RESEARCH ARTICLE

Hydrocortisone-loaded Lipid-Polymer Hybrid Nanoparticles for Macrophage Targeted Delivery in Chronic Obstructive Pulmonary Disease

Gaurav Tiwari[1], Saumya Singh[1], Shakkeela Y E Ahammed[2], Rajapandi Raju[3], Ashish A Jaiswal[4*]

[1]*PSIT-Pranveer Singh Institute of Technology (Pharmacy), Bhauti, Kanpur, India.*
[2]*Department of Pharmaceutical Chemistry, Unaizah College of Pharmacy, Qassim University, Unaizah, Kingdom of Saudi Arabia.*
[3]*Department of Pharmaceutical Analysis, Arulmigu Kalasalingam College Of Pharmacy, Krishnankoil, Viruthunagar, Tamil Nadu India.*
[4]*Technical Services, Upsher-Smith Laboratories, Minnesota, USA.*

Received: 15th August, 2023; Revised: 08th January, 2024; Accepted: 21st February, 2024; Available Online: 25th March, 2024

ABSTRACT

The multifaceted illness known as a chronic obstructive pulmonary disease (COPD) is characterized by a decline in post-bronchodilator pulmonary function in all individuals and is linked to local and systemic inflammation, attracting alveolar macrophages (AM). The objective of this study was to use carubinose-linked hydrocortisone-hybrid nanoparticles (HC-HNPs) to increase the glycotargeting effectiveness to AM. The emulsification solvent evaporation method was used to generate the HC-HCNPs, and various analytical techniques were used to characterize them, including %entrapment efficiency, analytical characterization and histopathology examinations. Results revealed that HC is entrapped inside the hybrid preparations, which contain specific signatures of molecules that interact with ligands and are expressed all through the cell surface, as well as the amorphous nature of HC-HNPs. Field emission scanning electron microscopy (FESEM) revealed the surface structure and the particle size diameter which is enough to reach the AM through the nasal route. The high-performance liquid chromatography (HPLC) showed no interference from excipients in determining HC, indicating that the method is specific. The *in-vivo* findings showed that HC-HNPs demonstrated proof that very small and cost-effective nanoparticles can be developed to effectively target AM by effectively delivering HC-HNPs to the lungs.

Keywords: Hydrocortisone, Carubinose-linked hydrocortisone-hybrid nanoparticles, Alveolar macrophages, *In-vivo* findings.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.1.22

How to cite this article: Tiwari G, Singh S, Ahammed SYE, Raju R, Jaiswal AA. Hydrocortisone-loaded Lipid-Polymer Hybrid Nanoparticles for Macrophage Targeted Delivery in Chronic Obstructive Pulmonary Disease. International Journal of Drug Delivery Technology. 2024;14(1):160-164.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a common preventable and curable condition characterized by elevated airflow limitations and a chronic inflammatory condition. It is a common illness that is mostly brought on by cigarette smoking and results in worsening airway blockage (chronic bronchitis and lung emphysema).[1] The main pathophysiological cause of COPD development is cigarette smoking (CS), which also contributes to other etiologic factors like pollution both indoors and outdoors, as well as world population aging.[2,3]

The recently developed lipid polymeric hybrid nanoparticle (LPHNP) provides the ability to target different cells or tissues. Because the LPHNP strategy uses carubinose (D-Mannose) to bind directly to GR in alveolar macrophages (AM), HC is able to reduce chronic inflammation and edema.[4] pharmaceuticals can be encapsulated into hybrid systems (HS) to stop drug escape and increase system stability. The HS preserves desired biological properties while lowering toxicity and prolonging drug release. It is possible to create site-specificity by applying additional surface modifications. It has a higher degree of Patient ease and conformity than injectables. This is a more practical way to give medicine to elderly, incapable, or sleeping people.[5] The current work aimed to create hybrid systems functionalized with carubinose that would deliver the hydrocortisone payload to alveolar macrophages. Higher patient compliance and a reduction in dosage are expected outcomes of the effective administration of hydrocortisone to AM *via* hydrocortisone-hybrid nanoparticles (HC-HNPs).

