-melts and lyophilized for sterile product applications. They are being envisaged for their potential to encapsulate and release drugs, vaccines, genes, proteins, etc.²⁶

Nanocarriers function on the principle of enhanced permeability and retention (EPR) by virtue of their smaller size range. There is an uncontrolled angiogenesis in

TABLE 4: Ongoing clinical trials of herbal drugs in cervical cancer therapy

Serial no.	Title and trial #	Brief description	Type	Drug	Sponsor
Herbal drugs alone or in combination					
1	NCT02944578 Curcumin in Treating HIV Infected and Uninfected Women with High Grade Cervical Squamous Intraepithelial Neoplasia	The study explores the effect of curcumin as a potential medical treatment in HIV-infected women with HSIL (high-grade squamous intraepithelial) lesions of the cervix. It is a type of randomized interventional trial.	Phase 2 November 20, 2017 to August 2021	Curcumin	Emory University, United States
2	NCT02020707 Targeted Complex Therapy for Advanced Melanoma and Gynecologic Cancers: Nab-Paclitaxel (Abraxane)/ Bevacizumab Complex (AB-Complex)	This phase I trial studies the side effects and best dose of paclitaxel albumin-stabilized formulation and bevacizumab in treating patients with stage IV melanoma that cannot be removed by surgery or with cancer of the cervix, endometrium, ovary, fallopian tube or peritoneal cavity.	Phase 1 February 3, 2014 to June 1, 2025	Paclitaxel + bevacizumab	Mayo Clinic, United States
3	CTR1/2018/02/012184 Study of Turmeric Extract in Prevention of Uterine Cervical Cancer	An open labeled clinical pilot study on safety and activity of holistic extract of <i>Haridra curcuma longa</i> Linn 600 mg extract capsule orally twice a day for 10 weeks for treatment of low grade squamous intraepithelial lesions of cervix (LSIL) in pap smears in women.	Phase 2	Curcumin	Kasturba Health Society Medical Research, Maharashtra, India
4	CTR1/2008/091/000131 Evaluation of the Efficacy of Curcumin in the Management of Advanced Cancer Cervix	A prospective, randomized, double-blind, placebo-controlled, multicentric, phase III study of the efficacy of curcumin in the management of advanced cancer cervix.	Phase 3	Curcumin	All India Institute of Medical Sciences, Delhi, India

TABLE 4: (continued)

Serial no.	Title and trial #	Brief description	Type	Drug	Sponsor
5	CTR1/2019/04/018382 Chemotherapy and Pelvic Radiation Therapy with or without Additional Chemotherapy in Treating Patients with High-Risk Early-Stage Cervical Cancer after Radical Hysterectomy	Phase III randomized study of concurrent chemotherapy and pelvic radiation therapy with or without adjuvant chemotherapy in high-risk patients with early-stage cervical carcinoma following radical hysterectomy.	Phase 3	Carboplatin + paclitaxel	Tata Memorial Hospital, Maharashtra, India
6	NCT03834571 A Randomized Phase II Trial of Concurrent Chemotherapy and Pelvic Radiation Therapy with or without Paclitaxel and Carboplatin in HIV-Positive Women with Locally Advanced Cervical Cancer (LACC)	This phase II trial studies how well standard chemotherapy and radiation therapy given with or without paclitaxel and carboplatin work in treating human immunodeficiency virus (HIV)-positive women with cervical cancer that has spread to nearby tissue or lymph nodes. It is randomized, parallel and interventional type of study.	Phase II	Paclitaxel + carboplatin	AIDS Malignancy Consortium, Sub-Saharan Africa

tumor microenvironment. The newly formed vessels are poorly aligned, have defective endothelial cells with wide fenestrations and leaky vasculature. The vessels lack smooth muscle layer, have impaired functional receptors and ineffective lymphatic system. It is because of these physiological conditions; nanoparticles not only gain easy access to tumor but are also retained at the target site. The EPR effect is further enhanced by many pathophysiological factors involved in enhancement of the extravasation of macromolecules in solid tumor tissues. The EPR effect forms the fundamental basis for passive targeting of active moiety to cancer tissue by use of nanocarriers. Nanocarriers offer larger surface area for multiple functionalization. In recent times, nanocarriers are rendered target specific by conjugating them with ligands such as folate, aptamers, peptides, and lectins which are recognized by antigens or receptors on tumor cells.²⁷ Carbohydrates, monoclonal antibodies, peptides, proteins, aptamers, vitamins and hyaluronic acid are some of the examples of conjugated ligands that have been reported in research literature. Ligands enable active targeting of anticancer molecules to target tissues.²⁸

Herbal drug nanocarriers are also being studied to help overcome multidrug resistance which is a major problem for success of cancer chemotherapy. Increased expression of P-glycoprotein by MDR-1 gene is a well characterized mechanism for chemoresistance. Punfa et al. conducted a study to overcome multidrug resistance by formulating PLGA nanoparticles of curcumin with anti-P-glycoprotein antibody. It was found that cells pretreated with curcumin and anti-P-gp antibody showed more sensitivity to chemotherapy, induction of cell death both *in vivo* and *in vitro* leading to inhibition of tumor growth. The study also stated that nanoparticles could enhance drug solubility and improve its safety and biocompatibility.²⁹

Nanoparticulate carriers with biocompatible lipids and materials have been found to improve patient survival by simultaneously increasing intracellular drug concentration and also reducing dose related toxicity.^{30,31} nanocarriers for herbal drugs ensure targeted delivery, increased stability of phytoconstituents, decreased toxicity, increased bioavailability, and increased penetrability, increased accumulation at the target site, increased therapeutic efficacy, protection of encapsulated active drug and easy uptake by the cancer cells.^{32,33} Various polymeric and lipid based³⁴⁻³⁶ nanocarrier approaches that have been reported for delivery of herbal constituents for management of cervical cancer are discussed in the following section (Fig. 2).

