

Human Growth Hormone Fragment 176–191 Peptide Enhances the Toxicity of Doxorubicin-Loaded Chitosan Nanoparticles Against MCF-7 Breast Cancer Cells

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Introduction: Numerous drugs with potent toxicity against cancer cells are available for treating malignancies, but therapeutic efficacies are limited due to their inefficient tumor targeting and deleterious effects on non-cancerous tissue. Therefore, two improvements are mandatory for improved chemotherapy 1) novel delivery techniques that can target cancer cells to deliver anticancer drugs and 2) methods to specifically enhance drug efficacy within tumors. The loading of inert drug carriers with anticancer agents and peptides which are able to bind (target) tumor-related proteins to enhance tumor drug accumulation and local cytotoxicity is a most promising approach.

Objective: To evaluate the anticancer efficacy of Chitosan nanoparticles loaded with human growth hormone hGH fragment 176–191 peptide plus the clinical chemotherapeutic doxorubicin in comparison with Chitosan loaded with doxorubicin alone.

Methods: Two sets of in silico experiments were performed using molecular docking simulations to determine the influence of hGH fragment 176–191 peptide on the anticancer efficacy of doxorubicin 1) the binding affinities of hGH fragment 176–191 peptide to the breast cancer receptors, 2) the effects of hGH fragment 176–191 peptide binding on doxorubicin binding to these same receptors. Further, the influence of hGH fragment 176–191 peptide on the anticancer efficacy of doxorubicin was validated using viability assay in Human MCF-7 breast cancer cells.

Results: In silico analysis suggested that addition of the hGH fragment to doxorubicin-loaded Chitosan nanoparticles can enhance doxorubicin binding to multiple breast cancer protein targets, while photon correlation spectroscopy revealed that the synthesized dual-loaded Chitosan nanoparticles possess clinically favorable particle size, polydispersity index, as well as zeta potential.

Conclusion: These dual-loaded Chitosan nanoparticles demonstrated greater anti-proliferative activity against a breast cancer cell line (MCF-7) than doxorubicin-loaded Chitosan. This dual-loading strategy may enhance the anticancer potency of doxorubicin and reduce the clinical side effects associated with non-target tissue exposure.

Keywords: anticancer potency, nanoparticles, cytotoxicity, docking analysis

Introduction

Nearly around 19.3 million new cases of cancer along with 10 million cancer-related deaths have been reported by the GLOBOCAN figures for 2020.¹ Moreover, it is also being predicted that cancer will soon eclipse cardiovascular diseases

as the leading cause of death.² According to these estimates, female breast cancer has overtaken lung cancer (11.7% vs 11.4% of all cases) as being the most commonly diagnosed cancer worldwide.¹

Many drugs as well as drug combinations with potent toxicity against cancerous cells have been developed for chemotherapy. However, these drugs can also damage non-cancerous cells, so a major challenge in cancer treatment is to deliver optimal amounts of drug precisely to the tumor sites without any exposure to the normal cells.³ In addition, bio-transformation, acquired drug resistance, and altered drug clearance can further diminish the efficacy of conventional chemotherapeutic drugs.³

To mitigate these problems, many novel drug delivery techniques have been developed for tumor-targeted chemotherapy.^{4–8} These include drug-loaded nanoparticles that which are able to bind with high affinity to cancer-associated proteins.^{6,8,9} For instance, targeting of a drug delivery vehicle such as a nanoparticle complexed with chemotherapeutic agent could be enhanced by co-loading the vehicle with peptide ligand to a receptor over-expressed on the tumor.¹⁰ Recent reports have also mentioned magnetic nanoparticles loaded with luteinizing hormone-releasing hormone (LHRH) to be able to aid in breast cancer diagnosis as well as enhancing its drug delivery.¹¹

Some other potential carriers against breast cancer include those studied by Azandaryani et al,¹² who studied the efficiency of Letrozole containing folate-conjugated polymer-lipid hybrid nanoparticles in the treatment of breast cancer. It was observed that the entrapment as well as therapeutic efficiency of letrozole within the amphiphilic carrier were significantly increased by the use of lipid nanoparticles along with surface modification, respectively.¹²

Alqaraghuli et al¹³ evaluated the effectiveness of the delivery of Epirubicin (Epr) through encapsulation with horse spleen apoferritin (HsAFr) cavity. Additionally, in this study, the surface of HsAFr-encapsulated Epr was also modified with folic acid (FA) for enhanced as well as optimal targeting of the breast cancer cells (MCF-7). It was also noticed in the drug release study that the HsAFr provided controlled drug release from Epr loaded with HsAFr, carried out at 37°C and at a pH of 7.4. The HsAFr–Epr–FA complex was tested on a human breast cancer cell line and the results demonstrated highly elevated toxicity against the MCF-7 cell line as compared to the free drug. It was also observed that 2 µM of free Epr, Epr-loaded HsAFr and HsAFr–Epr–FA demonstrated decreased cell viability.¹³