**Author for Correspondence: ajaiswal.1520@gmail.com*

MATERIALS AND METHODS

Materials

Hydrocortisone was purchased from Yarrow Chem Products, Mumbai. Carubinose (D-Mannose), soy lecithin and ethyl cellulose were purchased from Central Drug House. Milli Q water from Millipore (Bedford, USA) was utilized in the research facility for all high-performance liquid chromatography (HPLC) quantifications. In HPLC research, chromatographic grade solvents were employed. Analytical grade materials and reagents from Merck Germany were employed throughout the investigation.

Corticosteroid-loaded Hybrid Nanoparticle Preparation

Emulsion solvent evaporation

- *Step-1*

To create LPHNPs, polymeric nanoparticles and lipid-based particles, such as liposomes, blended together, and the polymer nanoparticles' exterior was adhered to by the lipid bilayer or lipid multilayer. These HC-HNPs vary based on the kind of LPHNs needed for a specific use. Using the two-phase method, the polymer core and lipid shell are made independently and then combined to have a PLN bilayer. Polyvinyl alcohol (PVA), which has both biocompatible and biodegradable qualities, makes up the polymer phase of these LPHNPs. PVA has the best oxygen barrier qualities of any known polymer; however, moisture must be kept away to prevent degradation of its gas permeability. PVA is a polymer that dissolves in water and resembles proteins. The physical properties, especially the film shape, and water solubility of PVA are significantly influenced by the level of crystal precipitating, molecular mass, and the process of hydrolysis. Soy lecithin, a phospholipid, is used in the lipid phase and is valued for its enhanced biocompatibility compared to polymerized particles. Hydrophobic cellulose ether-based ethylcellulose polymer is a biomaterial. HC, ethyl cellulose, and soy lecithin were dispersed in a different beaker during the lipid phase while being continuously stirred using a magnetic stirrer. PVA was dissolved in distilled water in the polymeric phase while being constantly stirred using a magnetic stirrer. Next, a magnetic stirrer and a syringe will be used, following the addition of the PVA solution (the aqueous phase) to the hydrocortisone-containing lipid phase while continuously stirred. The mixture was continuously stirred for thirty minutes and then sonicated. After filtering, the hybrid formulation was lyophilized and kept in a desiccator.[8-10]

- *Step-2 Lyophilized hybrid formulation coating*

Carubinose coating was done in acetate buffer pH 4.0 in order to achieve the ring-opening of carubinose. After an hour of continuous stirring, the lyophilized formulation was finally combined with the carubinose coating buffer solution. The carubinose-linked hybrid formulation subjected to filtration underwent additional lyophilization for storage and analysis.[9]

Hybrid Nanoparticles-loaded with Corticosteroids Lyophilized

Freeze drying, also referred to as lyophilization, is a process in which occurs when the sample is frozen and the water evaporates. It involves freezing water and then removing it from the sample. A Thermo Scientific Forma -86°C freezer was used to carry out the lyophilization process. The amorphous content of the nanoparticles remained in the interstitial area while pure water crystallized as a result of the nanoparticles freezing at a low temperature of -80°C for a full day. The sample is lyophilized for a period of 48 hours at an extremely high vacuum.[9,10]

Fourier-transform Infrared Spectroscopy Evaluation

The involvement of organic functional groups in HC, carubinose, HC-HCNPs, and mixtures of HC and carubinose was investigated using fourier-transform infrared spectroscopy (FTIR).[7,8] The Perkin Elmer Model No. 234 infrared spectrophotometer results were documented, and images of every formulation were provided in the 4000 to 400 cm^{-1} wavelength range.