A. Liposomes

Liposomes are lipid-based vesicular systems consisting of central aqueous phase enclosed by lipid bilayer of natural or synthetic phospholipids and cholesterol. Liposomes are one of the most extensively studied nanocarriers in cancer therapeutics.³⁷ Both hydrophilic and hydrophobic drugs can be incorporated into the liposomes. Lipid composition, drug to lipid ratio, vesicle size, manufacturing process parameters are some of the factors that are required to be optimized to attain desired pay drug load and encapsulation efficiency. The desired quality and functionality attributes of liposomes are enhanced permeability, stability, appropriate lamellarity, specific size and size distribution.³⁸ First

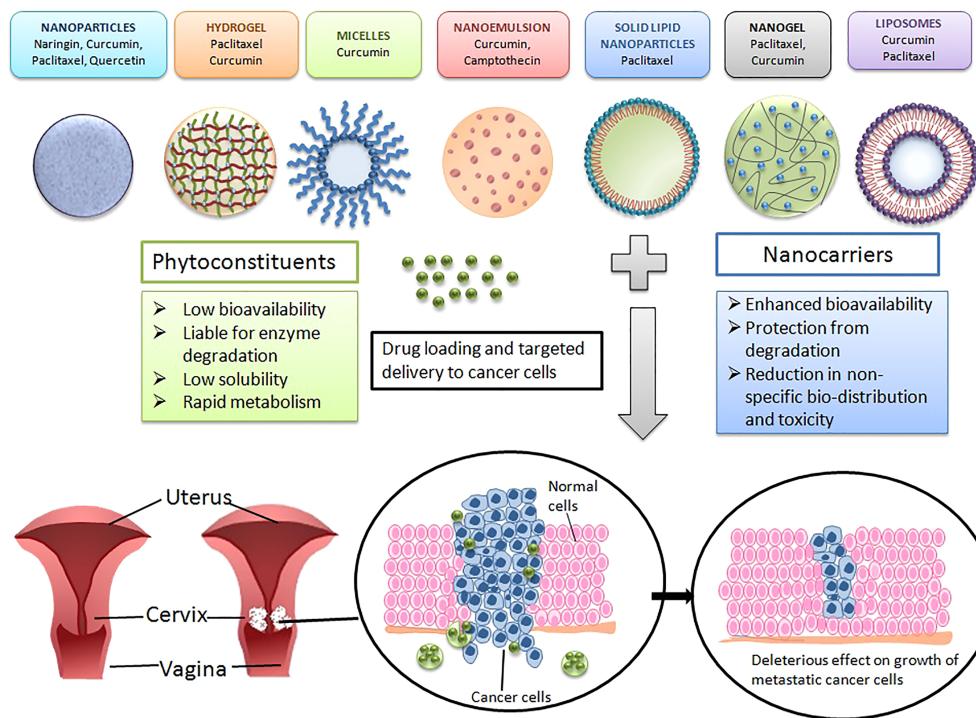


FIG. 2: Pictorial depiction of various nanocarriers involved in delivery of phytoconstituents. Phytoconstituents are loaded and delivered to target site with the help of nanocarriers (nanocarriers with reported phytoconstituents). After delivery to the cancer cells, the phytoconstituents act on cancer cells and exhibit deleterious effect without affecting normal healthy cells.

generation liposomes are the liposomes which are intended for localized delivery and stimuli-responsive liposomes which have the ability to provide site-specific and target oriented drug release are called second generation liposomes. Many herbal drugs have been tried for incorporation into the liposomes to improve therapeutic efficacy of the herbal moieties.³⁹

Curcumin is a natural product obtained from rhizomes of *Curcuma longa*. It has the potential to modulate multicellular signaling pathways including cell cycle, proliferation, metastasis, inflammation, angiogenesis, and apoptosis.⁴⁰ It has demonstrated many therapeutic properties including anti-oxidative effect and pleiotropic effect on cancer cells.^{41,42} Even though curcumin inhibits copious molecular cascades and biochemical processes, and also can inhibit growth of tumor cells, use of curcumin in clinical utility is limited. This is due to its poor solubility in aqueous solvents, instability in physiological pH and low bioavailability issues. To augment bioavailability and protect curcumin from degradation, use of nanocarriers for delivery of curcumin is considered as an efficient approach for cancer therapy. Of nanocarriers, liposomes have been studied extensively to deliver therapeutics to the target tumor site.

Cationic liposomes due to their biocompatibility, low immunogenicity and low cytotoxicity have been proven to enhance bioavailability and evade the degradation pathways. Cationic liposomes also preferentially target the angiogenic endothelium of tumor and disrupt the vascular function of tumor thus limiting the tumor growth and metastasis. Saengkrit et al. developed cationic liposomal formulation of curcumin in order to enhance its bioavailability by improving its solubility. Cationic liposomes of curcumin were formulated through surface modification of liposome prepared using cholesterol and a non-ionic surfactant (Montanov82). Various compositions of didecyldimethylammonium bromide (DDAB) were used to confer positive charge to the surface of liposomes and make them cationic in nature. DDAB-containing liposome and DDAB-free liposome were compared and evaluated for cytotoxicity and apoptosis against cervical cancer cells. The percent cell viability obtained for DDAB-containing liposomes was 20% and 80% for DDAB-free liposomes thus indicating that DDAB-containing liposomes exhibited more toxicity to cervical cells compared with DDAB-free liposomes. This cytotoxicity could be the result of charge-induced cell leakage, due to the presence of positively charged DDAB. The percent efficiency and loading capacity of curcumin in DDAB liposomes was 63.9% and was found to increase to 68–70% in presence of cholesterol. Curcumin release from liposomes at 48 hours was reported to be 54.0% for DDAB-free liposomes and 64.6% for DDAB liposomes. Overall it was observed that when curcumin was incorporated in liposome it significantly showed an increase in cytotoxicity against cancer cells as compared with cells treated with free curcumin. It was also observed that the incorporation of DDAB promoted apoptosis in both HeLa and SiHa cell lines.⁴³

