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Preparation, Characterization, and Evaluation of Physcion Nanoparticles for Enhanced Oral Bioavailability: An Attempt to Improve Its Antioxidant and Anticancer Potential

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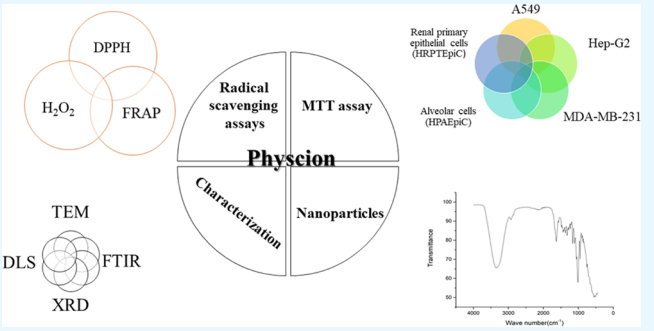
ABSTRACT: This study aims to enhance the dissolution rate of a poorly water-soluble drug physcion by producing its nanoparticles (NPs) using an antisolvent precipitation with a syringe pump (APSP) method and to assess its antioxidant and cytotoxic potential. The NPs were prepared using a simple and cost-effective APSP method and subsequently characterized by different analytical techniques including dynamic light scattering (DLS), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and X-ray powder diffractometry (XRD). They were also subjected to solubility and dissolution studies, and different parameters such as dissolution efficiency (DE), mean dissolution time (MDT), and difference (f_1) and similarity factors (f_2) were determined. Furthermore, physcion and its NPs were investigated for antioxidant and cytotoxic effects using various in vitro assays. SEM and DLS analysis indicated that the average size of physcion NPs was 110 and 195 ± 5.6 nm, respectively. The average ζ-potential and polydispersibility index (PDI) of the prepared NPs were −22.5 mV and 0.18, respectively, showing excellent dispersibility. XRD confirmed the amorphous nature of physcion NPs. The solubility and dissolution rates of NPs were significantly higher than those of the original powder. The antioxidant potential studied by the (DPPH), FRAP, and H_2O_2 assays was greater for physcion NPs than that for the raw powder. The IC_{50} values of physcion NPs against the aforementioned models were 57.56, 22.30, and 22.68 μg/mL, respectively. Likewise, the cytotoxic potential investigated through the MTT assay showed that physcion NPs were more cytotoxic to cancer cell lines A549 (IC_{50} 4.12 μg/mL), HepG2 (IC_{50} 2.84 μg/mL), and MDA-MB-231 (IC_{50} 2.97 μg/mL), while it had less effect on HPAEpiC (IC_{50} 8.68 μg/mL) and HRPTEpiC (IC_{50} 10.71 μg/mL) normal human epithelial cells. These findings have proved that the APSP method successfully produced physcion NPs with enhanced solubility, dissolution rate, and antioxidant and cytotoxic activities.



1. INTRODUCTION

Free radicals play a very important role in oxygen-dependent living systems. Being part of cellular processes, they are frequently involved in aging and disease development. Free radicals are highly reactive species and always attempt to pair up with other electrons. Subsequently, they remove electrons from other molecules and thus not only affect cell regulation but also damage molecules including DNA, proteins, and carbohydrates. Cell damage, especially the damage to DNA, may cause the development of cancer.[1,2] Antioxidants are capable of stabilizing reactive oxygen species (ROS) by donating electrons and thus inhibit their detrimental effects. Cancer is a complex, heterogeneous disease with approx. 9.5 million cancer-related deaths annually.[3] Therefore, there is an urgent need to develop more effective therapies for cancer. Traditional therapies for cancer include chemotherapy, surgery, and radiation; however, they are associated with unsatisfactory outcomes owing to the lack of specificity, drug resistance, and severe side effects. In order to overcome these limitations, more effective treatments such as gene therapy, chemodynamic therapy, immunotherapy, and nanomaterial-based chemotherapy are now available.[4−7] Among these, the latter has revolutionized cancer therapy because of its specificity, reduced toxicity, enhanced permeability and retention, and excellent bioavailability.[8,9] Typical nanomedicines are 1−100 nm in size and possess good permeability, high surface-to-volume ratio, and excellent biocompatibility.[10] The high surface-to-volume ratio enables the assembly of

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biomolecules that improve their specificity and efficacy, thereby reducing the side effects.[5] In addition to synthetic chemotherapeutic agents, many natural phytochemicals have been studied for their anticancer potential, at least in animal models.[11] Compounds that have been extensively studied for their cytotoxic properties include polyphenols, glycosides, alkaloids, and taxols.[12−14]

Physcion, a 1,8-dihydroxyanthraquinone glycoside, found in different plants, including *Rheum palmatum*, *Rhamnus alaternus*, *Frangula rupestris*, *Rhamnus sphaerosperma*, and *Rennoutria elliptica*.[15−18] It has been reported to induce apoptosis, block cell cycle progression, and suppress the metastatic potential of cancer cells. It shows potential antitumor effects against hepatocellular and nasopharynx carcinomas. The said agent has shown antiproliferative potential against colorectal cancer cells HCT116 and human breast cancer cells MDA-MB 231 through G0/G1 arrest.[19−21] The wide biological applications of physcion are, however, greatly limited due to its poor oral bioavailability. The systemic absorption of a drug primarily depends on the rate and extent of its dissolution from the dosage form. It has been reported that following oral administration, the plasma concentration (1 h) of physcion was only 0.018 μg/mL in rats.[22] The low oral bioavailability of physcion is attributed to its poor dissolution and aqueous solubility.[23]

The oral route is the most preferred route of drug administration, especially for mitigating chronic diseases. However, many drugs fail to achieve therapeutic levels due to their poor solubility, permeability, and absorption from the gastrointestinal tract. As a result, higher doses are required to achieve effective therapeutic levels, which may lead to more side effects.[24] Formulation scientists confront various challenges in formulation development because poorly soluble drugs are not easily wetted by water.[25] In order to meet these challenges, different techniques are adopted to enhance solubility and achieve maximum absorption in the gastrointestinal tract. Some of these techniques include solid dispersion, particle size reduction, and presentation of a drug in the form of nanoparticles (NPs).[26,27] Globally, NPs have been extensively investigated to improve the oral absorption of poorly water-soluble drugs, particularly BCS class II and IV.[28] Drug NPs are prepared using different techniques, including "bottom-up" and "top-down" approaches.[29] Top-down techniques include milling and homogenization, while bottom-up techniques include antisolvent precipitation and spray freezing. Antisolvent precipitation with a syringe pump (APSP) is the most simple and cost-effective method of achieving nanoscale materials.[30] Nanosized formulations have better pharmacodynamic and pharmacokinetic profiles. Due to its small size, the surface area, surface free energy, and surface-to-volume ratios are significantly enhanced, which may improve the solubility and dissolution rate of poorly water-soluble drugs.[31] As mentioned earlier, physcion has shown potential antitumor effects against different cell lines, including hepatocellular, breast, and colorectal cancer; however, its biological applications are greatly hindered due to its poor aqueous solubility and oral bioavailability.[19,20,32] The purpose of the present study is to prepare physcion NPs using the APSP method in order to improve the oral bioavailability of physcion. The prepared NPs will be investigated for parameters including dissolution, solubility, and in vitro antioxidant and cytotoxic potential.