Human growth hormone (hGH or somatotropin), is a 191-amino acid peptide hormone that is secreted by the anterior pituitary gland that stimulates longitudinal bone growth and tissue expansion.¹⁴ Receptors for hGH are widely expressed throughout the body, especially in the liver. In addition to tissue growth promotion, hGH modulates body fat composition as well as carbohydrate and protein metabolism.¹⁵ In adipose tissue, hGH induces breakdown of triglycerides by stimulating hormone-sensitive lipase and also inhibits glucose uptake which is essential for adipocyte differentiation.¹⁶ Moreover, hGH is an autocrine oncogenic factor that promotes the proliferation of breast cancer stem cells.¹⁷ Therefore, hGH-related peptides may be extremely beneficial in drug targeting and possibly for disrupting hGH-dependent breast cancer progression as well.

Human growth hormone is composed of 4 major α -helices which are arranged in an up-up-down-down topology required for receptor interaction along with three mini helices.¹⁸ A C-terminal hGH fragment 176–191 with a tyrosine to phenylalanine substitution at the last position has been reported to enhance lipid breakdown and fat utilization in mice.^{19–21} And hence, in the present study we examined if hGH fragment 176–191 peptide can facilitate the anticancer efficacy of doxorubicin-loaded nanoparticles.

Chitosan nanoparticles (CN NPs) are particularly efficient delivery vehicles for both peptides as well as for chemotherapeutic drugs that which are used in breast cancer treatment due to their good biocompatibility, susceptibility to enzymatic hydrolysis, and for their high molecular carrying capacity.¹¹ The topo isomerase inhibitor doxorubicin (Adriamycin) is frequently used alone or in combination for the eradication of solid tumors, but a major limitation of this agent is that it also induces non-cancer cell death, thereby resulting in numerous side effects including cardiovascular toxicity. Doxorubicin toxicity to healthy cells can be reduced by targeted drug delivery as well as by reducing the administered dose. Therefore, in the present study, we examined if the addition of hGH fragment 176–191 peptide to doxorubicin-loaded CN NPs can enhance *in silico* target binding as well as *in vitro* toxicity against breast cancer cells. Furthermore, we also examined if these dual-loaded CN NPs have physicochemical properties favorable for clinical application.

Materials and Methods

Part I: In silico Analysis

Two sets of experiments were performed in silico to determine (1) the binding affinities of hGH fragment 176–191 peptide to the breast cancer receptors, (2) the effects of hGH fragment 176–191 peptide binding on doxorubicin binding to these same receptors, (3) to evaluate the influence of hGH fragment 176–191 peptide on the anticancer efficacy of Doxorubicin.

Modeling of Doxorubicin Structure

The two-dimensional structure of doxorubicin (Figure 1) was downloaded (DB00997) in .mol format from Drug Bank database (<https://go.drugbank.com/drugs/DB00997>)²² and converted to Protein Data Bank (PDB) file format (.pdb) for 3D modeling using PEP-FOLD3.5 and docking analysis using Autodock 4.2 tools.²³ The doxorubicin model was also subjected to the CHARMM²⁴ energy minimization protocol in Discovery Studio Visualizer 2021 [BIOVIA, 2021].

Modeling of hGH Fragment 176–191 3D Structure

The hGH fragment 176–191 peptide sequence (YLRIVQCRSVEGSCGF) was submitted to the PEP-FOLD3.5 webserver. It uses the Hidden Markov Model sub-optimal conformation sampling approach for predicting small peptide 3D structures. After model generation using PEP-FOLD3.5, the 3D structure was further refined and assessed for quality using the MolProbity tool of the Swiss Model server. Finally, the suitability of the peptide structure for docking was analyzed using the Ramachandran plot function in Discovery Studio Visualizer.

Modeling of Target Protein Structures

The 3D structure files of the breast cancer biomarkers estrogen receptor (PDB:1QKU), progesterone receptor (PDB:2OVH), human epidermal growth factor receptor (PDB:3GOP), mind bomb (Mib) protein (PDB:4TSE), and Ki-67 (PDB:5J28) were then obtained from Protein Data Bank²⁵ (Figure 2; Table 1). Published structures were subsequently edited in order to remove non-standard residues by making use of the Discovery Studio Visualizer 2021. For energy minimization, the UCSF Chimera program (www.cgk.ucsf.edu/chimera) with the following conjugate gradient minimization settings was used: removal of steric collision with steepest descent steps set to 1000, steepest descent size to 0.02 Å, conjugate gradient steps set to 1000, as well as the conjugate gradient step size to 0.02 Å. Energy minimized structures were saved as PDB files for docking simulations.²⁶