Paclitaxel is one of the most potent anticancer drugs reported. Paclitaxel is an efficient anticancer agent which induces mitotic arrest by activating spindle assembly checkpoint. But, it is highly water insoluble.⁴⁴ Currently it is administered intravenously as 1:1 mixture of Paclitaxel-Cremophor EL as commercial Taxol®. However, the use of Cremophor EL is associated with severe side effects like nephrotoxicity, neurotoxicity, and hypersensitivity. To develop better tolerated and less toxic delivery systems for paclitaxel, liposomes were formulated. However, the phospholipids used in fabricating the liposomes were responsible for instability of liposomes during storage and in biological media. In order to overcome these limitations, numerous polyelectrolytes such as polyallyl amine hydrochloride, deoxyribonucleic acid, polyethylene glycol, polypeptides etc. were utilized to coat conventional liposomes and were scrutinized for improved stability, extended release and enhanced bioavailability of drugs encapsulated. Chen et al. conducted surface modification of liposomes by coating it with polyelectrolytes by layer-by-layer assembly. Liposomes encapsulating paclitaxel (PTX) were prepared by thin film hydration using soya lecithin, stearyl amine and cholesterol. These conventional liposomes were then coated with a layer of anionic polyacrylic acid (PAA) followed by a layer of cationic chitosan. The *in vitro* release studies of PTX from chitosan-PAA-PTX-liposomes were conducted by dialysis membrane method and studies showed that conventional liposomes showed a rapid release of 42% in 1 hour and a total of 69% release at the end of 4 hours. In contrast, PAA-PTX-liposomes showed a slow rate of release

with 36% released at the end of 4 hours. PAA-PTX liposomes with chitosan exhibited a more retarded release rate, only 31% released at the end of 4 hours. MTT assay was used to study the cytotoxicity of PTX-liposomes, PAA-PTX-liposomes and chitosan-PAA-PTX-liposomes and blank chitosan nanoparticles on HeLa cell lines. The percent cell viability decreased as concentration of formulation increased. 90% cell viability was observed in case of blank chitosan particles suggesting no significant cytotoxicity present. Chitosan PAA-PTX-liposomes showed a cell viability of 35% compared with 40% and 42% cell viability observed in PTX-liposomes and PAA-PTX-liposomes. This suggested that inclusion of multiple coatings of polyelectrolytes onto liposomes can enhance cytotoxicity of paclitaxel against cervical cancer cells. It was concluded that the chitosan surface modified liposomes had more cell affinity thus improving cellular uptake of paclitaxel and exhibiting more cytotoxicity.⁴⁵

Another phytoconstituent baicalein (BAI), a flavone compound has been reported to exhibit anticancer effect by inhibition of PI3K/Akt and its downstream proteins, up-regulation of tumor suppressor p38 and p53 and increased oxidative stress in the cells. It has shown anticancer activity against U14 cervical carcinoma cells and HeLa cells. However, its low bioavailability and poor absorptivity, limits its clinical application. In order overcome this limitation, researchers proposed that by encapsulating BAI into liposomes carrier, enhanced absorption of active moiety can be achieved. Li et al. formulated nanoliposomes of BAI by thin film hydration method using Soyabean phosphatidylcholine and cholesterol lipids. The average size of BAI liposomes was 194.6 ± 2.08 nm (PDI 0.17 ± 0.025) with zeta potential value of -30.73 ± 0.41 mV with the entrapment efficiency of $44.3 \pm 2.98\%$. An *in vitro* release study showed an initial burst release followed by slower release up to 65% at the end of 48 hours from BAI-LP, greater compared with 92% release of free BAI, indicating that the liposome formulation provided a drug depot effect. Cytotoxicity studies were conducted on HeLa cell lines. Placebo liposomes showed no effect on the cells. However, cells treated with BAI-LP showed higher rate of cell inhibition rate compared with non-encapsulated BAI. Tumor inhibition rate was conducted on Kunming mice model by inoculating U14 cervical cancer cells in the armpits. It was observed that BAI-LP treated group had improved inhibition rate when compared with the un-encapsulated BAI-treated group. Also, it was observed that BAI liposome could also resist rapid clearance because of their size being larger than spaces in endothelial junctions of normal vessels. The study concluded that baicalein nanoliposomes may serve as a promising approach for cancer therapy.⁴⁶

In another study, Xu et al. attempted co-delivery of resveratrol along with p53 tumor suppressor gene using a novel peptide-cationic lipid (CDO14) for better cancer inhibitory effect. Resveratrol is a lipophilic phytoconstituent and it induces apoptosis to inhibit tumor promoter-induced cell transformation through increased transactivation of p53 activity. Liposomes were prepared by thin-film and ultrasonic dispersing method using various weight ratios of CDO14 lipid and resveratrol (5/1, 10/1, 20/1, 100/1, and 200/1). The particle size increased from 65.9 nm to 220.7 nm for change in ratio from 200/1 to 5/1. The study indicated that particle size of the liposome had direct relationship with relative amount of resveratrol. The zeta potential values of CDOR5-CDOR200 were

between 81.4 mV and 109.8 mV which ensured 3 months stability. The encapsulation efficiencies of CDOR5, CDOR10, CDOR20, CDOR100, and CDOR200 were found to be 94.32%, 91.74%, 84.17%, 71.35%, and 65.13%, respectively. MTT assay for cytotoxicity on HeLa cells showed that cells treated with CDOR20/p53 lipoplexes exhibited stronger tumor growth inhibition effect than that of resveratrol alone thus illustrating the synergism between p53 and resveratrol. Apoptosis studies showed greater blue fluorescence with resveratrol liposomes compared with blank liposomes which suggested the role of resveratrol in apoptosis induction. The study concluded that co-delivery of genetic material and phytoconstituent resveratrol has potential in improving delivery of therapeutic agents to target cell.⁴⁷

From all the studies conducted by research groups mentioned in the preceding paragraphs, we can contemplate that Liposomes prove to be efficient delivery system for herbal drugs owing to their phospholipid bilayer mimicking the biological membranes. However, the large-scale production cost, sterilization complexity, batch to batch variability limits its use. Long term storage of liposomes is limited since the particle size and zeta potential of liposomes increases leading to agglomeration and instability.⁴³ Freeze drying with lyoprotectants or cryoprotectants can be useful to improve the stability but it adds to production costs.