2. MATERIAL AND METHODS

2.1. Chemicals and Reagents. Chemicals and reagents used in the present study were of analytical grade and included 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), phosphate and acetate buffers, DPPH (99%), FRAP (99%), H_2O_2, NaOH (98%), 1% penicillin−streptomycin solution, and Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS). The chemicals were purchased from Sigma-Aldrich, Germany. Deionized water was prepared using a Milli-Q system (Millipore, Milford). Physcion (Figure 1) was gifted by Dr. Mi−Jeong Ahn, Professor of Pharmacy at College of Pharmacy, Gyeongsang National University, Korea.

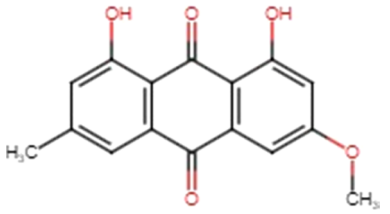

Figure 1. Chemical structure of physcion.

2.2. Preparation of Physcion NPs. Physcion NPs were prepared using the previously reported APSP method.[30] Briefly, in this method, a saturated solution of physcion was prepared using chloroform as a solvent at the predetermined concentration of 5−12 mg/mL. 20 mL of the prepared solution was injected using a syringe at a flow rate of 2−10 mL/min into a specific volume of deionized water (antisolvent) under mechanical stirring (3500 rpm). The same procedure was adopted for mixing a fixed ratio of drug solution with different volumes of deionized water (1:5, 1:10, 1:15, and 1:20 v/v) in order to obtain an optimum concentration of NPs. An opaque solution was achieved after mixing, which was quickly evaporated using a rotary evaporator to obtain nanosized drug particles.

2.3. Characterization of Physcion Nanoparticles. *2.3.1. Dynamic Light Scattering (DLS).* A Zetasizer (Malvern, U.K.) was used to determine the average particle size, ζ-potential, and polydispersibility index (PDI) of physcion NPs. The NPs were dispersed in distilled water and subsequently filtered through a filter paper (0.45 μm). The PDI and particle size were measured at ambient conditions using a scattering angle of 90. ζ-potential was measured using PALS technology.

2.3.2. Fourier Transform Infrared Spectroscopy (FTIR) Analysis. Different functional groups present at the surface of physcion NPS were analyzed with an FTIR (Version 10.5.1, PerkinElmer) spectrophotometer by adopting the KBr pellet method. Lyophilized NPs were directly placed on an ATR crystal and screened over the range of 500−4000 cm^{-1} with a spectral resolution of 1 cm^{-1}.

2.3.3. Scanning Electron Microscopy (SEM) Analysis. The crystalline structure and size distribution of physcion NPs were analyzed using SEM (Hitachi-7650, Japan) analysis. Briefly, the NPs were dispersed in distilled water, and a drop of it was put on a staining mat. A 'carbon-coated copper grid" was inserted into the drop, and after 15 min, it was removed, dried in air, and finally screened using the SEM technique.

2.3.4. X-ray Powder Diffraction (XRD) Analysis. The crystallinity of prepared NPs was investigated using XRD

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(JDX-3532 JEOL, Japan). The operating current and voltage were 30 mA and 40 kV, respectively, with Cu Kα radiation in $\theta-2\theta$ configurations. The crystal size was derived from the total width of peaks using the Debye−Scherrer's equation (eq 1).

$$D = \left(\frac{K\lambda}{\beta \cos\theta}\right) \tag{1}$$

where K is the Scherrer constant, λ is the wavelength of the X-ray beam used, β is the full width at half-maximum (FWHM) of the peak, and θ is the Bragg angle.

2.4. In Vitro Analysis. *2.4.1. Solubility Determination.* Briefly, a measured amount of the pure compound and its NPs were placed in separate vials. To each vial, 10 mL of distilled water was added, followed by vigorous shaking using an orbital shaker for 72 h (25 °C) at 3000 rpm. The solution was centrifuged (10,000 rpm) and filtered using Whatman filter paper. The filtrate was diluted and subsequently analyzed (430 nm) using an ultraviolet−visible (UV−vis) spectrophotometer (PerkinElmer, Lambda 35, Germany). The solubility of the test samples was also determined in 0.1 M HCl and PBS (pH 6.8) using the aforementioned procedure. The analysis was conducted in triplicate.[30]

2.4.2. Dissolution Studies. The dissolution studies were performed according to the United States Pharmacopeia (USP) method II (paddle method).[33] Distilled water, phosphate-buffered solution (pH 6.8), and 0.1 M HCl were used as dissolution media. 50 mg of the pure sample and its NPs were subjected to the dissolution studies. The operating conditions for the apparatus were as follows: the volume of the medium, 900 mL; paddle rotation, 50 rpm; and temperature, 37.0 °C ± 0.5 °C. 5 mL aliquots were drawn at predetermined intervals (0, 15, 30, 45, 90 and 120 min) and subsequently filtered through Whatman filter paper. In order to maintain the sink conditions, an equal amount of fresh media was replaced each time. The filtered samples were diluted and analyzed using a UV−vis spectrophotometer. The analysis was conducted in triplicate.

2.4.3. Dissolution Parameters. 2.4.3.1. Dissolution Efficiency (DE). This value was determined using the trapezoidal rule (eq 2)

$$\mathrm{DE} = \frac{\int_{t_1}^{t_2} y.\ \mathrm{d}t}{y_{100} \times (t_2 - t_1)} \times 100 \tag{2}$$

where y = % drug dissolved between times t_1 and t_2, and DE = percentage of the area of the rectangle described by the % dissolution value at the same time.

