Current Research & Reviews on Cervical Cancer

Chapter 1

Therapeutic action of curcumin loaded chitosan nanoparticles for cervical cancer

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Abstract

Cervical cancer is the second leading cancer in female globally and predominant etiological agent for cervical cancer is human papillomaviruses (HPVs). There is various conventional treatment strategies for the cervical cancer, such as surgery, anticancer drugs and chemoradiotherapy have been improved over a last few decades. However, they remain far from optimal treatment. Recently, cancer research is targeted on improving cancer diagnosis and nanotechnology based treatment methods, which engages the design, preparation, characterization and application on nanoscale drug carrier systems. In medicine, nanotechnologies, such as polymeric nanoparticles, solid lipid nanoparticles, nanostructured lipid (lipid nanaoprticles) carriers, gold & silver nanoparticles, hydrogels, cyclodextrin complexes, and liquid crystals are emerging tools for diagnostic probes and therapeutic devices. The aim of this review is to present a systematic knowledge of nanotechnology-based drug delivery systems for cervical cancer.

Keywords: curcumin; chitosan; nanoparticles HPV; cervical cancer

1. Introduction

Cervical cancer is one of the major health care problems worldwide in women. It is the second leading cause of cancer death in female in developing countries, although cytological screening programs have substantially reduced its toll in developed countries [1]. There are estimated approximately 493,000 new cases and 274,000 deaths globally due to cervical cancer in year 2002 but more than 80 % cases are obtaining in developing countries [2]. However, in

the United States, it is estimated that the occurrence of new infections ranges from 1 million to 5.5 million annually and the incidence is estimated to be much high as 20 million [3]. In India, the annual prevalence of cervical cancer is about 130,000 new cases and death 75-80,000 [4]. Thus, India shares globally one fourth burden of cervical cancer. The incidence of this disease due to involvement of various risk factors like, early age of marriage, multiple sexual partners, multiple pregnancies, poor genital hygiene, smoking, use of oral contraceptives and multiparity [5]. But the major cause of such malignancy due to the infection of human papilloma viruses (HPVs). Several studies have demonstrated that more than 99 % of cervical cancer worldwide is due to occurrence of HPV and persistent infection of oncogenic HPV types. Moreover, there are other factors such as, host cellular and genetic factors, immunodeficiency, HPV variants, viral load and viral integration are also considered to be help in the initiation and progression of cervical cancer [6].

It is well known that the initial causing agent of cervical cancer is human papillomaviruses [7]. It is reported that more than 99% of cervical cancers and greater than 90% of their precursor lesions, squamous intra-epithelial lesions (SIL) (also called cervical intraepithelial neoplasia [CIN]), contain HPV DNA [7]. Currently, more than 200 genotypes of HPV have been identified. HPVs can also be differentiated into two major grouped, one high-risk and other low-risk HPV types. A small number of genotypes are severely associated with cancer, named HPV16 and HPV18 [8]. Other High-risk HPV types include types 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70. These HPV types are very less participant in cancers but are often present in squamous intraepithelial lesions (SILs), the precursor lesions of cervical cancer called as low risk HPV types [9]. Low-risk 6, 11, 42, 43, and 44 subtypes are also occasionally present in cervical carcinomas.

HPVs are members of the *Papovaviridae* family. It is an icosahedral non-enveloped with double-stranded circular DNA genome viruses approximately 7.9kbp associated with histones proteins (Figure1). The HPV DNA codes two major categories of genes; one early gene and other late gene. The early gene products regulate viral DNA replication (E1, E2), viral RNA transcription (E2), cytoskeleton reorganization (E4) and cell transformation (E5, E6, E7), whereas the late gene products (L1, L2) proteins are structural components of the viral capsid. Expression of the viral proteins are strongly regulated and linked with the differentiation of infected basal epithelial cells. E2 is the key regulator that controls the expression of all the other viral genes and is particularly involved in the regression of E6 and E7. The E6 and E7 viral oncogenes are responsible for the transformation. During progression, the HPV genome integrates into the host chromosomal DNA, leading to the disruption of the E2 viral gene and an inability to express the late genes associated with high grade disease. Therefore, E2 is a transcriptional repressor of E6 and E7 gene and loss of E2 expression leads to upregulation of E6 and E7 genes. The over expression of E6 and E7 proteins results in the arrest of cell cycle

regulation and leads to genomic instability, thereby contributing to the progression of HPV-associated cervical cancer. Notably, their expression is necessary to maintain the transformed phenotype [10].