B. Micelles

Polymeric micelles, in general, have several advantages over other colloidal drug delivery systems, such as they have a smaller size compared with liposomes, which is important for percutaneous lymphatic delivery or extravasation from blood vessels into the tumor tissue. Micelles are colloidal systems consisting of surfactants (surface active agents) or amphiphiles with sizes in the range of 5-100 nm. They comprise of both hydrophilic head group and hydrophobic tail.⁴⁸ The amphiphiles exists as monomer units at low concentrations in aqueous medium. As the concentration increases, amphiphiles aggregate and self-assemble to form micelles. The concentration at which micelles are formed is termed as critical micelle concentration (CMC). Polymeric micelles are based on use of on block-copolymers having both hydrophobic and hydrophilic units that have the tendency to self-assemble in aqueous environment.^{49,50}

Most of the anticancer drugs are hydrophobic in nature. Therefore, it becomes difficult to incorporate them in soluble form into delivery systems. Because of this, the anti-cancer agents exhibit reduced absorption after administration. To solubilize this problem, hydrophobic drugs can be solubilized in micellar core.

To achieve a better therapeutic effect of curcumin in cervical cancer, Wang et al. formulated mixed polymeric micelles of curcumin using d- α -tocopheryl polyethylene glycol succinate (TPGS), Pluronic F127 and P123 by a thin-film hydration method. The apoptosis-inducing effect of free curcumin and curcumin mixed micelles was evaluated by Annexin V-APC/PI apoptosis detection kit. It was observed that the curcumin mixed micelles showed a stronger apoptotic effect on HeLa cells than the free curcumin. MTT assay was used to determine the *in vitro* cytotoxicity of free curcumin and curcumin

micelles. Free curcumin did not show very significant cytotoxicity against HeLa cells until the treated concentration reached 8 µg/mL. However, the curcumin micelles exhibited obvious cytotoxicity even at the low concentration of 2 µg/mL. After treatment with free curcumin or curcumin micelles at the high concentration of 8 µg/mL, cell viability was found to be 50% or 60%, respectively. It was demonstrated that the novel curcumin micelles enhanced the selective cytotoxic effect of the encapsulated drug, curcumin, on HeLa cells, especially at very low concentrations. The polymeric mixed micelles had the significant cervical tumor growth inhibition in the HeLa tumor xenograft mice. The tumor volumes from mice treated with curcumin micelles or free curcumin were reduced to 51.17% or 78.6%, respectively, of that in the negative control group. The studies confirmed that there is selective upregulation of apoptosis in HeLa cells through the mitochondria-mediated signalling pathway.⁵¹

Kumari et al. prepared curcumin-loaded methoxy-poly (ethylene glycol)-poly (D,L-Lactide) micelles conjugated with transferrin (Tf-PPC) via thin film hydration technique. To assess penetration efficiency, *in vitro* cytotoxicity and growth inhibition these micelles were tested in three-dimensional HeLa spheroids. Free curcumin demonstrated cell viability of $46.27 \pm 2.04\%$ and Tf-PPC showed cell viability of $30.60 \pm 1.97\%$. It was found that transferrin conjugated micelles accumulated more in spheroids, thereby resulting in higher cytotoxicity compared with free curcumin. Annexin V/PI fluorescence labeling kit was used to assay apoptosis. Spheroids treated with Tf-PPC illustrated 9.72% and 34.3% of early apoptotic and late apoptotic populations, respectively. The value was 10.8% for free curcumin treatment following 24 hours of treatment. In the study conducted for 48 hours it was observed that for early apoptotic population apoptosis was 9.5% for Tf-PPC, whereas it was 8.8% for free curcumin. It was similarly observed that apoptosis in late apoptotic population was 40.1% for Tf-PPC, whereas it was 30.3% for free curcumin. Thus, the data clearly indicated that delivery of curcumin by transferrin conjugated micelles resulted in enhanced apoptotic activity compared with free curcumin. Flow cytometry was used to study the uptake of Tf-PPC by HeLa spheroids. Free curcumin showed low intensity of fluorescence compared with fluorescence intensity of Tf-PPC micelles. The enhanced uptake of Tf-PPC by spheroidal cells compared with free curcumin indicated its targeting potential, which resulted in deep tissue penetration.⁵²

Sajomsang et al. synthesized novel pH-responsive amphiphilic N-benzyl-N, O-succinyl CS (BSCS) micelles using dialysis method. The release study of curcumin from the micelles was conducted in various media such as extracellular tumor cells (phosphate buffer, pH 5.5), simulated intestinal fluid (SIF, pH 6.8), simulated gastric fluid (SGF, pH 1.2) and, and normal blood (phosphate buffer, pH 7.4). The results obtained were compared with that of free curcumin. At low pH, it was observed that 30% curcumin released from micelles at the end of 24 hours while free curcumin released only 23% after 24 hours. At pH 5.5, 6.8, 7.4, the release rate of curcumin from micelles increased greatly with no burst release. Curcumin release rates from micelles were 38% at pH 5.5 and 50% release at both pH 6.8 and 7.4 within 10 hours, whereas about 70% of curcumin was released at pH 5.5, 6.8, and 7.4 after 24 hours, indicating that the release

behavior of curcumin from micelles is dependent upon pH. To investigate the cell uptake capacity of free curcumin and BSCS micelles, micelles were labeled with FITC. Encapsulated curcumin displayed a much higher anti-cervical activity against all cervical cancer cell lines. The IC₅₀ values of BSCS micelles were found to be lower than that of free curcumin. It was nearly 4.7-, 3.6-, and 12.2-fold lower for HeLa, SiHa and C33a cells, respectively. The study concluded that the curcumin encapsulated micelles showed high water solubility, and high apoptosis inducing capacity efficient cellular uptake, with strong cytotoxicity to cervical cancer SiHa, HeLa, and C33a cells in comparison to free curcumin.⁵³ Polymeric micelles thus can be used to increase the solubility of lipophilic herbal drugs⁵⁴ with tunable chemical and physical properties but their low drug loading capacity and dependency of critical micellar concentration, concentration of surfactant and related toxicity confines its utility on clinical front.