2.4.3.2. Mean Dissolution Time (MDT). This parameter was calculated using eq 3

$$\mathrm{MDT} = \frac{\sum_{j=1}^{n} t_j\, \Delta M_j}{\sum_{j=1}^{n} \Delta M_j} \tag{3}$$

where j = sample number, t_j = time at the midpoint between t_j and t_j-1 (calculated as $t_j + t_j$-1/2), n = number of dissolution sampling times, and ΔM_j = additional amount of drug dissolved between t_j and t_j-1. The MDT calculations were performed using Excel add-in DDSolver.

2.4.3.3. Difference (f_1) and Similarity Factors (f_2). The dissolution profile of the test samples was calculated using a model-independent approach. These factors were calculated using eqs 4 and 5

$$f_1 = \left\{\frac{\left[\sum_{t=1}^{n} R_j - T_j\right]}{\sum_{j=1}^{n} R_j}\right\} \times 100 \tag{4}$$

$$f_2 = 50 \times \log\left\{\left[1 + \left(\frac{1}{n}\right)\sum_{j=1}^{n} |R_j - T_j|^2\right]^{-0.5} \times 100\right\} \tag{5}$$

where R_j and T_j = % of reference and test drugs, respectively, dissolved into the dissolution medium at each time point j, and n = number of withdrawal points

2.5. Antioxidant Assay. *2.5.1. DPPḢ Assay.* DPPH free radical scavenging effects of physcion and its corresponding NPs were assessed using a conventional DPPH assay.[34] Stock solutions of physcion, NPs, and standard (1 mg/mL) were prepared in DMSO, followed by serial dilution to obtain various concentrations (31.5 62.5, 125, 250, 500, and 1000 μg/mL). Likewise, a 0.5 mM DPPH solution in ethanol was prepared. Thereafter, 100 μL from each working solution and 1 mL of DPPH were vortex-mixed and allowed to incubate for 15 min at 23 °C in a dark environment. DPPH solution without samples was considered blank. Subsequently, the reaction mixture was centrifuged at 3000 rpm for 5 min, and the absorbance of the supernatant layer was measured at a specific wavelength of 517 nm using a microplate reader against blank. Ascorbic acid was used as the reference standard. Using eq 6, the percent radical scavenging activity (RSA) was calculated.

$$\%\ \mathrm{RSA} = \frac{\mathrm{control}_{\mathrm{Abs}} - \mathrm{sample}_{\mathrm{Abs}}}{\mathrm{control}_{\mathrm{Abs}}} \times 100 \tag{6}$$

2.5.2. FRAP Assay. The FRAP assay was performed using a reported method.[35] This assay was used to determine the reducing power of physcion and its NPs using ascorbic acid as the reference standard. Compounds with antioxidant potential are considered to reduce Fe^{3+} to Fe^{2+} ions; the latter form a blue complex (Fe^{2+}/TPTZ), which enhances the total absorbance at 593 nm. The reaction mixture primarily consists of TPTZ solution (0.25 mL; 10 mM) in HCl (40 mM), FeCl3 (0.25 mL; 20 mM), acetate buffer (2.5 mL; 300 mM, pH 3.6), physcion, and/or NPs at different concentrations (31.5 62.5, 125, 250, 500, and 1000 μg/mL). The FRAP reagent (170 μL), NPs, and/or physcion (20 μL) were mixed in a 96-well plate and incubated for 30 min in a dark place. Following incubation for 30 min, the maximum absorbance was measured at 593 nm and the % RSA of the test samples was determined. The assay was performed in triplicate.

2.5.3. Hydrogen Peroxide Scavenging Assay. The method of Ruch et al. was adopted for performing the assay.[36] An aliquot of 0.1 mL of physcion and its NPs at different concentrations of 31.5, 62.5, 125, 250, 500, and 1000 μg/mL were mixed in separate tubes with 0.4 mL of phosphate buffer (50 mM; pH 7.4). The reaction was started by the addition of 0.6 mL of H_2O_2 (2 mM) solution. The mixture was vortexed, and after incubation for 10 min at room temperature, its maximum absorbance was recorded at 560 nm. Ascorbic acid was used as a reference drug, and the assay was performed in triplicate.

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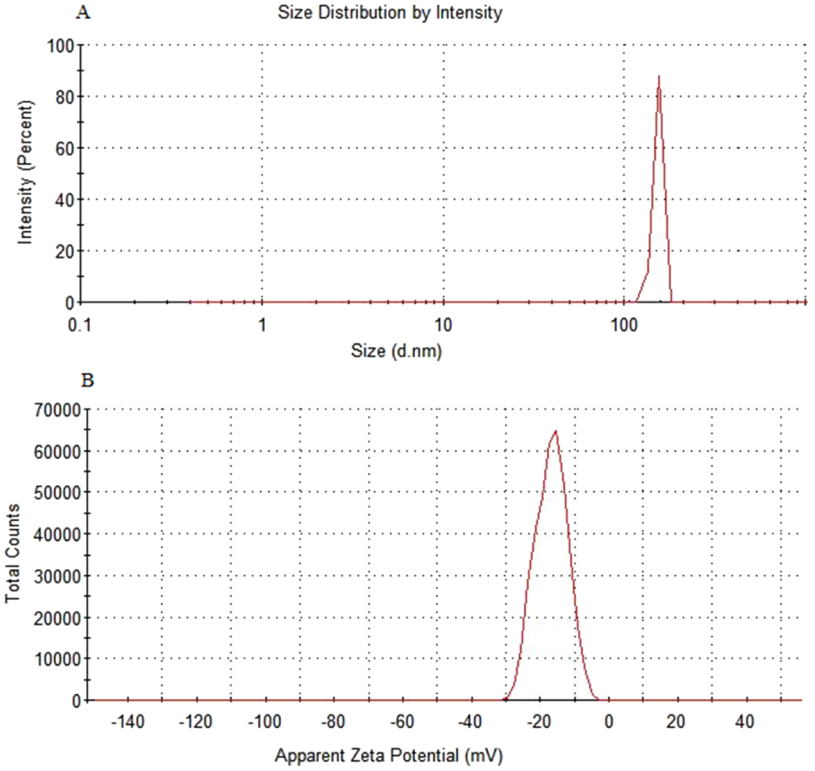

Figure 2. (A) Hydrodynamic size distribution and (B) ζ-potential of NPs.