Predicting Peptide–Receptor (Breast Cancer Biomarker) Interactions

Receptor–peptide complexes were modeled by the PatchDock online docking server (<https://bioinfo3d.cs.tau.ac.il/PatchDock/>), which makes use of a geometry-based molecular docking algorithm as a scoring function.²⁷ After docking analysis, complexes were additionally refined by using the FireDock online server.²⁸

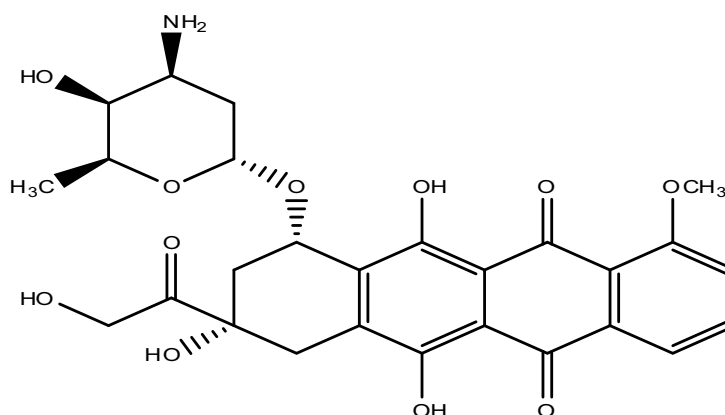


Figure 1 Two-dimensional (2D) structure of doxorubicin.

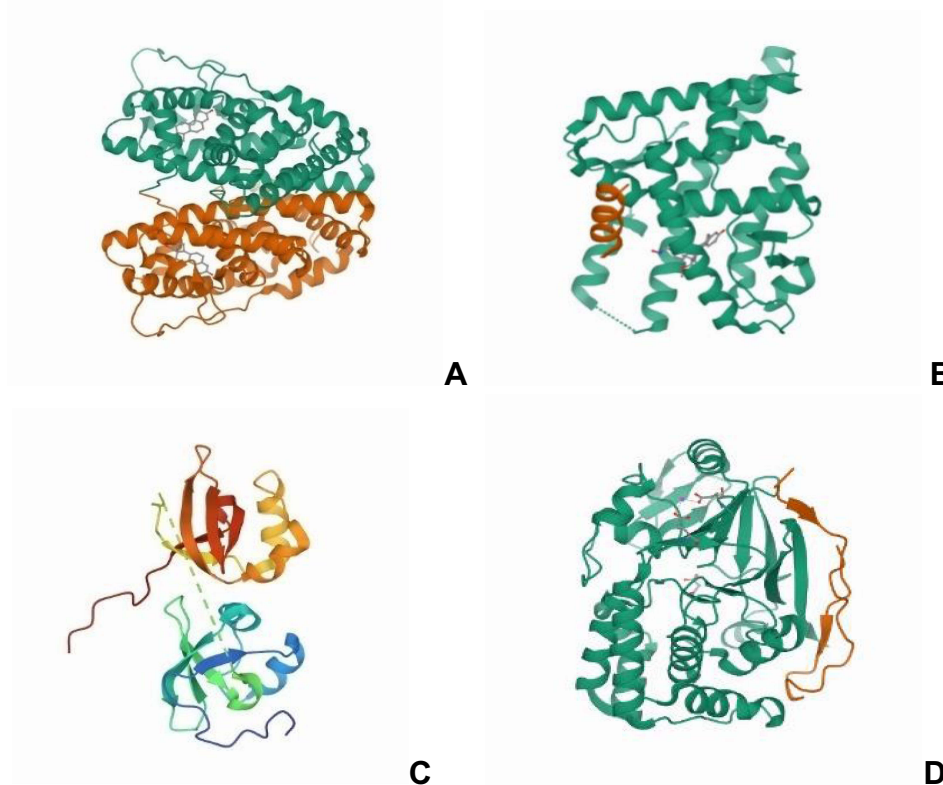


Figure 2 Crystal 3D structure of (A) estrogen receptor (PDB:1QKU), (B) progesterone receptor (PDB: 2OVH), (C) human epidermal growth factor receptor (PDB:3GOP), and (D) mind bomb (Mib) protein (PDB:4TSE), Ki-67 (PDB:5J28). All 3D graphics were obtained from RCSB-PDB.

Molecular Docking Analysis

Docking of doxorubicin with tumor biomarkers (receptors) was simulated using Autodock Version 4.2.^{23,29} Autodock searches for the best conformation of receptor and doxorubicin were on the basis of its binding energy. Water molecules were subsequently removed from the 3D files of receptor molecules prior to docking and then later on hydrogen atoms were added to all the target proteins. Gasteiger charges were also added to the receptors. A 60×60×60 Å grid box was also set for covering the entire surface of the receptor with a default grid point spacing of 0.375 Å. The Lamarckian genetic algorithm (LGA)²⁵ was then used for making receptor-drug flexible docking calculations. The LGA parameters were set as follows: population size (ga_pop_size) = A, energy evaluations (ga_num_generation) = B, mutation rate = C, crossover rate = D, step size = E, and number of LGA runs = 10. Molecular docking simulations of doxorubicin to peptide-bound receptors were also conducted using a 75×75×75 Å grid and default parameters.