Pathogenesis of HPV infection may be occurred at basal cells of stratified squamous epithelium and other host cell likely to be resistant because replication is associated with the differentiation of keratinocytes and difficulty in regeneration of stratified structure of the epithelium *in vitro*. The cell cycle of HPV replication starts with the entrance of the virus into the host cells of basal layer (stratum germinativum) of the epithelium [11]. HPV replication initiates with the factor of host cell which interact with the LCR region of the HPV genome and start transcription of the viral oncogene E6 and E7. The E6 and E7 oncogene products deactivate the cell cycle of host cell by binding and suppressing the tumor suppressor proteins such as cell cyclins, and cyclin dependent kinases (Figure 2). Cell growth is controlled mainly by two cellular proteins, the tumor suppressor protein (p53) and the retinoblastoma protein (pRB). In the differentiated keratinocytes, the supra-basal layers of the host cell epithelium, the virus switches to a rolling-circle mode of DNA replication and amplification of itself DNA to high viral load, synthesize capsid proteins and lead to assemble in new virus form [12].

Most of the HPV-infected cervical cell changes are transient and more than 90% regress spontaneously by host immune system to eliminate the virus [13]. The first line of immune defense against HPV infection is a cell-mediated and humeral response induced at local lymph nodes. Early invasive cervical cancers are managed with radical hysterectomy or external-beam high-energy radiotherapy and implants loaded with radioactive element iridium, Ir192. The aim of this therapy is to destroy/kill malignant cells in the cervix, para-cervical tissues and regional lymph nodes. In additional by the surgical and cytodestructive procedures, several antiviral and immunomodulatory agents have been implicated in the treatment of HPV-associated cervical lesions.

Vaccination might be another important implemented in the form of preventive vaccines, which generate neutralizing antibodies to block/inhibit HPV viral infection or in the form of therapeutic vaccines, which eliminate infection by elicit virus specific T cell-mediated immune response. Now a day, in market the newly licensed by US Food and Drug Administration, preventive HPV vaccine, Gardasil® (Merck and Co, Inc.) and Cervarix™ (GlaxoSmith-Kline). Gardasil and Cervarix both are L1 VLP based prophylactic vaccine against HPV types 16 and 18 because these two major serotypes that are engaged with cervical cancer and both are remarkable safety profile and clinical efficacy against the HPV genotypes.

In current therapies strategies, some anticancer drugs have been used alone or in combination for the treatment of cervical cancer, such as cispla¬tin, cetuximab, fluorouracil, paclitaxel, docetaxel (DTX), and methotrexate [14,15]. Furthermore, another way for oral ad-

ministration facilitates may be prolonged exposure to a cytotoxic agent [16]. However, low solubility in aqueous solvent, low apparent permeability and poor bioavailability are noted as limitations for oral chemotherapy [17]. Cyclodextrins is a drug-like chemical compounds to establish a complex in solution, result in the improvement of pharmacokinetic parameters and physicochemical properties of the guest component, such as higher stability, increased aqueous solubility, decreased plasma protein binding and cellular toxicity [18]. Intravenous administration is the most direct effective and its overcome the variable absorption patterns of the gastrointestinal tract. Intravenous administration leads to immediate and complete bioavailability. Thus, this route has the potential to be hazardous because high concentrations of drugs are delivered to normal tissues causing greater effect to normal tissues and increased adverse reactions [19]. For that region to overcome disadvantages of current treatment procedure for cancer, the scientific community has switched toward nanotechnology to develop new, modern and more effective nanotechnology-based drug delivery systems to optimize oral, buccal and intravenous treatment routes.