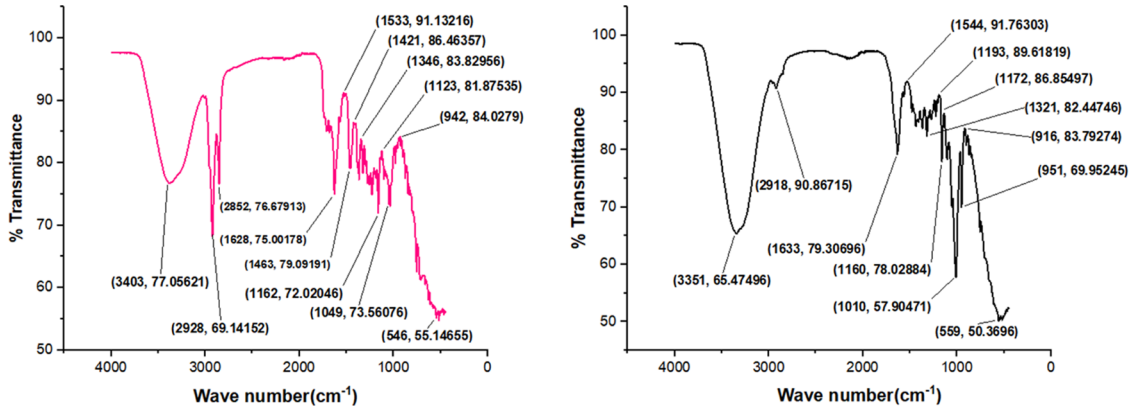

Figure 3. FTIR spectra of unprocessed physcion (pink line) and physcion NPs (black line).

2.6. Cytotoxicity Assay. The cytotoxic potential of powdered physcion and its NPs was investigated using the MTT assay.[37] Different cancer cell lines including HepG2 (hepatocellular carcinoma cells), MDA-M231 cells (breast adenocarcinoma), and A549 (lung adenocarcinoma cells) were used to investigate the cytotoxic potential of the studied samples. Dulbecco's modified Eagle's medium supplemented with FBS (10%) and penicillin−streptomycin (1% v/v) was used to culture the selected cell lines. They were incubated at 37 °C with 5% CO_2. Cells with 90% confluency were suspended in the RPMI-1640 medium. This activity was performed at Jeju National University, Korea, where the cell lines, e.g., human hepatocellular carcinoma cell line HepG2, human lung adenocarcinoma cell line A549, human breast adenocarcinoma cell line MDA-MB-231, and alveolar (HPAEpiC) and renal primary epithelial (HRPTEpiC) normal human cell lines, were obtained from the Korea Cell Line Bank, Seoul, Korea.

2.6.1. Assessment of Cytotoxic Potential on Cancer Cell Lines. Prior to treatment, the cells were incubated overnight at 37 °C with 5% CO_2 in a 96-well flat-bottom plate containing 10^4 cells/well. They were then treated with different concentrations of physcion and/or physcion NPs. Following incubation for 48 h (37 °C; 5% CO_2), the MTT reagents (25 μL) in phosphate buffers were then added to each well and incubated at 37 °C for 30 min. Using a microplate reader, the absorbance was recorded at 490 nm. The % cell viability was then calculated using the following formula (eq 7)

$$\%\ \text{viable cells} = \left(\frac{\text{abs}_{\text{sample}} - \text{abs}_{\text{blank}}}{\text{abs}_{\text{control}} - \text{abs}_{\text{blank}}}\right) \times 100 \quad (7)$$

In addition, the selectivity index (SI) was calculated to find out the specificity of test samples toward normal cell lines.

2.7. Statistical Analysis. The results were statistically evaluated by one-way analysis of variance (ANOVA) using

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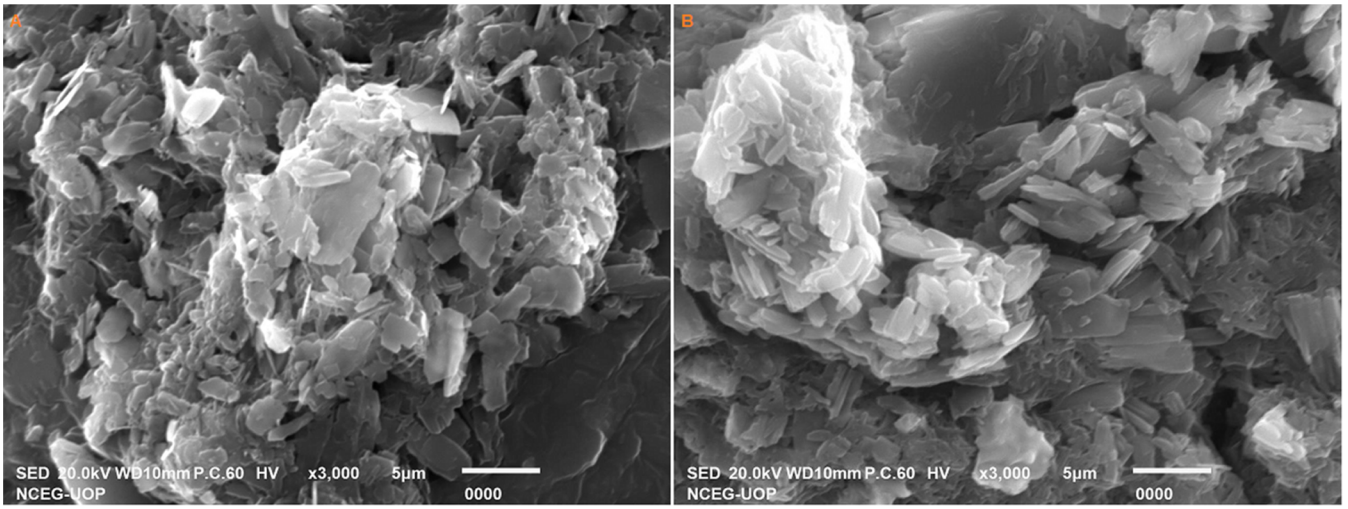

Figure 4. SEM images of (A) unprocessed physcion and (B) physcion NPs.