Percentage Entrapment Efficiency (%EE)

To determine drug entrapment, 5 mg of formulation was mixed in pH 6.8 using a vortex. It was centrifuged at 4000 rpm for 15 to 20 minutes. After being removed, the supernatant layer was evaluated at a wavelength of 246 nm.[8]

Differential Scanning Calorimetry Evaluation

When analyzing polymeric materials, differential scanning calorimetry (DSC) is a strong analytical instrument for determining a variety of physical characteristics and temperature changes. It calculates the energy that flows to or from a sample that is changing chemically or physically.[11] Using Thermo gravimetric Analyzers, Q600, TA Instruments, US, the thermal properties of hybrid nanoparticles loaded with corticosteroid and HC were examined. DSC measurements was conducted in a nitrogen-filled environment with a weight corresponding to 1 to 5.25 mg.

Thermogravimetric Analysis Studies

As a sample's temperature rises, its weight is recorded. The two main components of the apparatus are a programmed furnace that regulates the sample's heat-up rate and an extremely sensitive scale that measures weight variations.[12] Thermogravimetric analysis **(**TGA**)** was used to analyze the thermal properties of the hybrid nanoparticles loaded with

Figure 1: Cigarette smoking-induced COPD in wistar rats

corticosteroid and HC. The sample was sealed into an alumina pan and heated to a temperature of 25.84°C.

X-ray Diffraction Pattern Studies

X-ray diffraction (XRD) is a non-destructive method for identifying a material's chemical makeup, crystallographic structure, and physical characteristics. It operates by employing a crystalline sample. Shorter wavelength electromagnetic radiation, or X-rays, are created when electrically charged particles accelerate to a high enough energy.[10-12] A 1.52 mm high divergence fixed slit was employed. Sample recordings were made with parameters: diffraction angle of 2θ and a maximum step size of 0.001.

Scanning Electron Microscopy Evaluation

Scanning electron microscopy evaluation (SEM) imaging is an electron-based imaging technique. Backscattered or secondary electrons are identified by scanning the sample's surface with the incident electron beam in a pattern called a raster.[23] With TESCAN from the Indian Institute of Technology, Kanpur, the surface morphology of the HC-HNPs was examined utilizing a field emission scanning electron. Images of the prepared hybrid nanoparticles loaded with corticosteroids were captured at two distinct magnifications: 25.0 and 50.0 kx.

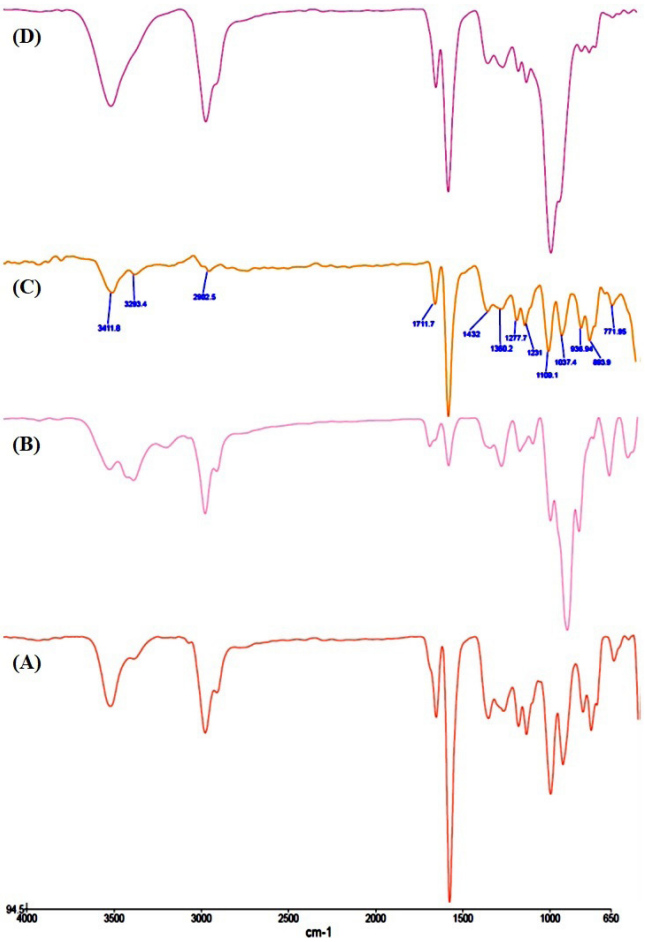

Figure 2: FTIR images of (A) HC; (B) carubinose, (C) physical mixture of HC and carubinose; (D) HC-HCNPs