Nanoparticles may be explained as ultra dispersed solid supra¬ molecular structures with a sub-micrometer size ranging from 10 to 1,000 μm [20]. The drugs can be dissolved, entrapped, encapsulated, or attached to a nanoparticle matrix, which acts as a reservoir for particulate systems and therefore plays an important role as a drug carrier system for clinical applications, particularly in the area of oncology [21]. Nanoparticles formulated from polysaccharides, pro¬teins, lipids and biocompatible/biodegradable polymers, such as polyethylene glycol (PEG), poly(γ-benzyl l-glutamate) (PBLG), poly(D,L-lactide), poly(lactic acid) (PLA), poly(D,L-glycolide), poly(lactide-co-glycolide), polycyano¬acrylate, liposome, chitosan, gelatin, and sodium alginate are called nanopartcles preparation[22]. The nanoparticles (NPs) are mainly fomulated *via* the dispersion of preformed polymers, the polymerization of monomers, ionic gelation, or the co-acervation of hydrophilic polymers, but other methods for their preparation have also been reported, such as supercritical fluid technology and particle replication in non-wetting templates[23,24]. NPs can also improve the stability of drugs and control their targeted delivery, allowing for a constant and uniform concen¬tration at the site of a lesion and facilitating drug extravasation into the tumor system and finally reducing side effects [25].

Curcumin is a great ancient medicinal phenolic compound purified from the rhizome of turmeric plant (*Curcuma longa*) and primarily used as a natural yellow pigment, extensively used in Ayurveda, Unani and Siddha which can absorb the visible light at a wavelength between 420–425 nm. Curcumin are usually occurs in three molecules namely: 1,7-bis (4-hydroxy-3 -methoxyphenyl)-1,6-heptadiene-3,5-dione (curcumin); 1,6-Heptadiene-3,5-dione,1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl) (demethoxy curcumin) and (1E,6E)-1,7-bis (4-hydroxy phenyl) hepta-1,6-diene- 3,5-dione (bis demethoxy curcumin). It has a variety of biological activities and pharmacological actions, such as anti-inflammatory, anti-carcinogen-

ic and anti-virus properties, as well as promising clinical applications due to its low toxicity [26].

Several researchers have pointed on the safety and efficacy of curcumin including transcription factors, growth factors and their receptors, cytokines, enzymes, and genes at the molecular level exhibiting drug and gene interaction. It has a daily requirement even up to 1600mg/kg/day. Unfortunately due to limited aqueous solubility, the curcumin cannot be readily absorbed from the gut region imparting very low concentration in serum and poor bioavailability to exhibit therapeutic activity at the targeted tissues [27]. Recently, reviewers are switching their attention towards the use of drug-loaded nanoparticles for targeted carrier applications [28]. Today, due to technological innovations nontoxic, biocompatible, inexpensive and biodegradable nanoparticles with various colloidal dimensions are being developed to enhance the penetration ability, reduce the frequency of doses, toxicity and to improve the therapeutic efficacy [29].

For this study, we have to use polysaccharide, chitoson for the preparation of nanoparticles. Chitosan [β-(1–4)-2-amino-2-deoxy-d-glucose] is a natural linear polycation polysaccharide obtained by partial *N*-deacetylation of chitin. Chitosan has many advantages as a carrier in nanoparticulate drug delivery system. It is nontoxic, biocompatible and biodegradable and has been proven to control the release of drugs. It is soluble in aqueous media, avoids the use of organic solvents and does not require further purification of NPs [30]. With the presence of free amine group in its line structure, Chitosan has a cationic nature and can interact with various cross linkers to anionic molecules to form NPs. The positive charge of Chitosan caused by the primary amino groups in its structure is responsible for its mucoadhesive properties and therefore prolongs the residual time at the absorption site. Chitosan NPs are expected to be appropriate carriers for oral absorption of drugs [31]. The purpose of this study was to synthesize Curcumin encapsulated nanoparticles (CCN) to improve the solubility and stability of the Curcumin in gastrointestinal (GI) conditions by evaluating their particle size, encapsulation efficiency, drug release and haemocompatibility aspects.