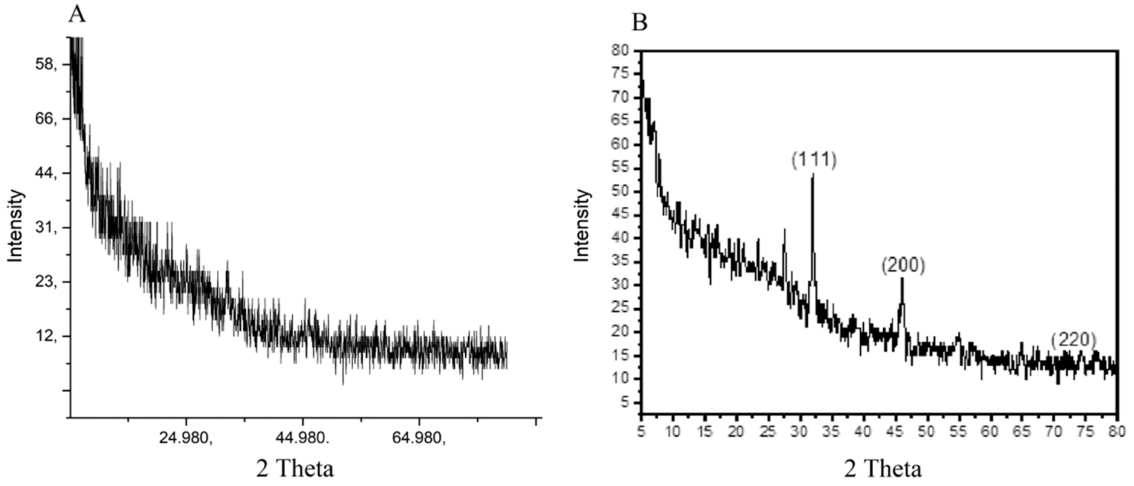

Figure 5. XRD spectra of (A) physcion NPs and (B) unprocessed physcion.

GraphPad Prism software. The data were presented as mean ± SEM.

3. RESULTS AND DISCUSSION

3.1. Characterization of Physcion Nanoparticles. *3.1.1. Determination of Particle Size Using the Dynamic Light Scattering (DLS) Technique.* This is one of the most widely used techniques for measuring the nanoparticle size. In this procedure, a monochromatic light laser illuminates a colloidal dispersion, and as the incident light impinges the NPs, its direction and intensity alter, which is related to the size of particles according to a correlation function.[38] This procedure has many advantages for sizing NPs in a short duration of time. In this study, the observed mean particle size was recorded to be 195 ± 5.6 nm, while the average ζ-potential was −22.5 mV (Figure 2). Moreover, the PDI value of prepared NPs was 0.18, which shows excellent dispersibility. The negative potential may be due to the presence of −OH groups at the surface of NPs, which contribute to its overall stability. According to the literature, higher ζ-potential results in greater stability due to electrostatic repulsion. Moreover, the small size and low PDI of NPs make them suitable for uptake by the cells. Moreover, the size of particles is very important in drug dissolution, absorption, and cellular transportation. Nanosized particles can easily pass through biological membranes, including the plasma membrane of the cell. It has been reported that particle sizes <100 nm show excellent properties in drug delivery systems.[39]

3.1.2. FTIR Analysis. FTIR spectra of the raw powder of physcion and its corresponding NPs are presented in Figure 3. The results indicate that the spectra of the studied samples have little difference, which shows that the chemical composition of physcion is unaffected by particle size reduction. The spectra of powdered physcion and its NPs showed slightly different stretching vibration patterns of the functional groups. The broader bands at 3361 and 3403 cm^{-1} are due to the stretching vibration of the −OH groups, whereas the bands at 2928 and 2931 cm^{-1}, 1628 and 1633 cm^{-1}, and 716−1366 cm^{-1} represent C−H, C═O, and C−C vibrations, respectively. Similarly, the peaks at 1009 and 1049 cm^{-1} are due to CH_2 wagging from CH_3 groups. The peak observed at 1533 cm^{-1} is due to C−H bending vibrations.

3.1.3. SEM Analysis. SEM analysis showed that the average particle size of physcion was greatly reduced after processing by the APSP method with an average diameter of approx. 110 nm (Figure 4). The high surface area and energies associated with these NPs greatly enhanced the dissolution rates, solubility, and bioavailability of physcion NPs.

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3.1.4. XRD Analysis. The XRD technique was used to determine the crystalline nature of the formed NPs. The APSP-created NPs exhibited almost no diffraction peaks as compared to raw powder, which shows the amorphous nature of NPs (Figure 5). Amorphous materials possess distinct physicochemical characteristics compared to their crystalline counterparts. One of the properties, the enhanced solubility of amorphous drugs, is exploited in the pharmaceutical industry as a means of improving the bioavailability of poorly water-soluble drugs.[40]

3.2. In Vitro Analysis. *3.2.1. Solubility Determination.* The solubility of unprocessed physcion and its corresponding NPs in different solvents is shown in Figure 6. The solubility of physcion NPs is comparatively high in all three solvents. The solubility of NPs nearly 100% increased in distilled water. Similarly, in PB, its solubility was significantly enhanced from 0.038 to 0.105 μg/mL, while in HCL, its solubility increased from 0.049 to 0.112 μg/mL. The improvement in solubility may be attributed to the formation of nanoscale particles and their conversion from crystalline to a more soluble amorphous form. According to the literature, amorphous materials lack a 3-dimensional structure as compared to crystalline materials. Both forms possess the same molecular composition but different physicochemical properties. These properties are successfully exploited in many areas, including pharmaceutical industries. The increased solubility of amorphous materials is of great value in improving the oral bioavailability of poorly water-soluble drugs.[41]

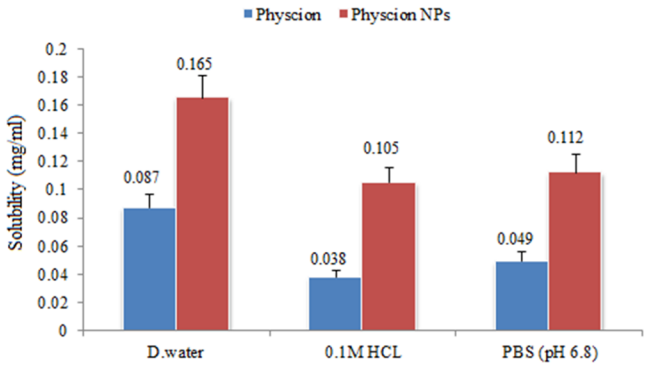

Figure 6. Solubility studies of unprocessed physcion and its NPs.