Table 1 Structural Information on Selected Breast Cancer Biomarkers

Receptor Name	UniProt ID	PDB ID	Reference
Estrogen receptor (ER)	P03372 (ESR1_HUMAN) https://www.uniprot.org/uniprot/P03372	1QKU	[40]
Progesterone receptor (PR)	P06401 (PRGR_HUMAN) https://www.uniprot.org/uniprot/P06401	2OVH	[41]
Human epidermal growth factor receptor (HER2)	P00533 (EGFR_HUMAN) https://www.uniprot.org/uniprot/P00533	3GOP	[42]
Mind bomb (Mib) protein	Q86YT6 (MIB1_HUMAN) https://www.uniprot.org/uniprot/Q86YT6	4TSE	[43]

Part - II: Nanoparticle Preparation

Doxorubicin, low molecular weight (LMW) Chitosan (95% deacetylated) and glacial acetic acid were purchased from Sigma-Aldrich (Merck group, U.S.A.) while gum Arabica (Gum Acacia) was purchased locally from Gizan, Jazan Province. Other pharmaceuticals along with analytical grade materials were used in the present study as received.

Preparation of Dual hGH Fragment 176–191 Peptide- and Doxorubicin-Loaded Chitosan Nanoparticles (Ch-hGH-DOX)

Chitosan nanoparticles were synthesized using the ionic gelation method of Avadi et al,³⁰ with slight modification.³¹ Negatively charged gum Arabica was dissolved in water at normal room temperature under constant stirring, while positively charged LMW Chitosan was dissolved in 0.1% aqueous acetic acid also at normal room temperature, and under constant stirring. The pH of both solutions was subsequently adjusted to 5.5 with the addition of 0.5 M sodium hydroxide. The indicated concentrations of doxorubicin and hGH 176–191 fragment were added to the Chitosan solution. Nanoparticles were then prepared by making use of dropwise addition of gum Arabica to LMW Chitosan solution under constant magnetic stirring (200–300 rpm) at normal room temperature for three hours. Composition of the optimal dual-loaded nanoparticles is presented below in Table 2.

Measurement of Particle Size, Polydispersity Index, as Well as the Zeta Potential

Photon correlation spectroscopy was used for measuring the mean particle size (PS), polydispersity index (Pdl), as well as the zeta potential (ZP) (Malvern, Nano ZS90, UK). All formulations were dissolved in double-distilled water (Millipore) prior to the analysis. To avoid multi-scattering effects, the appropriate particle concentration was obtained for each dilution. All measurements were made in triplicate.

Part - III: Effects of Ch-hGH-DOX on Breast Cancer Cell Viability

Human MCF-7 breast cancer cells were procured for the present study from American Type Culture Collection (ATCC, Manassas, VI, USA) and were grown in RPMI-1640 medium (pH 7.4). It was further supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 g/mL streptomycin. For both propagation as well as the treatment, cells were maintained in an incubator (Heraeus, GmbH, Germany) at 37°C; humidity (90%) along with CO₂ (5%).

Cell Viability Assay

The cytotoxicity profiles of the indicated formulations were analyzed by making use of the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) microculture tetrazolium viability assay.³² Cells were then seeded in multi-well plates and later incubated for 48 hours with the indicated concentration of Ch-hGH-DOX, Ch-DOX, or DOX (0–2.5 µg/mL). Each concentration was tested in triplicate, with each plate also including untreated controls along with a blank cell-free control. Subsequent to the incubation, MTT (5 mg/mL) was then added to each of the wells and the plates were further incubated for another 4 hours. The medium was then removed and DMSO (100 µL/well) was added for solubilizing the formazan crystals formed from MTT by viable cells. The optical density (OD) at 490 nm was recorded as an estimate of remaining viable cell number by making use of a microtiter plate reader (BioTek Instruments).

Table 2 Composition of Dual-Loaded Chitosan Nanoparticles

Factor	Concentration
Chitosan	1 mg/mL
Gum Arabica	10 mg/mL
hGH (176–191) fragment	50 nm/mL
Doxorubicin	1 mg/mL

Growth inhibition (%) was calculated from the OD according to the following formula:

$$\text{Growth inhibition} = (\text{OD control} - \text{OD treated}) / \text{OD control} \times 100$$

The cytotoxicity of the sample on cancer cells was expressed as the IC₅₀ (the concentration that reduces the OD by 50% relative to untreated cells).

Statistical Analysis

Statistical Package for the Social Sciences (SPSS-10 Inc., Chicago, IL., USA) version 23 was used for data analysis. All data are presented as mean \pm standard deviation (SD). One-way ANOVA was used for comparing treatment group means. $p < 0.05$ was considered statistically significant.