2. Materials & Methods

2.1 Synthesis of curcumin loaded chitosan nanoparticles (curcumin nanoparticles)

Chitosan nanoparticles (CsNPs) were synthesized by an ionic gelation method as described previously by Calvo *et al*., 1997 [32]. Briefly, an aqueous solution of two compounds (positively charged chitosan and negatively charged sodium tripolyphosphate) were added with and without curcumin load to the formation of nanoparticles without any aggregation and stored at 4°C. This formulation was selected for further studies and characterization as well as for entrapment of curcumin.

2.2 Particles size and zeta potential analysis

Particle size and zeta potential were determined by DynaPro-TC–04 dynamic light scattering equipment (Protein Solutions, Wyatt Technology, Santa Barbara, CA) worked on quasi-elastic light scattering [33].

2.3 Transmission electron microscopy:

The internal structure of chitosan nanoparticles (CsNPS) and curcumin loaded chitosan nanoparticles (CLCsNPs) was evaluated by TEM (Morgagni-26AD: FEI Company, Netherland at All India Institute of Medical Sciences, India). The TEM image capture and analysis was done using Soft Imaging Viewer software for further analysis.

2.4 Characterization of curcumin bound nanoparticles

The Curcumin was extracted from curcumin loaded chitosan nanoparticles and characterized through High Performance Liquid Chromatography (HPLC) [34], Maldi-TOF mass spectroscopy [35], Fourier transform infrared (FTIR) [36], X- ray diffraction (XRD) [37]. The release of drug from CLCsNPs carried out separately at pH 5 by dissolving of nanoparticles in 10 ml PBS (0.01 M) [38]. The released curcumin was re-suspended in 10% ethanol and recoded the absorbance at 430 nm by a UV–vis spectrophotometer.

The entrapment efficiency of curcumin within CLCsNPs was determined by Anitha, *et.al.*, 2011 [39] and calculated by the standard curve of known amount of curcumin. Entrapment efficiency [EE] was calculated based on the ratio of amount of curcumin present in the nanopartcles to the amount of curcumin used in the loading process.

$$EE\% = \frac{\text{Total amount of curcurmin with in the pellet}}{\text{Initial amount of curcurmin taken for loading studies}} \text{ X } 100$$

Loading efficiency (LE) of the drug-loaded system was also calculated with respect to the yield of the nanoparticles obtained after centrifugation [39].

$$LE\% = \frac{\text{Total amount of curcurmin entrapped with in the pellet}}{\text{Yield of curcurmin loaded NPs}} \text{ X } 100$$

In vitro release profile of curcumin from CLCsNPs was performed by direct dispersion method [38,39] and curcumin release were monitored separately up to a period of 120 hrs at two different pH, 5 and 7.4 respectively.

2.5 Procurement of Cell lines and maintenance

All cervical cancer cell lines were procured from NCCS Pune, India. SiHa, Hela, Caski and C33a, cultured and maintained in Dulbecco's Modified Eagle's Medium (DMEM), supple-

mented with 10% Fetal Bovine Serum and antibiotics in a humidified atmosphere of 5% CO2 at 37°C in culture dishes/flasks. The cellular uptake of curcumin (CLCsNPs) was performed in cervical cancer cell lines SiHa, HeLa, CasKi and C33A described previously [40].