C. Nanoparticles

Nanoparticles are solid colloidal particles in the size range of 10–100 nm. The drugs can be dissolved, entrapped, encapsulated or coated on the surface of nanoparticles. It is also possible to use nanoparticles for loading molecules like DNA, RNA, and antibodies and make them target specific. Their small size enables better cellular and tissue targeting, improved oral bioavailability, protection from enzyme induced degradation, sustained delivery and better solubilization for intravascular delivery. A wide range of natural or synthetic polymers can be used to fabricate nanoparticles for better tumor targeting ability.⁵⁵

Naringenin (NAR) is a naturally occurring flavanone found in citrus fruits. Naringenin has exhibited many pharmacological activities such as anti-mutagenic, anti-atherogenic, anti-inflammatory, hepatoprotective, etc. It has been investigated to show cytotoxicity and induce apoptosis by both intrinsic and extrinsic pathways in various human cancer cell lines. Krishnakumar et al. developed polymeric nanoparticles of naringenin with nanoprecipitation technique. Eudragit E (EE) and polyvinyl alcohol (PVA) were used as carriers. The *in vitro* studies showed a very rapid initial burst release which was followed by slow release. The initial burst release could be attributed to the release of NAR adsorbed on surface of nanoparticles and slow release due to NAR embedded inside the matrix of nanoparticles. Nanoparticles showed controlled release with 72% drug released at the end of 24 hours. MTT assay was conducted on free NAR and NAR nanoparticles for evaluating their cell viability on HeLa cell lines. It was observed that free NAR had no significant effect on cell viability while NAR nanoparticles exhibited high cytotoxicity. As the concentration of NAR in nanoparticles increased, cytotoxicity also increased. It was suggested that nanoparticles help in better binding and internalization of drug thus enhancing cytotoxicity. Nanoparticles could escape endolysosomes and enter cytoplasm and remain in the cytoplasmic compartment to release the encapsulated drug in a sustained manner. Thus it was concluded that nanoparticle could provide enhanced therapeutic efficiency compared with free drug and dose dependent cytotoxicity by altering mitochondrial membrane potential, increased ROS production, apoptotic morphological changes and reducing glutathione levels intracellularly.⁵⁶

It has been reported that oral bioavailability of curcumin can be increased up to 9-fold by encapsulating it within nanoparticles. Polymers like PLGA has been used extensively in the development of nanoparticles for anticancer drug delivery.⁵⁷⁻⁶⁰ Nair et al. suggested that PLGA nanoparticles of curcumin could provide better anti-cervical cancer activity compared with free curcumin. PLGA nanoparticles of curcumin were prepared by solvent evaporation technique. Two PLGA combinations, 50:50 and 75:25 (different lactide to glycolide ratios) were used to encapsulate the phytoconstituent. The release studies showed an initial burst release followed by sustained release for over a week. It was observed that PLGA 50:50 nanoparticles showed a release of 62% curcumin and PLGA 75:25 nanoparticles showed a release of 48% at the end of 24 hours. At the end of a week study it was seen that 85% of curcumin released from 75:25 nanoparticles in contrast to 94% curcumin from 50:50 nanoparticles. Enhanced intracellular fluorescence compared with free curcumin was seen during cellular uptake studies in HeLa cell lines. MTT assay cells proved that curcumin nanoparticles using PLGA 50:50 had better antitumor activity. The results indicated that the nanoparticles enhanced aqueous solubility thus increasing the anticancer efficacy of curcumin.⁶¹

Danhier et al. developed nanoparticles of PTX without using Cremophor to avoid the side effects associated with its use and also to improve the therapeutic index of the drug. PLGA was used as the polymer and the nanoparticles were PEGylated to obtain better target ability. Paclitaxel loaded PEGylated PLGA nanoparticles were formulated using simple emulsion and nanoprecipitation technique. It was observed that encapsulation efficiency was higher in nanoparticles made using nanoprecipitation technique. Release studies showed initial burst release because of drug on the surface of nanoparticles followed by sustained release because of drug embedded inside. MTT assay was conducted to assess the *in vitro* anti-tumoral activity against HeLa and the results were compared with marketed formulation Taxol and Cremophor EL. Taxol and PTX-nanoparticles both induced same percentage of apoptosis in HeLa cell lines. With 25 µg/mL of paclitaxel, the cell viability was lower for PTX-loaded nanoparticles than for Taxol (IC₅₀ 5.5 vs. 15.5 µg/mL). Flow cytometry studies suggested that uptake of PTX nanoparticles was time and concentration dependent. Also, PTX nanoparticles illustrated greater tumor growth inhibition effect *in vivo* compared with Taxol. Therefore, PTX-nanoparticles may be considered as an efficient anticancer drug delivery system for cervical cancer chemotherapy.⁶²

Kim et al. developed paclitaxel-loaded nanoparticles made using biodegradable poly (ethylene oxide) and poly (ϵ -caprolactone) (PEG/PCL) amphiphilic block copolymers conjugated to biotin ligands for better targeting to cervical cancer cells. *In vitro* release study showed sustained release of paclitaxel from biotin-conjugated PEG/PCL nanoparticles with no initial burst release. Biotin conjugated nanoparticles showed much higher cytotoxicity for cancer cells than the nanoparticles devoid of biotin group. These results revealed that the biotin conjugation could improve the selective delivery of paclitaxel into cancer cells. This is attributed to presence of overexpression of biotin receptors on the cancer cell surface. Similarly, Gu et al. prepared paclitaxel loaded nanoparticles. The IC₅₀ of PTX nanoparticles and PTX for HeLa cells were found to be 5.0 ± 0.3 and 8.0 ±

0.3 ng/mL at 48 hours and 2.0 ± 0.1 and 6.5 ± 0.3 ng/mL at 72 hours respectively. Thus, PTX-loaded nanoparticles were found to be an efficient carrier for site specific delivery.

