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Citation on the Research Work

Background of Research

Alzheimer's disease is a type of neurodegenerative disease involving memory loss and loss of patients' cognitive ability. At the end of 2017, there were 121,404 deaths from AD patients were recorded officially, and AD emerged as 6th prominent death cause in US and 5th prominent death cause in patients with age above 65. Americans with the population of 5.8 million recently are living with AD in 2019. However by the end of 2050, 14 million people were projected to be affected. Moreover, the drugs approved by USFDA exhibits Acetylcholinestarase inhibitors as pharmacological effect and these drugs too have limitations to reach brain due to its physicochemical properties and biological barriers to reach brain. In addition, flavonoids and carotenoids are another group of natural drugs of origin that showed antioxidant activity and attracted much attention from researchers due to its multipurpose nature and possible pharmacological action. As aforementioned these drugs also come across biological barriers through which they are unable to reach brain. Thus to overcome these barriers, it was hypothesized to develop core shell nanoparticles which can efficiently load both lutein and curcumin. Additionally, intranasal route was preferred to overcome the blood brain barrier by directly transferring drugs from nose to brain.

Aim and Objectives:

Aim was to develop and characterize core shell nanoparticles for the treatment of Alzheimer's disease using intranasal route. So the objectives of the investigation was to develop lutein and curcumin loaded core shell nanoparticles for increasing targeting efficiency to brain and was also compared with individual core nanoparticles.

Materials and Methods

In the present investigation, several types of nanoparticles were developed viz. chitosan nanoparticles, PLGA nanoparticles and chitosan coated PLGA core shell nanoparticles loaded

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with lutein and curcumin individually and including their respective blank nanoparticles. All six different formulations were compared with each other through characterization, in-vitro, ex-vivo and in-vivo evaluation. Briefly, chitosan nanoparticles of each drug were fabricated using ionic gelation method. The PLGA nanoparticles of each drug were fabricated using nanoprecipitation method. The coating or surface functionalization of chitosan over the PLGA nanoparticles was done using electrostatic interaction. All the formulations and their relative materials including pure form of drugs were characterized for FT-IR, DSC, pXRD and TEM. The in-vitro release study was performed to investigate ability of individual nanoparticles to sustain drug release. The Ex-vivo permeation study was carried out to investigate the ability of individual nanoparticles to cross nasal mucosal membrane. The investigation of cytotoxicity of prepared nanoparticles was performed on two distinct cell lines SH-SY-5Y and RPMI-2650 cell line using MTT assay. ROS generation study was carried out to study whether prepared formulations exhibit any ROS generation or not. Cellular uptake and cellular uptake mechanism studies were perform to investigate cell uptake efficiency of individual nanoparticles and type of pathways followed. Blood brain barrier permeation study was carried out using co-culture modal to investigate permeation efficiency of nanoparticles to reach brain; which entered the blood circulation when administered intranasally. The antioxidant activity of prepared nanoparticles was performed using three different assays viz. SOD, CAT and MDA. The stability study in the form of photostability and thermal stability was performed to investigate the capability of individual nanoparticles to protect both lutein and curcumin when individually loaded into the nanoparticles. The amount of drug distributed in various visceral organs was evaluated using biodistribution study. Drug targeting efficiency and drug transport percentage was evaluated using pharmacokinetic study with the help of SD rats.

Result and Discussion:

The blank chitosan nanoparticles were developed using quality-by-design approach using ionic gelation method. TPP was utilized as cross linker in the preparation of chitosan nanoparticles. In the preparation of chitosan nanoparticles several critical quality attributes and critical process parameters were processed which can influence dependent variables to obtain optimized nanoparticles. With selection of ranges of numerous factors including factors related to drugs, plackett-burman design and followed with box-behnken design was carried out to obtain

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optimized chitosan nanoparticles. Optimized chitosan nanoparticles were obtained in the range of target product profile. Blank PLGA nanoparticles and lutein and curcumin (individually) loaded PLGA nanoparticles were developed using nanoprecipitation method. The coating or surface functionalization of chitosan over the surface of PLGA nanoparticles was carried out using electrostatic interaction. For the same several distinct concentrations of chitosan solutions were used to optimize coating. The DSC, FT-IR, pXRD and TEM images demonstrated that all the materials utilized in the preparation of nanoparticles were compatible with each other. The change in the crystallinity of both pure drugs when loaded into nanoparticles indicated that drugs are efficiently entrapped into the nanoparticles. The TEM images showed that prepared nanoparticles were with smooth surface and round with size within the range i.e. below 200 nm. The in-vitro release study demonstrated that all the prepared nanoparticles were able to sustain the release of both lutein and curcumin, individually. The chitosan nanoparticles and PLGA nanoparticles exhibited biphasic release i.e. initial burst release followed by sustain release and this may be due to adsorption of drugs at the surface of nanoparticles. However, core shell nanoparticles were able to attend more sustained release as compared to chitosan nanoparticles and PLGA nanoparticles. Moreover, the ex-vivo permeation study revealed that prepared nanoparticles were efficiently transporting both drugs across the membrane as compared to their respective pure drug suspension. The MTT assay revealed that all the prepared nanoparticles were found safe to be administered via intranasal route as RPMI 2650 cell line indicating nasal epithelial cells and SH-SY-5Y cells indicating neuronal cells exhibited greater than 90% of cell viability. The cellular uptake and cellular uptake mechanism study demonstrated that all the prepared nanoparticles were efficiently internalized into cells efficiently as compared to control following cavaeloe pathway as one of the pathway for the internalization. None of the prepared nanoparticles exhibited enhancement in the ROS generation indicating its utilization as effective carriers for drug delivery. The BBB permeation study also revealed that nanoparticles can effectively get transported through BBB to reach brain. The three different antioxidant assays demonstrated that drug loaded nanoparticles were able to exhibit greater antioxidant activity as compared to pure drug. The stability study indicated that all the prepared nanoparticles were efficiently protecting the drugs encapsulated within them as compared to free drug suspension. In-vivo biodistribution and pharmacokinetic study demonstrated that maximum amount of drug

was found in the brain revealed the nanoparticles targeting to the brain followed by intranasal administration.

Conclusion

The overall results strengthen the present concept that utilizing core shell nanoparticles as a drug delivery platform can enhance the targeting efficiency of drugs with higher stability and photo and thermal-protection. The intranasal route exhibited as potential route to enhance targeting efficiency of drugs by bypassing several biological barriers and BBB at greater extent. Thus the developed delivery platform can serve as a potential for targeting numerous other therapeutic moieties for many other neurological disorders including Alzheimer's disease.

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