3-(4,5-dimethyl-2-yl)-2,5-diphynyl tetrazolium bromide (MTT) is a metabolic substrate, which is reduced by the mitochondrial succinate dehydrogenase enzyme and forms formazan crystal. Cellular metabolic assay was carried out in curcumin, and curcumin loaded chitosan nanoparticles treated cervical cancer cell line (Caski, C33a, Hela and SiHa) by MTT assay [41]. Briefly, cells were seeded overnight with cell count 1× 104 per well and then incubated with various concentrations of curcumin and curcumin formulations for 24, 48 and 72 hr respectively. At the end of the treatment, media was removed and cells were incubated with 20µl of MTT (5 mg/ml in PBS) in fresh medium (50µl) for 4 hr under CO2 incubator. After 4 hr, formazan crystal formed by mitochondrial reduction of MTT were solubilized in DMSO (150µl/well) and the absorbance was read at 570 nm after 10 min incubation on ELISA reader (Bio-Rad, USA). Percent cytotoxicity was expressed as IC50. Percentage of cell viability was calculated and result was also expressed as percent mean of viability ± standard error of mean (SEM).

Apoptosis was identified by the presence of fragmented DNA in cells. After treatment of HeLa, SiHa, CasKi and C33A cells (2×106 cells) with curcumin loaded chitosan nanoparticles for 24 hrs, fixed and stained with DAPI as described before [42]. The expression level of pro-apoptotic Bax and anti-apoptotic Bcl-2 proteins was estimated in control and treated with CLCsNPs by Western blotting as described earlier [43] and further confirmed apoptosis by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay [44]. The apoptotic index (AI) was monitored randomly of selected area containing at least 500 cells under microscope.

3. Results and Discussion

3.1. Biophysicochemical characterization of curcumin loaded chitosan nanoparticles

Chitosan nanoparticles (CsNPs) were synthesized between the interaction of positively charged chitosan and negatively charged sodium tripolyphosphate (TPP). The nanoparticles were formed with varying concentrations of chitosan and TPP and the best possible formulation was selected for loading of the anti-cancer drug curcumin. In this regard, a noble formulation for obtaining small size, maximum entrapment and for enhanced bioavailability, maximum solubility and stability of curcumin. The particle size of CsNPs and CLCsNPs were demonstrated by Dynamic light scattering (DLS). While particle size governs the distribution of nanoparticles in the body; zeta potential determines their stability in the system. For a stable nano-suspension, the zeta potential of the nanoparticles should ideally be more than ±30 mV

[45]. This was further confirmed by TEM, which indicated that CsNPs have distinct, spherical particles with a dense structure; however they appeared considerably smaller as compared with the average particle size of CLCsNPs [46]. Binding of curcumin to chitosan nanoparticles was pH dependent and maximum binding of active form of curcumin (curcuminoids) at acidic pH 5. The binding at lower pH is additional advantage for the stability of curcumin [47].

In vitro release of curcumin was done *via* the direct dispersion method at pH dependent with different pH acidic & basic. The rate of curcumin release pattern presented a burst release in the first few hrs followed by a controlled release of curcumin over a longer period. Curcumin is adsorbed on to the NPs surface and this entrapment near the surface could be the reason for initial burst release, as the dissolution rate of the chitosan polymer near the surface is higher; therefore released the amount of drug is also higher. Furthermore, we found that the drug release was to be faster at lower pH than that of the higher pH because in acidic environment the polymer matrix swells due to protonation of amine group of chitosan [48]. Thus, it was clear indicated that the entrapment of curcumin in chitosan nanoparticles has significantly retained prolong and greater release at acidic pH than basic medium. *In vitro* cellular uptake of curcumin from curcumin loaded chitosan nanoparticles was measured by spectrophotometrically. The cellular uptake of curcumin (curcumin loaded chitosan nanoparticles) by cervical cell lines like, SiHa, HeLa, CasKi and C33A cells was higher at first few hrs and then slow down and starts to inhibit the cellular proliferation and later cell death. Furthermore, it was confirmed by DAPI

3.2. Characterization of curcumin loaded chitosan nanoparticles

The entrapment of curcumin has been further characterized by different biophysical techniques like, HPLC, MALDI-TOF, FTIR, and XRD and extraction of curcumin from the curcumin loaded chitosan nanoparticles was further analyzed in respect to native curcumin.