Results and Discussion

In silico Analysis

The estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Mib1/Ki-67 proliferation index are firmly established biomarkers used for the diagnosis, prognosis, and treatment guidance of primary, recurrent, and metastatic breast cancer patients. Additionally, these biomarkers are established therapeutic targets for breast cancer as well.

The 3D Structure of hGH Fragment 176–191 Peptide

In total, 63.64% of peptide residues were in the favorable zone for docking simulation according to the Ramachandran plot, while only 9.09% were in Ramachandran Outlier and 7.69% in Rotamer Outlier regions (Figure 3A).³³ In addition, the MolProbity score was 2.00, and no C-Beta deviations, bad angles, or bonds were found³⁴ (Figure 3B).³⁵ Thus, the peptide 3D structure proved suitable for docking analysis.

The docking simulations in our study depicted that the hGH fragment 176–191 peptide binds with high affinity to Ki-67, MiB protein, and the estrogen receptor, but not with the progesterone receptor or HER2 (Table 3). The peptide also influenced simulated doxorubicin binding to these breast cancer receptors (Table 4). For example, the binding energy score for doxorubicin to the progesterone receptor was -10.09 kcal/mol in the absence of hGH fragment 176–191 peptide, but was -11.31 kcal/mol after peptide binding. Furthermore, peptide binding reduced the doxorubicin inhibition constant for the progesterone receptor from 139.01 to 14.41 nM. In addition to the progesterone receptor, hGH fragment 176–191 peptide binding also augmented doxorubicin binding to HER2 and MiB protein, but slightly reduced binding to the estrogen receptor and Ki-67.

There is compelling evidence indicating that progesterone receptors (PRs) playing a hierarchical role in breast cancer growth and therefore they might potentially be useful in improving the success of endocrine treatments.³⁶ Likewise, Ki-67 and MiB proteins promoting the proliferation in breast cancer can be the choice of targeting breast cancer treatment (Soliman and Yussif, 2016). Increased affinity of doxorubicin to the progesterone receptor, Ki-67 and MiB protein hence proves worthiness of the hGH fragment 176–191 peptide in targeting breast cancer cells with increased efficacy.

In silico work presents with a good predictive model of what may actually happen in the cellular environment. Therefore, the efficacy of the hGH fragment 176–191 peptide was further evaluated in in-vitro condition.

Nanoparticle Preparation

Ch-hGH-DOX was successfully prepared by the ionic gelation technique developed by Avadi et al to produce Chitosan nanoparticles from a mixture of Chitosan and gum Arabica.³¹ The electrostatic interactions occurring between the positively charged Chitosan as well as the negatively charged Arabica gum also facilitate the incorporation of other charged compounds such as peptides. The formula for the synthesis of Ch-hGH-DOX (Table 3) was optimized on the basis of PS, uniformity of dispersion, and ZP.