In-vivo Study

Wistar rats were used for whole-body (WB) or nose-only (NO) exposure to cigarette smoke (CS). Three categories—control (n = 3), negative (n = 3), and test (n = 3) were randomly assigned to the rats. The rat's mouth was attached to the cigarette chamber while restrained softly for the nose-only exposure.[12-14] In this protocol:

- The daily CS exposure started at two cigarettes and increased to six cigarettes (three in the morning and three in the evening), with a five-minute gap between each cigarette and a pace of 15 minutes per cigarette. After four weeks of exposure, coughing and sneezing were seen. The animal started to lose weight at 24 weeks; by 36 weeks, they had significantly decreased in weight.
- About 20 smokes a day, twice a day for an hour, five days a week, for up to 16 weeks (Figure 1).

Lung tissue was removed and preserved in 4% formalin solution after the necessary amount of time had passed. A percentage was assigned to each degree of inflammation. The study on lung histology was completed at the Kanpur Diagnostic Centre located in Kalyanpur, Kanpur.[14]

Weight and Mortality Changes

Three groups of wistar rats weighing 170 mg each received a nasal delivery of a dosage of 1000 mg/kg. Using the Organization for Economic Co-operation and Development (OECD) guidelines 423,[15,16] morbidity and responsiveness were recorded for each wistar rat every 24 hours throughout a 14-day period.

RESULTS AND DISCUSSIONS

Evaluation of HC-HNPs

HC exhibits a significant, broad stretching of O-H at 3422 cm^{-1}, alkane (C-H) stretching (medium) at 2924 cm^{-1}, a strong ketone (C=O) stretching at 1710 cm^{-1}, a strong alkene (C=C) stretching at 1640 cm^{-1}, and carboxylic acid (O-H) bending at 1435 cm^{-1}, a potent stretching of secondary alcohol (C-O) at 1108 cm^{-1}, whereas carubinose also exhibits an associated category of powerful, O-H stretching at 3297 cm^{-1}, medium C-H stretching at 2921 cm^{-1} (Figure 2(B)). The hybrid formulation's HC and carubinose groups are slightly moved toward 2902 cm^{-1}; the HC and carubinose groups of the alkane (C-H) are linked with the carubinose group at 1639 cm^{-1}, resulting in a medium-intense peak; the secondary alcohol group of the carubinose is attached to the vinyl ether group at 1107 cm^{-1} and has a high-intensity show stretching at 1019 cm^{-1}, which indicates a bending of the secondary alcohol and vinyl ether group and shows a potent bond formation (Figure 2(C)).

A strong, broad stretching of amine salt (N-H) at 2924 cm^{-1} also indicates the presence of carubinose binding with HC and other excipients, strong stretching; in HC-HNPs, strong bond formation at 3424 cm^{-1} indicates the existence of alcohol (O-H) bond, indicating that the HC is associated with carubinose as well as other excipients (Figure 2(D)). It was discovered that 79% of HC-HNPs had %EE of HC. Figure 3(A) displayed the

DSC thermogram of HC, which showed a sharp endothermic peak at 200°C, which corresponds to its melting point of 195 to 200°C. As seen in Figure 3(B), HC-HNPs lost their sharp appearance and instead showed a broad endothermic peak at that temperature.

According to TGA analysis, the HC-HNPs are shown in Figure 4(B), while the HC Figure showed weight loss and melting point at about 278.7°C Figure 4(A). According to the graph, the HC-HNPs weighed 100% at 21.66°C. At the glass transition temperature of 297.6°C, the sample's weight began to decrease at a rate of approximately 90.79% as the temperature increased. In the third stage, a 33.92% sudden loss of weight at 413.11°C. The weight has dropped to 8.61% by the end. Consequently, the observed total weight fluctuation was 92.36%, or 4.710 mg of residue Figure 4(B).