Quercetin is a flavanoid found in apples, red onions, berries, citrus fruits, red wine, green tea, etc. Quercetin exhibits potent anti-oxidant, anti-obesity, anti-inflammatory, and anti-cancer properties. The poor water solubility of quercetin however limits its clinical use. In order to study the effect of quercetin on cervical cancer progression, Luo et al. formulated gold nanoparticles of quercetin targeting Janus kinase 2 (JAK2), a non-receptor tyrosine kinase which is over expressed cervical cancer cells. Quercetin inactivates JAK2 and prevents cervical cancer progression by inducing apoptosis, inhibiting proliferation and autophagy. Quercetin nanoparticles down regulated JAK2 expression in both CaSki and HeLa cell lines. The study indicated that quercetin gold nanoparticles could suppress cervical cancer progression by STATs-regulated Bcl-2/Caspase-3 signaling pathway and PI3K/AKT-related GSK and mTOR pathways.⁶³

Silver nanoparticles (AgNPs) are one of the most promising forms of nanoparticles displaying unique and effect anti-tumor effect on various cancerous cell lines. Jeyaraj et al. synthesized silver nanoparticles of *Podophyllum hexandrum*. *P. hexandrum* reports presence of polyphenols, lignans and nitrate reductase. AgNPs of podophyllum were evaluated for anti-cancer activity against HeLa cell lines. MTT assay was used to monitor cytotoxicity. With 50 microg/mL concentration of AgNP, 100% cell death was observed. The data suggested that AgNPs induced cell death in HeLa cells by increasing the production of reactive oxygen species. ROS disrupts mitochondrial membrane potential thus increasing apoptotic morphological changes in the AgNPs treated cells.⁶⁴ Furthermore, crystalline gold nanoparticles of *P. hexandrum* were also found to exhibit an effective anticancer activity by inducing oxidative stress, cell cycle arrest, oxidative stress and activation of caspase cascade, leading to apoptosis of cancer cells.⁶⁵

Pentacyclic triterpenoids such as Ursolic acid shows number of pharmacological activities such as antifungal, antibacterial, anti-inflammatory, anti-atherosclerotic, hepatoprotective, anti-mutagenic, anti-oxidative, antiangiogenic, etc. Ursolic acid has been known to induce apoptosis response in various human cell lines including cervical cancer. Wang et al. developed nanoparticles loaded with Ursolic acid to provide a controlled and sustained release of Ursolic acid and enhance its therapeutic efficacy. Poly lactic co-glycolic acid copolymer NPs are biodegradable and biocompatible in nature thus eliminating carrier related toxicity. The spherical Ursolic acid loaded NPs showed diameter of 80 nm. SEM studies revealed that nanoparticles displayed spherical surface without cracks or pinholes. Cell viability studies showed that Ursolic acid nanoparticles decreased the viability of cancer cells in a time-dependent manner and dose-dependent manner over 40 μ M without cytotoxicity to normal cells, which suggested that NPs were specific for cancer cells. Apoptosis assay showed that ursolic acid nanoparticle treatment significantly accelerated apoptosis in cervical cancer cell lines, especially at the highest concentration of 100 μ M compared with the control groups. Western blot techniques revealed that ursolic acid NPs induced apoptosis through caspase dependent pathways in both SiHa and HeLa cell lines. Ursolic acid nanoparticles also suppressed cervical cancer growth and progression in a mouse xenograft model. The study accomplished that

ursolic acid nanoparticles targeted caspases and p53, caused downregulation of BCL-2 and cIAP, induced apoptosis in cervical cancer cell line and thus can be used a potential treatment for cervical cancer.⁶⁶

Genistein, a flavonoid, possesses anticancer activity by inhibiting cell growth by modulating MAPK and PK-B/AKT pathway in cancer cells. However, its poor aqueous solubility limits its clinical use. In order to enhance its solubility, Zhang et al. fabricated genistein-incorporated biodegradable TPGS-*b*-PCL (poly(ϵ -caprolactone)co-polymerized with d- α -tocopheryl polyethylene glycol 1000 succinate) nanoparticles by a modified nanoprecipitation method. Genistein-incorporated TPGS-*b*-PCL NPs showed a size of 181.83 nm and zeta potential of -14.70 mV, which indicated good stability. Encapsulation efficiency and drug loading capacity was about 95.56% and 8.69% respectively. TPGS-*b*-PCL NPs showed an initial burst release of 14.58% and after 15 days a cumulative release of 58.94%. *In vitro* cellular uptake studies on HeLa cell line at the concentrations of 100, 250, and 500 μ g/mL showed that the TPGS-*b*-PCL NPs exhibited 1.2- to 1.3-fold higher cellular uptake efficiency than PCL NPs. *In vitro* cytotoxicity studies on HeLa cell line showed that IC₅₀ values for genistein-loaded TPGS-*b*-PCL nanoparticles was lower compared with cells treated with unencapsulated drug after treatment for 24, 48, and 72 hours suggesting better cytotoxicity by NPs. The study concluded that genistein-loaded TPGS-*b*-PCL NPs are an excellent low-toxic aqueous formulation of genistein with improved anticancer activity, which may have potential application in cervical cancer therapy.⁶⁷

Nanoparticles offer controlled and sustained drug release with the ability to use biodegradable and biocompatible polymers to avoid toxicity and also incorporate both hydrophilic and hydrophobic drugs. The surface of nanoparticles can be coated with ligands or PEGylated to make them defensive against reticuloendothelial system.