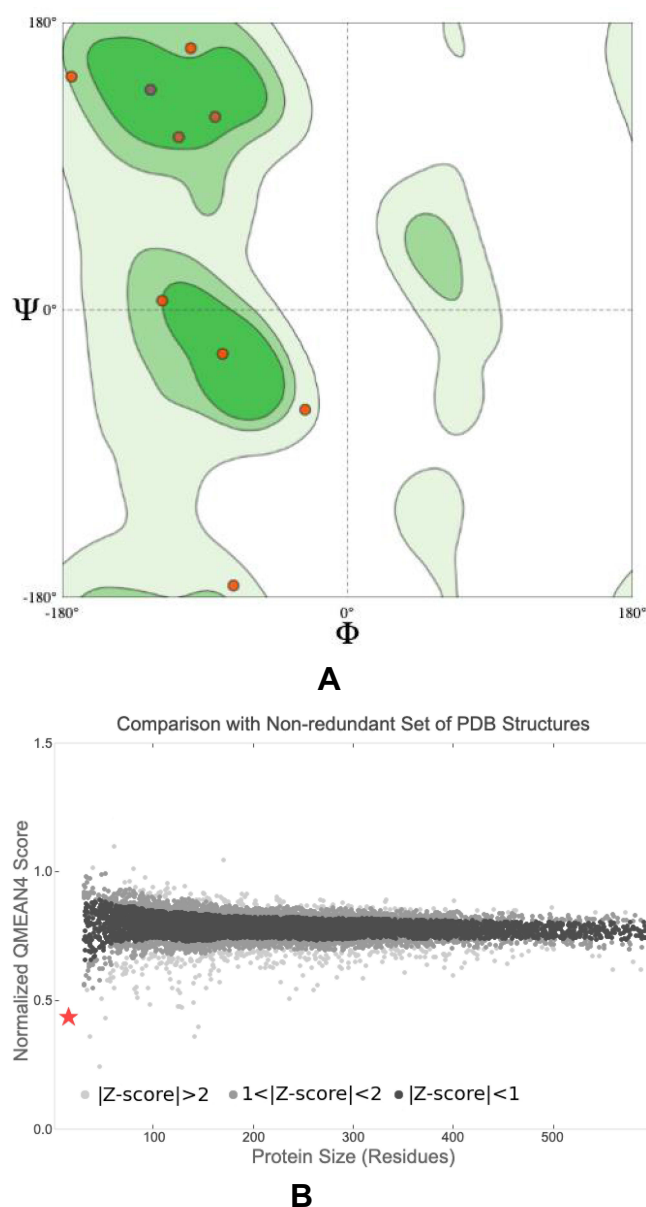


Figure 3 Quality of the hGH fragment 176–191 peptide 3D-model structure **(A)** Ramachandran plot showing distribution of peptide residues -phi (Φ) is the N(i-1), C(i), Ca(i), N(i) torsion angle and psi (Ψ) is the C(i), Ca(i), N(i), C(i+1) torsion angle. **(B)** The QMean score of modeled peptide after comparing with Non redundant set of PDB structures (★) is showing model on Z score plot when comparing it with structure available in PDB database.

PS, Polydispersity Index, and ZP

These Ch-hGH-DOX nanoparticles were first examined for PdI value, ZP, and PS distribution using a Malvern Zetasizer. Mean PS (90.25 nm), PdI (0.197), and ZP (31.7 mV) were all considered acceptable for subsequent testing in biological preparations (Table 5; Figure 4A and B).

In the present study, we found the particle size to be less than 100 nm and hence we can suggest that these nanoparticles can be considered suitable for the effective delivery of the chemotherapeutic agents. According to Danaei et al 2018,³⁷ nanocarriers having a particle size lesser than 150nm (or 200 nm) are considered to be extremely beneficial and effective for the treatment of cancers as they passively target the tumor cells by means of improved permeability as well as retention.³⁷ The polydispersity index (PdI) of Ch-hGH-DOX nanoparticles was found to be less than 0.2 this indicates that our formulation not only has a monodispersity conduct but it also has a much lesser tendency

Table 3 Docking Results of the hGH Fragment 176–191 Peptide Model with Breast Cancer Receptor Models After Refinement Using the FireDock Server

Receptor Names	PDB IDs	Global Energy	Attractive VdW	Repulsive VdW	ACE	HB
Estrogen receptor (ER)	1QKU	−48.29	−30.94	22.87	−11.63	−3.04
Progesterone receptor (PR)	2OVH	−21.78	−23.16	10.44	−0.43	−1.03
Human epidermal growth factor receptor (HER2)	3GOP	−31.65	−17.47	2.92	−4.21	−2.26
Mind bomb (Mib) protein	4TSE	−51.27	−25.50	18.61	−11.15	−6.86
Ki-67	5J28	−56.62	−25.30	11.74	−17.16	−2.50

Notes: Global Energy - The binding energy of the solution. Attractive and Repulsive VdW - The contribution made by the van der Waals forces to the global binding energy. ACE - The contribution made by the atomic contact energy (ACE) to the global binding energy. HB - The contribution made by the hydrogen bonds to the global binding energy.

Table 4 Docking Results for Doxorubicin with Unbound Breast Cancer Receptors and hGH Fragment 176–191 Peptide-Bound Breast Cancer Receptors

Breast Cancer Biomarkers Receptor	Receptor vs Doxorubicin		Receptor + Peptide vs Doxorubicin	
	Final Intermolecular Energy (Kcal/mol)	Inhibition Constant (Ki)	Final Intermolecular Energy (Kcal/mol)	Inhibition Constant (Ki)
Estrogen receptor (PDB:1QKU)	−2.77	NA	−1.58	NA
Progesterone receptor (PDB:2OVH)	−10.09	139.01 mM	−11.31	14.41 mM
Human epidermal growth factor receptor (PDB:3GOP)	−6.55	39.10 mM	−8.02	6.02 mM
Mind bomb (Mib) Protein (PDB:4TSE)	−5.76	158.20 mM	−6.11	74.65 mM
Ki-67 (PDB:5J28)	−7.62	2.81 mM	−6.84	20.54 mM

Table 5 Physiochemical Characterization of Ch-hGH-DOX by Photon Correlation Spectroscopy