The X-ray diagrams of HC Figure 5(A) and HC-HNPs Figure 5(B) showed higher and lower peak intensities, indicating that the crude drug is found in a crystalline state. In contrast, HC-HNPs are found in an amorphous state, which is necessary for the formulation to be delivered *via* the nasal route. The size and shape of HC-HNPs before (Figure 6(A)) and after (Figure 6(B)) lyophilization were examined through field emission scanning electron microscopy (FE-SEM). It were observed that HC-HNPs was irregular and porous while the particle size was 2 µm.

In-vivo Study

A histological examination of the lungs reveals that the typical rat has emphysematous alveolar walls with few lymphocytes, plasma cells, and little fibrosis. Plasma cells and lymphocytes are seen in the peribronchiolar tissue. This section describes the 40% lung tissue destruction in the negative rat, including polymorphs, lymphocytes, plasma cells, and alveolar

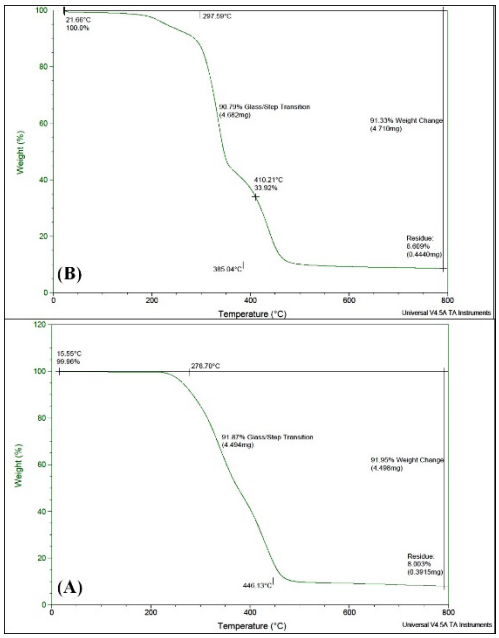

Figure 4: TGA analysis of (A) HC; (B) HC-NPs

macrophages. However, no granulomas or malignant cells were seen, indicating moderate chronic lung inflammation. Hemostasis and bleeding in large regions accompanied by hemosiderin-loaded macrophages. Plasma cells and lymphocytes are seen in the peribronchiolar tissue. There are no granulomas visible. There are no cancerous cells visible. The section shows how polymorphs, lymphocytes, and plasma cells destroyed 10% of the test rat lung tissues with alveolar macrophages; this suggests that HC-HNPs were responsible for 30% of the gain in lung function. Comparing the test rat to the negative control, minor necrosis and hemorrhages were