3.2.2. In Vitro Dissolution Studies. Such studies are mostly used as an alternative to in vivo studies in investigating the bioavailability and absorption of poorly water-soluble drugs for both cost reduction and ethical considerations.[42] These assays embrace the general principle of "No unnecessary human testing should be performed." The present study aims to assess the in vitro dissolution profile of physcion and its NPs that can be used as a surrogate for its absorption. The dissolution studies of the test samples were conducted in different media including distilled water, 0.1 M HCl, and phosphate buffer (pH 6.8) over time periods of 0, 15, 30, 45, 90, and 120 min. Figure 7 shows the enhanced drug dissolution of physcion NPs compared to unprocessed physcion in different media. In this work, the dissolution of physcion and its NPs was assessed in terms of the fit factors. Moreover, the MTD and DE were also applied. It has been documented that if a drug possesses high DE, the drug is expected to remain in contact with the absorbing membranes for a prolonged period of time and thus exhibit high bioavailability. MTD is used to statistically determine the mean dissolution time for a drug, which provides an accurate drug release profile.[43] Physcion NPs rapidly dissolved in distilled water with a release of >85% in 30 min. It showed slightly lower dissolution in 0.1 M HCl (75%) and phosphate buffer (80%). On the other hand, the crystalline drug showed very low and incomplete dissolution, where <20% was dissolved during the complete assay duration (120 min). Our results showed that the prepared NPs exhibit a 4-fold increase in the dissolution profile compared to the plain drug during the first 15 min of the release duration. The improved dissolution profile of the NPs can be attributed to several

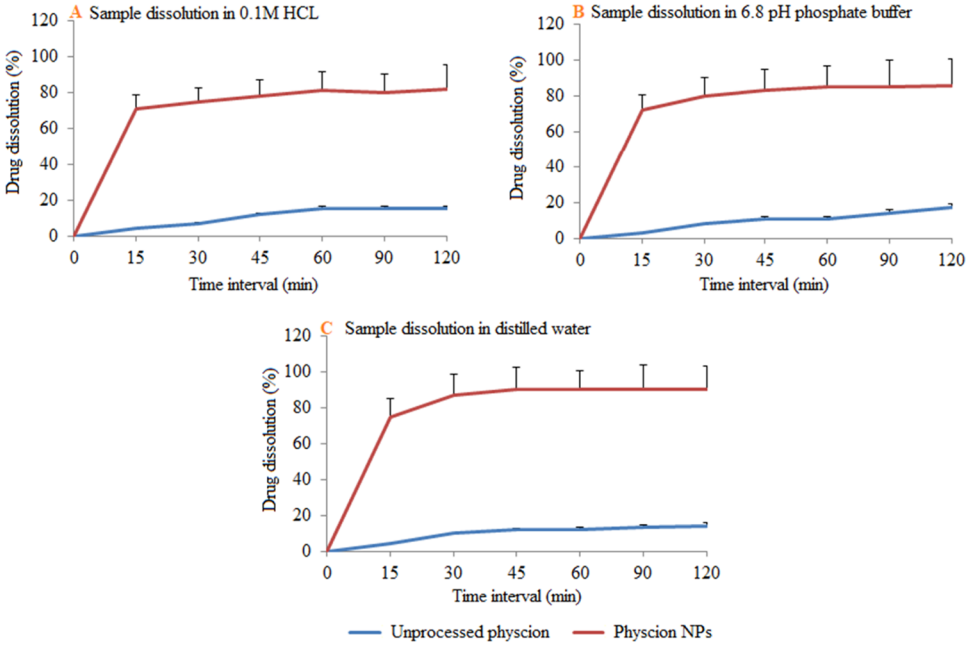

Figure 7. Drug dissolution profiles at various time intervals for unprocessed physcion and physcion NPs at (A) gastric pH, (B) intestine pH, and (C) distilled water under a physiological environment (37.0 ± 0.5 °C).

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Table 1. Different Dissolution Parameters[a]

dissolution media	distilled water				0.1 M HCl				phosphate buffer (pH 6.8)			
parameters used	MDT, (min)	DE (%)	f_1	f_2	MDT, (min)	DE (%)	f_1	f_2	MDT, min	DE (%)	f_1	f_2
physcion	41.5	10			38.25	07			37.0	08		
physcion NPs	15.2	87	93.6	20.2	17.8	75	24.3	38.5	20.5	80	20.3	41.5

[a]MDT: mean dissolution time; DE: dissolution efficiency; f_1: dissimilarity factor; f_2: similarity factor.

factors, including high surface area, polydispersibility, reduction in crystallinity, and agglomeration. According to the literature, the crystalline form is more rigid and possesses stronger intramolecular forces than its amorphous counterpart. Therefore, it does not form hydrogen bonds with water easily and shows less dissolution.[44]

f_2 is used to compare the dissolution profiles of pure physcion and its NPs in different media. A value of <50 shows a significant difference between their dissolution profiles. In the present study, f_2 was recorded to be 20.2, 38.5, and 41.5 in distilled water, 0.1 M HCl, and phosphate buffer, respectively (Table 1), which shows the enhanced dissolution of the NPs. Similarly, the MDT of physcion NPs was significantly reduced in all test media. The MDT was reduced from 41.5 to 15.2 min in distilled water, while in HCL and PBS, it was reduced from 38.25 to 17.8 min and from 37 to 20.5 min, respectively. Moreover, the DE of NPs was significantly improved. It was enhanced from 10 to 87%, 7 to 75%, and 8 to 80% in distilled water, HCL, and PBS, respectively. In the present study, the results of MDT and DE support the conclusion drawn from the f_2 analysis.

3.3. Antioxidant Assays. *3.3.1. DPPH Scavenging Assay.* The results of the DPPH radical scavenging assay are summarized in Table 2. The antioxidant potential of test samples was investigated at various concentrations, e.g., 31.5, 62.5, 125, 250, 500, and 1000 μg/mL. At a concentration of 1000 μg/mL, the antioxidant activity of physcion and its NPs was 51 and 75%, respectively, whereas at the same concentration, the standard ascorbic acid showed 80% activity. The corresponding IC_{50} values of physcion, NPs, and standard were 33.31, 27.56, and 23.70 μg/mL, respectively. Physcion NPs showed comparable results to ascorbic acid at 1000 μg/mL. In this assay, the NPs exhibited a concentration-dependent scavenging activity against the DPPH model.