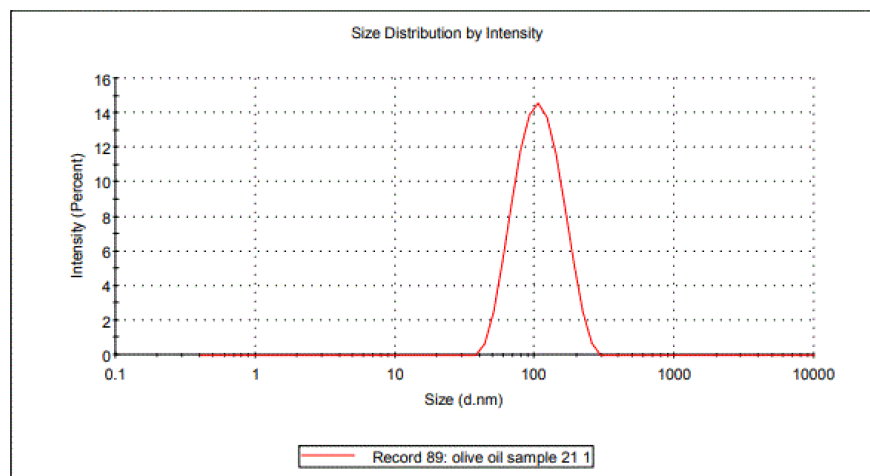
Formulation Code	Size (Z-Average) (d.nm) (n = 3) (±SD)	PdI (n = 3) (±SD)	Zeta Potential (mV) (n = 3) (±SD)
Ch-hGH-DOX nanoparticles	90.25 ± 0.94	0.197 ± 0.01	31.7 ± 2.36

towards aggregation.³⁸ With regard to the Zeta potential it was observed in the present study that the Ch-hGH-DOX nanoparticles showed high positive values. With a ZP greater than 30 mV it can be said that Ch-hGH-DOX nanoparticles have a tendency of stabilizing colloidal preparations and thus preventing particle aggregation.³⁹

With the aforementioned findings, we can conclude that the Ch-hGH-DOX nanoparticles prepared by the present method have shown to possess characteristics that are favorable for cellular uptake as well as having colloidal stability, including suitable diameter, surface charge along with a low polydispersity index that which is indicative of a reasonably homogeneous size distribution.

Results

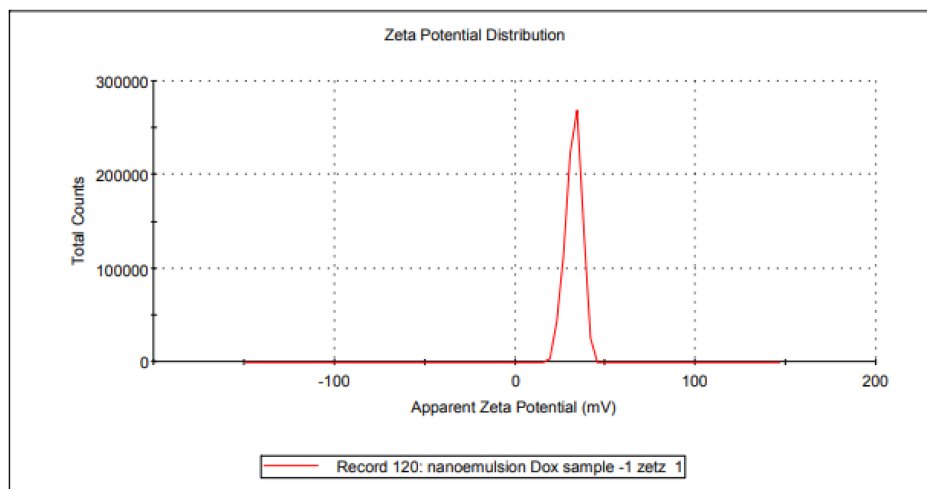
	Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 91.33	Peak 1: 112.8	100.0	41.96
Pdl: 0.196	Peak 2: 0.000	0.0	0.000
Intercept: 0.937	Peak 3: 0.000	0.0	0.000
Result quality: Good			



A

Results

	Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): 32.4	Peak 1: 32.4	100.0	4.50
Zeta Deviation (mV): 4.50	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 0.0131	Peak 3: 0.00	0.0	0.00
Result quality: Good			



B

Figure 4 (A) Size distribution of Ch-hGH-DOX nanoparticles (size = 91.33 d.nm) (n = 1), (B) Zeta potential distribution of Ch-hGH-DOX nanoparticles (ZP = 32.4 mV) (n = 1).

Anticancer Efficacy of Ch-hGH-DOX Nanoparticles

Finally, we compared the cytotoxic effects of these Ch-hGH-DOX nanoparticles to Ch-DOX nanoparticles. MCF-7 breast cancer cells when treated with varied concentrations of Ch-hGH-DOX nanoparticles for 48 hours yielded an IC₅₀ value