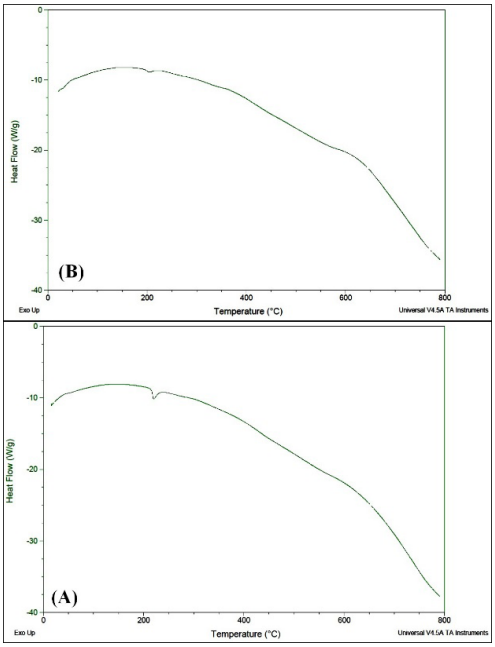

Figure 3: Endothermic peaks of (A) HC; (B) HC-NPs

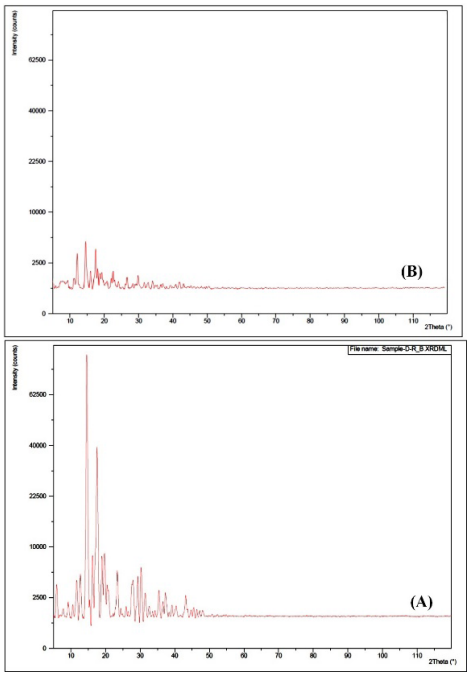

Figure 5: X-ray diffractogram of (A) HC; (B) HC-NPs

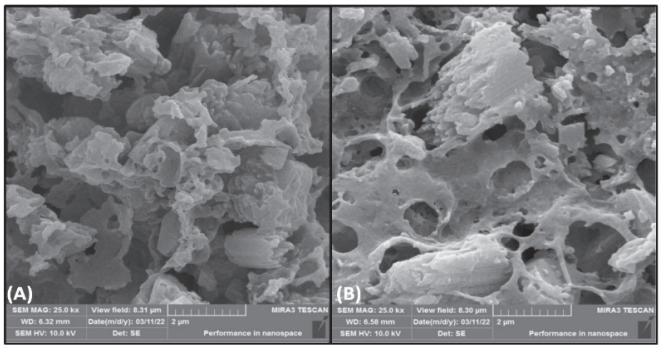

Figure 6: SEM images of (A) HC; (B) HC-NPs

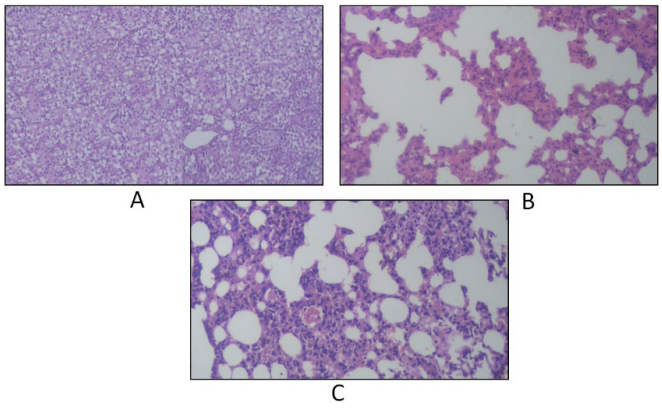

Figure 7: Histopathology images of (A) Control, (B) Negative and (C) Test

associated with hemosiderin-loaded macrophages. Plasma cells and lymphocytes are seen in the peribronchiolar tissue. There are no granulomas visible. No cancerous cells are visible (Figure 7).

CONCLUSION

Polymeric nanoparticles frequently offer controlled release to the needed location, involve both hydrophilic and hydrophobic drugs, and offer much greater stability. However, inadequate loading abilities and scaling up are drawbacks. The advantages of lipid-based systems include improved entrapment, prolonged drug release, biodegradability and biocompatibility, as well as increased drug solubility and scalability. The drawbacks include unstable drug loss and limited surface modifications by carefully choosing lipids and polymers with the right properties. It was possible to successfully regulate the release properties of hydrophobic HC using HC-entrapped hybrid systems. The hybrid technique's glycotargeting approach makes direct target drug delivery advantageous when interacting with ligands (e.g., carubinose). In terms of their effectiveness, the hybrid particles that have been reported were predicted to show themselves to be appealing substitutes for single nanoparticulate systems.