Table 2. DPPH Free Radical Scavenging Activity of Physcion, Physcion NPs, and Standard

	DPPH scavenging activity (%)		
concentration (μg/mL)	physcion	physcion NP	ascorbic acid
31.5	4.19 ± 0.26	7.50 ± 2.10	11.7 ± 0.90
62.5	16.78 ± 1.10	31.75 ± 4.468	39.3 ± 3.54
125	36.79 ± 2.36	45.0 ± 5.833	50.0 ± 4.93
250	39.81 ± 2.98	60.25 ± 5.21	68.3 ± 6.82
500	46.90 ± 4.27	66.74 ± 4.48	75.3 ± 7.35
1000	51.18 ± 5.55	75.0 ± 2.90	80.7 ± 9.57
IC_{50}	33.31	27.56	23.70

3.3.2. FRAP Scavenging Assay. The FRAP radical scavenging potential of the test samples is depicted in Table 3. At a concentration of 1000 μg/mL, the antioxidant activity of physcion, NPs, and standard was 44, 84, and 90%, with IC_{50} values of 28.0, 22.30, and 21.28 μg/mL, respectively. The results demonstrate that the scavenging activity of physcion NPs was approximately equal to that of standard at 1000 μg/mL. In the FRAP model, both the NPs and standard had almost similar IC_{50} values, indicating that the potency of crude physcion was significantly improved by its conversion into NPs.

Table 3. FRAP Scavenging Activity of Physcion, Physcion NPs, and Standard

	FRAP scavenging activity (%)		
concentration (μg/mL)	physcion	physcion NP	ascorbic acid
31.5	3.0 ± 0.12	15.45 ± 2.15	28.34 ± 1.65
62.5	8.34 ± 0.98	38.70 ± 3.40	45.25 ± 3.80
125	26.78 ± 2.0	59.56 ± 5.85	70.80 ± 4.45
250	30.80 ± 2.52	68.20 ± 4.34	78.45 ± 6.89
500	39.72 ± 4.11	78.85 ± 6.50	83.65 ± 6.30
1000	44.95 ± 4.90	84.0 ± 6.90	90.78 ± 7.75
IC_{50}	28.0	22.30	21.28

3.3.3. Hydrogen Peroxide Scavenging Assay. The hydroxyl radical scavenging activity of physcion, NPs, and standard is presented in Table 4. Physcion NPs had higher antioxidant potential than unprocessed physcion at all studied concentrations. At a concentration of 1000 μg/mL, the inhibitory potential values of physcion, NPs, and standard were 37, 70 and 88%, respectively. The corresponding IC_{50} values were 29.80, 22.68, and 16.42 μg/mL, respectively. It is evident from our results that the antioxidant potential of physcion powder was almost doubled by its conversion into NPs.

Table 4. Hydrogen Peroxide Scavenging Activity of Physcion, Physcion NPs, and Standard

	H_2O_2 scavenging activity (%)		
concentration (μg/mL)	physcion	physcion NP	ascorbic acid
31.5		11.22 ± 1.0	18.73 ± 1.0
62.5	5.66 ± 0.88	21.56 ± 2.40	33.51 ± 2.34
125	9.67 ± 0.93	30.0 ± 2.88	44.0 ± 3.31
250	20. 37 ± 1.22	42.23 ± 4.14	56.31 ± 5.94
500	27. 73 ± 2.0	59.71 ± 5.50	70.50 ± 7.0
1000	37. 69 ± 3.28	70.10 ± 7.11	88.95 ± 10.12
IC_{50}	29.80	22.68	16.42

A number of pathophysiological conditions such as cancer, neurodegenerative disorders, and diabetes are associated with the production of free radicals in the body.[45] Free radicals are atoms or molecules that possess unpaired electrons and are, therefore, extremely unstable. They have a tendency to pair with biological macromolecules including proteins and DNA in normal cells, thus causing their damage.[46] Such cell damage usually becomes more widespread due to the weaker cellular antioxidant defense system. There are several innate antioxidant defense mechanisms in the human body that clear damaged cells. However, these mechanisms may be inefficient. Therefore, the consumption of natural antioxidants is necessary for the protection of normal cells.[47] It has been

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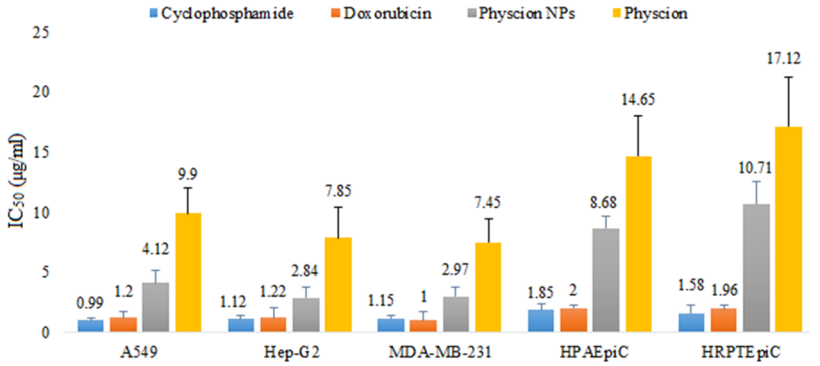

Figure 8. IC_{50} values of the standards, physcion NPs, and physcion against selected cancer and normal human cell lines.

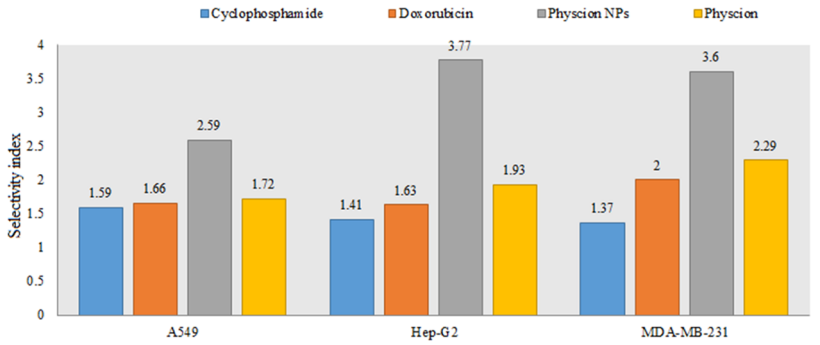

Figure 9. Selectivity indices of standards, physcion NPs, and physcion against renal primary epithelial cells.