D. Nanoemulsions

Nanoemulsions are transparent, optically isotropic colloidal dispersions composed of lipids, surfactant and co-surfactant designed to incorporate both hydrophilic as well as lipophilic drugs used for cervical cancer.⁶⁸ The drug encapsulated within the nanoemulsion is protected from degradation. An increased plasma half-life, large surface area, specific targeting, superficial charge, imaging capacity of formulation are the characteristics that make nanoemulsions an efficient therapeutic drug delivery system. Cancer cells are highly vascularized; hence nanoemulsions can easily accumulate in tumor microenvironment owing to their size and their ability to cross the barriers. Also, nanoemulsions can be conjugated with ligands, antibodies or their fragments for better targetability. Studies have shown that this conjugation helps to internalize drug-loaded nanoemulsions more successfully inside cancer cells.⁶⁹

Curcumin inhibits the expression of COX-2, VEGF, and EGFR in CaSKi cells thus exhibiting antiangiogenesis and antitumor activity. de Matos et al. formulated curcumin nanoemulsions by interfacial prepolymer deposition and spontaneous nanoemulsification method using medium-chain-triglycerides and natural soy phospholipid and

poloxamer 188 as anionic surfactant. MTT assay was used to determine curcumin cytotoxicity against HaCaT, SiHa, and CaSKi cells. After 24 hours of incubation, curcumin nanoemulsion showed cell viability of 7.8% for CaSKi cells, 8.6% for SiHa cells and 16.5% for HaCaT cells. Fluorescence microscopy was used to perform cellular uptake studies which showed that cells were able to internalize the curcumin loaded nanoemulsion with better localization at cytoplasm as observed in intracellular environment of SiHa cells (36 hours after incubation) and CaSKi and HaCaT cells (48 hours after incubation). The study concluded that curcumin nanoemulsion helped to increase curcumin concentration at the target site and protecting curcumin against degradation.⁷⁰

Camptothecin is a potent anticancer agent active against a broad spectrum of cancers. Camptothecin binds to topoisomerase I enzyme and DNA resulting in a ternary complex that prevents DNA religation and leads to DNA damage resulting in cell death. However, its clinical application is limited because of its insolubility, instability and toxicity. Also, the derivatives of Camptothecin could not surpass camptothecin in potency of action. Therefore, in order to improve solubility, targeting ability and prolong its duration of action, Fang et al. suggested developing an acoustically active nanoemulsion composed of perfluorocarbon and oil as camptothecin-encapsulated carriers. The camptothecin loaded nanoemulsions showed cytotoxicity of 60% against cervical cancer cells with encapsulation of > 90% camptothecin.⁷¹ Nanoemulsion helps to solubilize lipophilic drugs, and also ensures rapid and efficient penetration of drugs however use of surfactant and cosurfactant needed to stabilize nanoemulsion if higher, can be toxic to normal cells.

E. Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) are submicron-sized lipid-based particles with a diameter between 50 and 1000 nm. They consist of solid lipid matrix, surfactants and co-surfactants. They are less toxic and more biocompatible compared with inorganic or polymeric nanoparticles. Also because of their small size, they offer large surface area and better cellular uptake. The drug release is prolonged since the drug has to permeate through solid matrix of nanocarriers. The SLNs fabricated using biodegradable excipients are able to incorporate both hydrophilic and lipophilic drug, thus serving as a viable option for controlled and targeted drug delivery.^{72,73}

There is a profound need to develop localized and targeted paclitaxel incorporating delivery systems in order to reduce its toxicity and side effects associated with the concomitant use of Cremophor EL for intravenous (IV) infusion. Büyükköroğlu et al. synthesized SLNs of paclitaxel to be incorporated with siRNAs in vaginal suppositories against cervical cancer. HeLa (human cervical cancer adenocarcinoma) cell line was used for cell culture studies. Paclitaxel from siSLN showed a gradual release of nearly 100% at the end of 45 hrs. MTT assay showed that PTX-incorporated siSLNs showed less cell viability at all concentrations (0.5–3 µL/100 mL) compared with free paclitaxel or placebo SLN. Free paclitaxel showed cell viability of more than 90% while PTX-loaded siSLNs showed cell viability of 40%. This showed that paclitaxel siSLNs are more cytotoxic to cervical cancer cells *in vitro*.⁷⁴

In another study, Liu et al. attempted co-delivery of paclitaxel and TOS-Cisplatin via trans-activating transcriptional activator (TAT)-targeted SLNs with synergistic anti-tumor activity against cervical cancer cells by formulating SLNs. TOS is a succinic acid ester of α -tocopherol, and has been found to possess antiproliferative activity against cervical cancer cells, breast cancer cells, prostate cancer cells, gastric cancer cells, etc. TAT is a cationic classical CPP and enhances cellular uptake of various moieties such as drugs, genes, peptides, etc. SLNs of TAT PTX/TOS-CDDP were synthesized using emulsification and solvent evaporation method using glyceryl monostearate and Tween 80 as stabilizer. The paclitaxel drug loading was between 3.5% and 5.9% which indicated that PTX were successfully loaded into the SLNs. Plasma stability studies indicated that over 24 hours 70 percent of drug was still encapsulated in SLNs. The drug release was sustained with complete release occurring at 96 hours. Also, drug release at acidic pH (pH 5) was faster than at neutral conditions (pH 7.4). Cytotoxicity studies were used to study synergistic effect using Chou and Talalay's method on HeLa cell lines. It showed CI_{50} value of TAT PTX/TOS-CDDP SLNs was found to be 0.646 and that of PTX/TOS-CDDP SLNs was 0.861 which suggested a significant synergism effect exhibited by TAT. The study concluded that the administration of TAT PTX/TOS-CDDP SLNs can lead to a dramatic increase of drug accumulation in the tumor tissue, as compared with the free drug solutions with the fact that solid tumors have leaky vasculature and nano-sized particles could passively target the tumor cells.⁷⁵

These studies imply that SLNs have emerged as promising drug delivery system for cancer cell targeting. They are well tolerated by the *in vivo* systems since they are aqueous dispersions of solid lipids.⁷¹ SLNs provide better control over rate of drug delivery and also protect the drug inside solid lipid core.