REFERENCES

1. Al–Tufaily Y, Hussein QN, Hussein KF. Zinc Status in Children with Bronchial Asthma. International Journal of Drug Delivery Technology. 2022;12(2):851-854. DOI: 10.25258/ijddt.12.2.69
2. Al-Mustafa SS, Al-Sultan RG. Histological Changes of Liver and Lung Induced by Bisphenol a in Pregnant Mice. International Journal of Drug Delivery Technology. 2022;12(2):678-681. DOI: 10.25258/ijddt.12.2.37
3. Kadhim HM, Mosawi AMTA. Effects of Emodin and Salvianolic Acid on Carbon Tetrachloride (CCl4)-induced Lung Fibrosis in Mice Model. International Journal of Drug Delivery Technology. 2021;11(4):1269-1274. DOI: 10.25258/ijddt.11.4.25
4. Srivastava N, Tiwari G, Tiwari R. Polyherbal preparation for anti-diabetic activity: a screening study. Indian Journal of Medical Sciences. 2010;64:4:163. DOI: 10.4103/0019-5359.97356
5. Tiwari R, Pathak K. Local Drug Delivery Strategies towards Wound Healing. Pharmaceutics. 2023;15:2:634. DOI: 10.3390/pharmaceutics15020634
6. Tiwari G, Tiwari R, Srivastava B, Bhati L, Pandey S, Pandey P, Bannerjee SK. Drug delivery systems: An updated review. International Journal of Pharmaceutical Investigation. 2012;2(1):2-11. DOI: 10.4103/2230-973X.96920
7. Tiwari R, Singh I, Gupta M, Singh LP, Tiwari G. Formulation and Evaluation of Herbal Sunscreens: An Assessment Towards Skin Protection from Ultraviolet Radiation. Pharmacophore. 2022;13(3):41-9. DOI: 10.51847/svzLRFMP5F
8. Gupta A, Tiwari G, Tiwari R, Srivastava R. Factorial designed 5-fluorouracil-loaded microsponges and calcium pectinate beads plugged in hydroxypropyl methylcellulose capsules for colorectal cancer. International Journal of Pharmaceutical Investigation. 2015;5(4):234-46. DOI: 10.4103/2230-973X.167688
9. Mishra AP, Chandra S, Tiwari R, Srivastava A, Tiwari G. Therapeutic Potential of Prodrugs Towards Targeted Drug Delivery. Open Medicinal Chemistry Journal. 2018;12:111-123. DOI: 10.2174/1874104501812010111
10. Tiwari G, Tiwari R, Rai AK. Cyclodextrins in delivery systems: Applications. Journal of Pharmacy & Bioallied Sciences. 2010;2(2):72-9. DOI: 10.4103/0975-7406.67003
11. Al-Rrubaei HA, Al-Jboori MJ, Hameed NM, Al-Terehi MN. Impact of Smoking and Passive Smoking Habit in the XRCC1 Arg399Gln and RAD-18 Arg302Gln Gene Polymorphisms in Type 1 Diabetes Mellitus Patients. International Journal of Drug Delivery Technology. 2021;11(4):1119-1122. DOI: 10.25258/ijddt.11.4.1
12. Lopez-Campos JL, Tan W, Soriano JB. Global burden of COPD. Respirology. 2016;21:14-23.
13. Ma P, Li T, Xing H, Wang S, Sun Y, Sheng X, Wang K. Local anesthetic effects of bupivacaine loaded lipid-polymer hybrid nanoparticles: *In-vitro* and *in-vivo* evaluation. Biomedicine & Pharmacotherapy. 2017;89:689-695.
14. Tiwari R, Tiwari G, Parashar P. Theranostics Applications of Functionalized Magnetic Nanoparticles. In: Jain, K., Jain, N.K. (eds) Multifunctional And Targeted Theranostic Nanomedicines. Springer, Singapore. 2023. DOI: 10.1007/978-981-99-0538-6_15
15. Tahir N, Madni A, Correia A, Rehman M, Balasubramanian V, Khan MM, Santos HA. Lipid-polymer hybrid nanoparticles for controlled delivery of hydrophilic and lipophilic doxorubicin for breast cancer therapy. International Journal of Nanomedicine. 2019;14:4961-4974.
16. Tiwari R, Khatri C, Tyagi LK, Tiwari G. Expanded therapeutic applications of Holarrhena antidysenterica: A review. Comb Chem High Throughput Screen. Published online August 21, 2023. DOI:10.2174/1386207326666230821102502