reported that antioxidants stabilize the damaged cells by providing electrons to these cells. According to the literature, thioredoxin-1 (Trx-1) and glutathione might be the dominant antioxidant systems that convert reactive oxygen species (ROS) to non-harmful compounds. They also control the nitrosylation and glutathionylation of proteins and act on transcription factors and signaling pathways, resulting in the prevention of cell apoptosis.[48] They also convert free radicals into water-soluble byproducts that are then eliminated from the body. The regular consumption of fruits and vegetables lowers the risk of many diseases caused by free radicals. Several phytochemicals such as vitamins, polyphenols, and anthraquinone glycosides are considered to have strong antioxidant properties. The antioxidant potential of vitamins and polyphenols is well documented; however, scientific information on the antioxidant properties of physcion, an anthraquinone glycoside, is scarce.[49] Therefore, the investigation of such properties remains an interesting task, particularly to find new sources of natural antioxidants for nutraceuticals and/or consumption foods.

It has been well documented that the rate and extent of drug absorption are affected by its aqueous solubility, dissolution, and gastrointestinal permeability. The water solubility of a drug is an important parameter in the absorption of drugs after oral administration. In recent years, the number of BCS class II and IV drug molecules used in the mitigation of different diseases has been comparatively higher, and their GIT absorption and biological activity depend on water solubility. To address the limitations of these drugs, several techniques have been developed, including particle size reduction, antisolvent co-precipitation, nanoparticle production, salt formation, complexation with cyclodextrins, and APSP methods.[50] In the present study, physcion NPs depicted improved antioxidant activity as compared to raw powder. The enhanced activity of the former may be attributed to its greater solubility and fast dissolution rate in different dissolution media.

3.4. Cytotoxicity Assay. In the MTT assay, physcion NPs showed considerable and dose-dependent growth inhibition of A549, HepG2, and MDA-MB-231 cancer cell lines. The % cell viability of physcion NPs, unprocessed physcion, and standards against cancer cell lines is depicted in Figures S1−S3. Physcion NPs revealed considerable cytotoxic potential against A549 (12%), HepG2 (10%), and MDA-MB-231(18%) at a concentration of 10 mg/mL. The unprocessed form showed relatively less effect, while the standard drugs cyclophosphamide and doxorubicin showed 3 and 4%, 3 and 6%, and 2 and 8% cell viability against A549, HepG2, and MDA-MB-231, respectively, at the same concentration. On the other hand, the % cell viability of physcion NPs against the normal cell lines HPAEpiC (21%) and HRPTEpiC (23%) was relatively high at 10 mg/mL concentration, which shows the safety of the test drug toward non-cancerous cells (Figures S4 and S5). In the present study, the test samples were further investigated for their IC_{50} values in order to determine their effectiveness level against the studied cell lines.

The IC_{50} values of test samples along with standard drugs are shown in Figure 8. Physcion NPs revealed significant cytotoxic potential against MDA-MB-231 cell lines with an IC_{50} of 2.97 μg/mL compared to cyclophosphamide (1.15 μg/mL) and doxorubicin (1 μg/mL). Similarly, the NPs showed considerable activity against A549 and HepG2 with IC_{50} values of 4.12 and 2.84 μg/mL, respectively. On the other hand, physcion NPs showed higher IC_{50} values against the normal cell lines HPAEpiC and HRPTEpiC (IC_{50} of 14.65 and 17.12, respectively), which reveals the safety of the test drug.

To further validate the specificity of physcion NPs toward cancer cell lines, the data were further analyzed to determine the SI values (Figures 9 and 10). SI values of 2 or more

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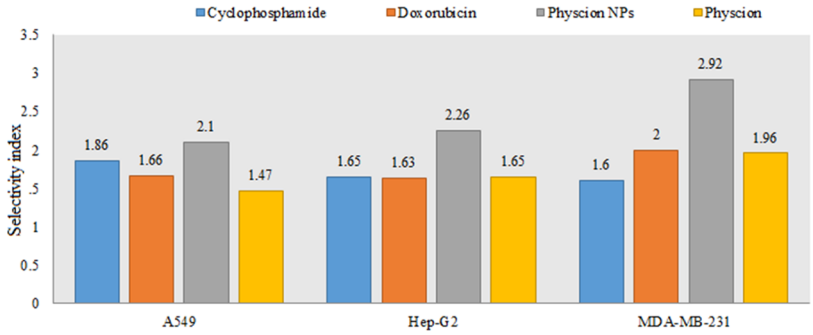

Figure 10. Selectivity indices of standards, physcion NPs, and physcion against alveolar primary epithelial cells.

indicate high specificity of the test samples.[51] The SI values of physcion NPs were 2.59 (A549), 3.77 (HepG2), and 3.6 (MDA-MB-231) using HRPTEpiC as normal cells. Likewise, they demonstrated high SI values against the said cell lines using HPAEpiC as normal cells. These results suggest that physcion NPs exhibited considerable cytotoxicity without affecting the normal cell lines.

Several studies have been reported in order to address the poor water solubility of cytotoxic drugs, including the use of prodrugs, surfactants, nanocolloids, polymeric NPs, and lipoidal microspheres.[52] However, these approaches are limited by several challenges including but not limited to instability, low drug loading capacity, potential material toxicity, complex physical structures, altered drug distribution, and clearance.[53] APSP is a formulation technique in which a saturated solution of a poorly water-soluble drug is slowly injected into a specific volume of an antisolvent under mechanical stirring. Subsequently, the turbid solution is evaporated to obtain nanosized drug particles. This method aids the dissolution of poorly water-soluble drugs primarily by presenting them in an amorphous form, thereby lowering the total energy required for the solubilization of the crystalline drug. The APSP method has produced tremendous benefits for therapeutically potent, poorly water-soluble drugs such as silibinin and berberine.[27,54]

4. CONCLUSIONS

The APSP technique has been used in this study to improve the dissolution rate and solubility of physcion. Owing to the reduction in particle size and crystallinity, physcion NPs revealed a high dissolution rate as compared to unprocessed physcion. Furthermore, physcion NPs demonstrated strong antioxidant and antiproliferative potential as compared to the raw counterpart. Due to their unique size-dependent properties, physcion NPs could offer the possibility to develop new site-specific antiproliferative formulations.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c04821.

Cell viability (%) of physcion, NPs, and standards against different cell lines (PDF)

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Notes

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