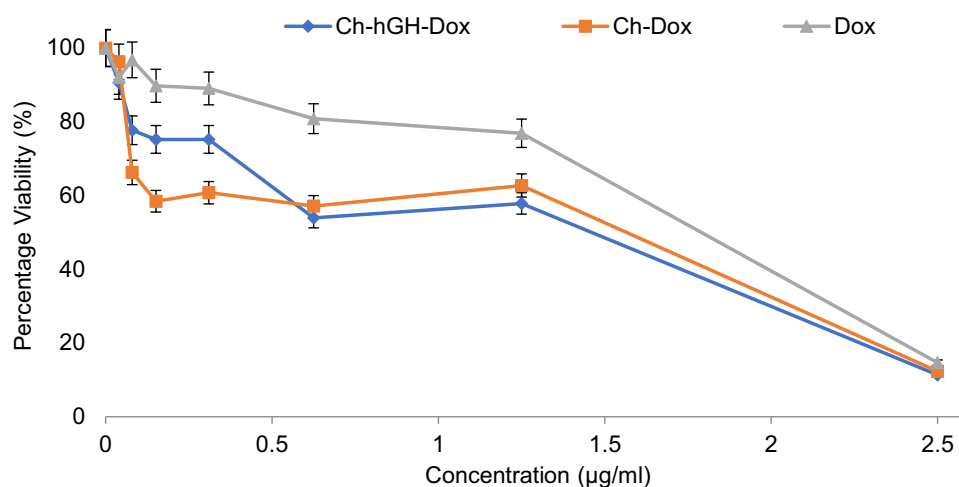


Figure 5 Cytotoxicity of the different Chitosan nanoparticle formulations against MCF-7 cells. Cells were treated for 48 hours and remaining viable cells estimated by MTT assay.

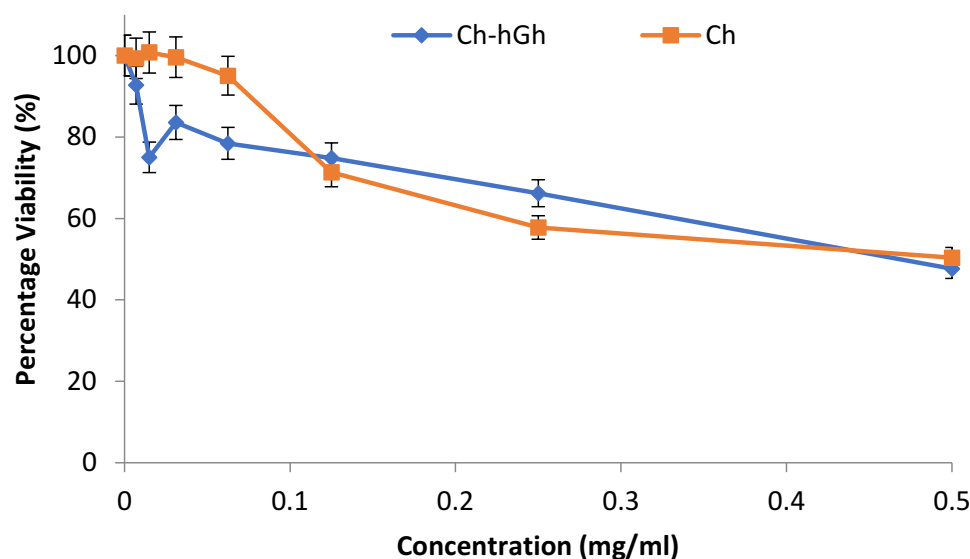


Figure 6 Cytotoxicity of different blank formulations against MCF-7 cells. Cells were treated and viability measured as described in Figure 4.

lower than those for doxorubicin alone and doxorubicin-loaded Chitosan nanoparticles (1.5 µg/mL vs 1.85 µg/mL as well as 1.7 µg/mL, receptively) (Figure 5). In contrast, neither unloaded Chitosan nanoparticles nor peptide-loaded Chitosan nanoparticles demonstrated substantial cytotoxicity (Figure 6). Furthermore, within the intermediate dose range (0.15 to 1.25 mg/mL), the proportion of viable MCF-7 cells remaining was significantly lower after 48 hours of Ch-hGH-DOX treatment than after Ch-DOX or DOX treatment (Figure 5). Therefore, hGH fragment 176–191 peptide appears to substantially enhance the toxicity of doxorubicin-loaded Chitosan nanoparticles, possibly by enhancing doxorubicin binding to breast cancer target proteins (Table 4).

Some of the potential limitations of the study are its inability to presently conduct any morphological studies, entrapment efficiency, as well as in-vitro release study.

Conclusion

Chitosan nanoparticles loaded with both doxorubicin and hGH fragment 176–192 peptide demonstrate both favorable physicochemical properties and enhanced cytotoxic efficacy against breast cancer cells. Molecular docking simulations

suggest that the enhanced cytotoxicity stems from greater binding of doxorubicin to peptide-bound target proteins compared to unbound targets. Moreover, the inclusion of hGH fragment 176–192 peptide may promote accumulation of doxorubicin-loaded Chitosan nanoparticles in breast tumors while reducing non-target tissue exposure, thereby enhancing therapeutic efficacy and reducing side effects.

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Disclosure

The authors report no conflicts of interest in this work.

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