The HPLC profile of standard curcumin showed at specific retention time. Curcumin extracted from CLCsNPs also showed similar HPLC pattern at required retention time. Furthermore, the entrapment of curcumin in curcumin loaded chitosan nanoparticles was confirmed by MALDI-TOF. As we know that the standard mass of curcumin about 369, 339 for demethoxycurcumin and 309 for bisdemethoxy curcumin. Similarly, the results were obtained in curcumin extracted from CLCsNPs with a same mass as standard curcumin. Further to confirms the loading of drug in curcumin loaded chitosan nanoparticles formulation, FTIR analysis was observed in Fig. 3 shows the FT-IR spectra of (a) chitosan nanoparticles and (b) curcumin loaded chitosan nanoparticles. In Fig. 3 (a), a peak at 1640cm−1 was observed and this corresponds to amide I bending vibration. In the CsNPs spectrum, the wave number shifted from 1640 to 870cm−1. In Fig.3 (b), spectra of CLCsNPs, a peak at 1640cm−1 was observed which corresponds to amino deformation and a change in the peak at 1089cm−1 cor-

responds to -keto group of the curcumin [38,49]. From the FT-IR data, it is confirmed that the NPs were formed due to the interaction between the phospho groups of TPP and amino groups of chitosan. There was an interaction between the -keto group of curcumin and amine group of chitosan that resulted into drug loading.

As curcumin is a highly hydrophobic molecule, it tends to form crystals when added to an aqueous solution. The nano sized crystals are formed inside the matrix of the NPs hindering drug and release from the NPs. Similarly, The X-ray Diffraction (XRD) was used to confirm the nature of crystal structure of the formed curcumin loaded chitosan nanoparticles. The CLCsNPs exhibited strong reflections with the characteristic intensity (cps). In addition, curcumin has only one reflection with characteristic intensity (cps). In chitosan nanoparticles, however no such crystalline peak was observed. This result clearly indicates that curcumin encapsulated in NPs is in the amorphous or disordered-crystalline phase or in the solid-state solubilized form in the polymeric matrix. This disordered-crystalline phase of curcumin inside the polymeric matrix helps in sustained release of the drug from the nanoparticles. Presence of drug in crystalline form inside nanoparticles hampers its release as such large sized molecules cannot diffuse from the small pores of the nanoparticles. However, if the drug is in amorphous or in disordered-crystalline phase easy diffusion of drug molecules can occur through the polymeric matrix, leading to a sustained release of the encapsulated drug.

3.3. Cell Viability assay

Cytotoxicity of the curcumin loaded chitosan nanoparticles (CLCsNPs) and curcumin were evaluated by the assessment of its *in vitro* impact on the HPV positive (Caski, HeLa and SiHa) and HPV negative human cervical cancer cells and (C33a) for 24, 48 and 72 hr respectively. MTT assay revealed a IC50 for CLCsNPs was lower than native curcumin However, cell lines incubated with chitosan nanoparticles up to 200µg/ml, demonstrated no any significant cytotoxicity for 24, 48 and 72 hrs respectively. Results indicated that the chitosan nanoparticles are nontoxic at the given dose and time of exposure. Anticancer drugs act through the induction of apoptosis [50]. Microscopic examination of the curcumin loaded chitosan nanoparticles treated cervical carcinoma cells revealed cell death with characteristics of apoptosis was revealed to stain with DAPI (fluorescent DNA-binding dye). Treated cells demonstrated the altered nuclear morphology seems to have nuclear condensation, nuclear blebbing and nuclear fragmentation. Untreated (control) cells showed no significant change in normal/regular morphology. CLCsNPs was found to suppress proliferation and induce cell death in various cancer types, including cervical cancer, breast cancer, prostate cancer, melanoma, neuroblasoma, lymphoblasic leukemia and lymphoma [51].