F. Hydrogels and Nanogels

Hydrogels are three-dimensional semi-solid gel network systems comprising of hydrophilic polymers dispersed into aqueous environment in form of a mesh entrapping drug moiety in the cross-linked network. They have a tendency to swell so that the drug releases through the expanded polymeric mesh. The drug release can be modulated by controlling the degree of cross linking and porosity of the gel matrix. Hydrogels also possess physical properties like better permeability, biocompatibility, mechanical resistance which can be layered with structural modifications. Nanogels are three-dimensional hydrogel materials in the nanoscale size range (20–200 nm)⁷⁶ formed by cross linked swellable polymer networks with high capacity to hold water, without actually dissolving into aqueous medium. Nanogels, are also called macromolecular micelles, have gained attention due to several advantages, such as low toxicity, high stability and small size, which may allow passive targeting of solid tumor tissues by enhanced permeation and retention (EPR) effect.

To achieve better passive targeting ability of paclitaxel, Perez et al. formulated a pH and glutathione (GSH) responsive, PTX-loaded nanohydrogel by microemulsion polymerization method. Poly-N-isopropylacrylamide (NIPA), N-hydroxyethyl acrylamide

(HEAA), and tert-butyl 2-acrylamidoethyl carbamate (2AAECM) were used as monomers and N,N'-cystamine bisacrylamide (CBA) as cross-linker. By varying the number of monomers NIPA/HEAA/2AAECM in two different ratios of 80:15:5 and 80:10:10, NHA 80/15/5-CBA and NHA 80/10/10-CBA were the two different nanohydrogel formulated. NHA 80/10/10-CBA nanogels showed loading capacity of 42.3 ± 0.6 µg/mg and encapsulation efficiency of 84.9%. NHA80/15/5-CBA displayed a loading capacity of 33.6 ± 1.3 µg/mg and encapsulation efficiency of 67.8%. 64.0% of PTX was released from NHA 80/10/10-CBA and 58.8% drug was released from NHA 80/15/5-CBA at the end of 50 hours. Cell viability studies were performed on unloaded nanogels, pure paclitaxel and paclitaxel loaded nanogels using HeLa cell lines. Unloaded-NHA 80/10/10-CBA had cell viability of 70% and PTX-loaded nanogels had cell viability of 25%. Similarly, unloaded-NHA 80/15/5-CBA had cell viability of 80% and PTX-loaded nanogels had cell viability of 20%. It was found that PTX-nanogels were much cytotoxic toward HeLa cells compared with pure PTX. This suggested that nanogel formulation had a synergistic anti-tumor activity. PTX effects were enhanced after its encapsulation since drug was more stable and protected against enzyme degradation. The results of the study suggested the use of nanogels as a novel nanocarrier of anticancer agents.⁷⁷

Hydrophobic drug delivery with hydrogel is not very commonly reported because of incompatibility of hydrophobic drug with hydrophilic polymer network. In order to overcome this limitation, semi-interpenetrating networks can be prepared to tune the hydrophilicity of hydrogel. Site specific delivery can be achieved by developing a pH-sensitive and biocompatible hydrogel.

Deepa et al. prepared curcumin-entrapped PEG cross-linked acrylic hydrogel by inverse emulsion polymerization technique. Two hydrogels were prepared with varying levels of cross-linking (0.5% and 1%). The entrapment efficiency of 0.5% cross-linked hydrogel was found to be 71.6% and that of 1% cross-linked hydrogel was found to be 67.5%. Cytotoxicity studies on HeLa cell lines revealed that 0.5% cross-linked curcumin hydrogel showed cell viability of 40% and 1% cross-linked hydrogel showed cell viability of 35% compared with blank showing 90% cell viability. This exhibited that curcumin loaded hydrogels showed more cytotoxic effect towards HeLa cell lines compared with free curcumin. As the degree of cross-linking increased, the gel showed a more sustained release of curcumin *in vitro*. Hydrogels with 0.5% cross-linking showed more rapid release compared with hydrogels with 1% cross-linking. The study concluded that the developed gel system was found to be favorable for pH-sensitive controlled delivery of curcumin.⁷⁸

Hydrogels are known to have excellent biocompatibility and biodegradability and lower toxicity than nanoparticle carriers. They also provide *in situ* gelation and controlled release which greatly enhances the efficiency and convenience of drug delivery. Smart hydrogels can be formulated which release drug in response to stimuli in the environment, e.g., heat, pH, light, and ultrasound.⁷⁹ Similarly nanogels have high drug loading capacity and show better permeation due to smaller size. Their drug release profile can also be modulated by thermosensitive, pH responsive, photochemical internalization, and photoisomerization mechanism.

VI. CONCLUSION AND FUTURE PROSPECTS

Cervical cancer has been reported to be fourth most common type of cancer prevalent in women with HPV 16 and HPV 18 infections being major causative factor. The current treatment modalities include chemotherapy, surgery, radiation therapy, and targeted therapy. However, these therapies lack specificity and ability to differentiate between normal and cancerous cells. Also, conventional therapy is associated with development of drug resistance. To overcome these limitations researchers have investigated the potential of herbal drugs as an alternative approach to chemotherapeutic agents. Herbal drugs are associated being comparatively less toxic and exhibits more target-oriented activity. The poor solubility of herbal drugs however hinders the delivery of herbal phytoconstituents at optimum concentration to the target tissues. To enhance the solubility profile of such drugs, newer delivery approaches are studied. The design of nanocarriers for delivery of herbal drugs has the potential to overcome this limitation. Nanocarriers, owing to their size range can evade the barriers and reach the tumor microenvironment to provide better distribution of drugs. nanocarriers also improve the stability and retention of drug at the target site for better therapeutic activity. Liposomes, micelles, nanoemulsions, SLNs, nanoparticles, hydrogels and nanogels are some nanocarrier delivery systems that have been exploited for delivery of herbal drugs to cervical cancer tissues. These approaches can be used in conjunction with other therapies such as gene therapy, immunotherapy, and photothermal radiation for effective eradication of cervical cancer. The herbal nanocarriers however have the potential to be exploited for use at clinical phases.

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