Up-regulation of pro-apoptotic proteins (Bax) and down-regulation of anti-apoptotic proteins (Bcl-2) was studied by curcumin loaded chitosan nanopartcles (CLCsNPs) in treated

and untreated cervical cancer cells. Expressional analysis of Bax and Bcl-2 were performed to demonstrate the expression level of apoptotic and anti-apoptotic protein through western blot using protein specific monoclonal antibody. Beta-actin used as positive control for each experiment of western blot. Our results indicated that pro-apoptotic protein level enhanced after the treatment of CLCsNPs. This was accompanied by a simultaneous decrease in anti-apoptotic proteins. We have also standardized other apoptotic/anti-apoptotic and/or oncoprotein expression study through western blot. CLCsNPs induced apoptosis was further measured by TUNEL assay. Treatments with IC50 values of CLCsNPs revealed apoptosis after 2 hr.

4. Conclusion

The entrapment of curcumin in chitosan nanoparticles is water soluble as well as stable to the forefront of existing anticancer therapeutic agents. In this regard, the encapsulation of curcumin within nanoparticle brought a new avenue to improve the bioavailability of curcumin and can make the drug responsive for the treatment of cancer. Most importantly, the observed results justified the curcumin loaded chitosan nanoparticles was more effective under in vitro condition with time due to greater cellular uptake, sustained intercellular drug retention and enhanced anti-proliferative effect by inducing apoptosis. Most importantly, the enhanced cellular internalization and sustained release of entrapped curcumin in our formulation results its enhanced systemic bioavailability. Thus, the nanoparticulate curcumin provided an efficient delivery for encapsulated curcumin and proved a promising carrier candidate by increasing its water solubility and improving its stability for tumor therapeutic treatment in near future.

5. Acknowledgement

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6. Figures

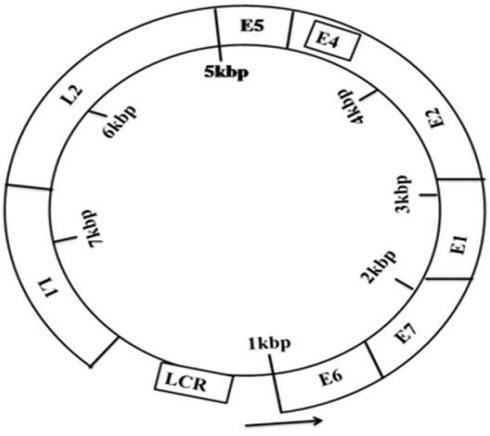

Figure 1: Systemic presentation of HPV genome

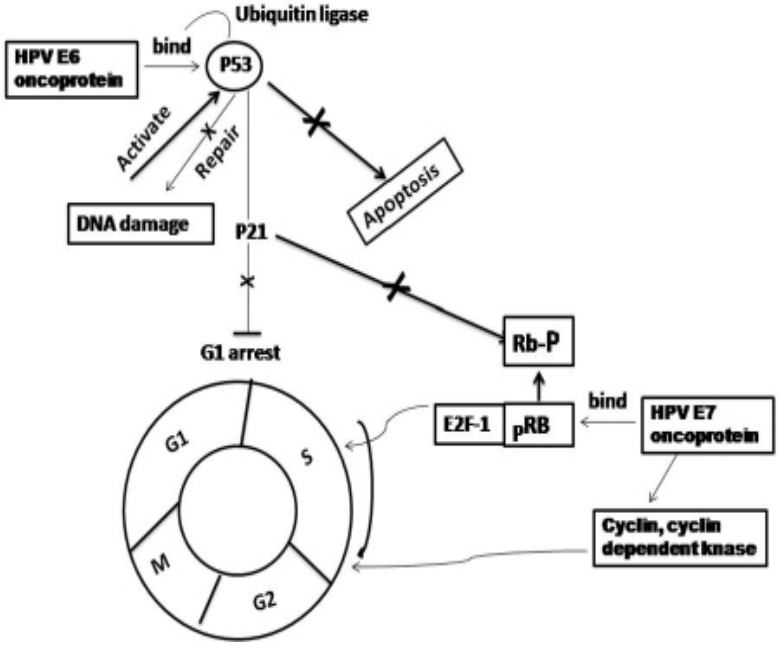

Figure 2: Pathogenesis of oncogenic genes in HPV

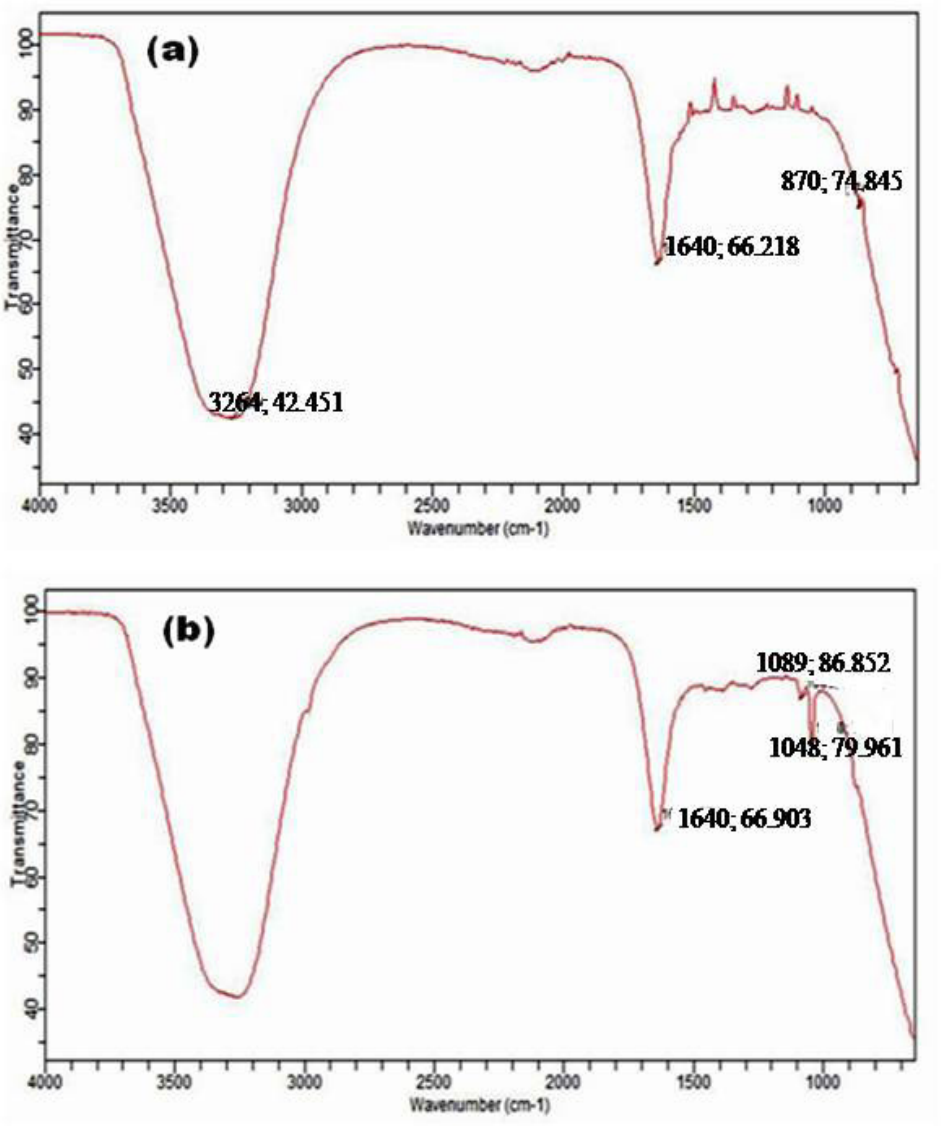

Figure 3: FTIR analysis of **(a)** Chitosan nanoparticles and **(b)** Curcumin loaded chitosan nanoparticles

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