INTRODUCTION

Recently, interest in intranasal drug delivery has increased [1]. A non-invasive strategy to deliver APIs for local, systemic, and CNS action is through the nasal passages [2]. Brain is complex organ that controls thought, memory, emotions, touch, motor skills etc. Together brain and spinal cord that extends from it makeup Central Nervous system (CNS). A major obstacle in treating neurological problems is his BBB. Blood Brain Barrier(BBB) is semi permeable membrane barrier at interface between blood and brain tissue composing endothelial cells, striated cells, pericardium and perivascular mast cells. BBB strictly control the transport of various substances from blood to brain. The penetration efficiency is hard to attain therapeutic concentration in brain parenchyma for effective treatment.. If oral or parenteral administration is considered, the drug must cross her BBB to reach the brain, with some limitations such as: Molecules with high molecular weight, permeability and solubility [3].

The mechanism underlying intranasal delivery to CNS is increasing evidence that neural pathways that connect the nasal passages to the brain and spinal cord. Pathways involving the vascular, CSF and lymphatic systems are used to transport Molecules from nasal passages to the CNS. The olfactory nerve pathway and trigeminal nerve pathway are alternative to treat neurological disorders bypassing BBB. There are 5 stages associated with AD: Alzheimer’s preclinical disease, mild dementia due to Alzheimer’s, moderate cognitive impairment, moderate dementia, Severe dementia.

Central nervous system (CNS) disorders such as Alzheimer's disease, migraines, depression, Parkinson's disease and Huntington's disease are expected to become more prevalent in the 21st century [4]. Defects in cholinergic transmission and low levels of acetylcholine (ACh) are symptoms of Alzheimer's disease (AD), characterized by synaptic loss and degeneration of cholinergic cells in the cortex and other brain regions [5]. ACh levels are elevated to moderate AD levels by cholinesterase inhibitors (ChEI), which catalyze the degradation of AChE in the synaptic cleft [6].

AD is thought to be caused by the abnormal buildup of proteins in and around brain cells.The drugs in current use for treating AD are given orally [7]. Therefore the bioavailability of drug as well as their transport to brain crossing BBB is limited and thus may not achieve effective concentration to treat AD effectively. Delivering drugs through intranasal route to achieve desired concentration at the site of action rapidly and for prolong period could be an effective strategy for managing AD [8]. Clinical manifestations of Alzheimer's disease (AD) include gradual memory loss, impaired judgment, altered decision-making, apraxia, and ultimately death from complete brain failure [9]. About 25 million people worldwide have Alzheimer's disease (AD). There is a growing demand for innovative brain lipid nanoparticles for macromolecular drugs to treat CNS disorders like AD [10].

There are various AChE inhibitors on the market, including rivastigmine, galantamine, memantine, and pioglitazone, which have photostability issues. Therefore, it is of great importance to develop long-term non-gastrointestinal delivery systems for these cholinesterase inhibitors for the treatment of AD. An alternative route, IN administration, has advantages over oral administration and improves patient compliance [14]. Donepezil is the most suitable and commonly used drug due to its safe and stable properties. The over-the-counter tablet Aricept (Donepezil HCL) is a reversible inhibitor of the enzyme acetylcholinesterase. [15]

Nanostructured lipid carriers are novel pharmaceutical formulations composed of physiologically and biocompatible lipids, surfactants and co-surfactants Thus this study attempts has been made to deliver Donepezil HCl to brain through nasal route by formulating Nanostructured Lipid Carrier(NLC). NLC are modified versions and next generation of solid lipid nanoparticles. NLC's are binary mixture of solidified and liquid lipid in formulation typically range between 50-50 upto 90-10.The surfactant present in composition are about 15%(w/v). Surfactant has major role in stability of NLC as well as in preparation of stable formulation by reducing surface tension between lipid phase and aqueous phase. The drug loaded into solid lipids and liquid lipids as dual protection from external degradation factor. Method for preparing NLC have different types: Hot homogenization, cold homogenization, emulsification with ultrasonication, solvent diffusion, solvent emulsification evaporation, film ultrasonication and phase inversion technique. In this research we have made an attempt to prepare a NLC by hot high pressure homogenization followed by ultrasonication method to increase the stability of formulation and reduce the particle size of NLC [11]. NLCs are second-generation lipid nanocarriers with average sizes ranging from 10 to 500 nm[12]. Advantages: low tendency to particle growth, high drug loading capacity. Overcomes problems such as unpredictable gelling tendency, drug release after polymer conversion during storage, and high-water content in dispersions observed at dosages of 5-10 mg/day [13].

In this study Nigella Sativa oil has been used as liquid lipid which is also having antioxidant property and thus may give synergistic action with donepezil for efficient management of AD.

It has been found that essential oils with different chemical constituents have many properties and are also useful in treating various ailments (natural medicine). used. Such as Nigella sativa (NS) oil, which has neuroprotective properties [16] . The chemical component of NS oil is a thymoquinone derivative, which means that nigellon has powerful effects on the brain [17].This oil is also used as an antioxidant. The purpose of this oil is to have a synergistic effect with synthetic drugs like Donepezil for the treatment of Alzheimer's disease.

In this era, contrast agents and therapies for CNS diseases rely heavily on nanomedicine for its promising role in CNS drug delivery [18]. Nanomedicine has been shown to cross BBB and penetrate deep into diseased brain tissue actively and effectively. In addition, nanomedicine is also associated with increased strength, stability, surface area, and sensitivity [19] .

MATERIALS AND METHODS:

Donepezil hydrochloride was obtained as a gift sample from Lupine Limited (Verna, Goa, India). Nigella sativa oil was received as a gift sample from his Rmayra Naturals Impex in Delhi. GMS, Tween 80 and stearic acid are from Mohini Organics Pvt. Ltd Bombay. Compritol 888ATO, Precirol ATO5 and Transcutol P were received as gift samples from Gattefosse, Mumbai. SLS, DPPH were obtained from Loba chem. All other chemicals and reagents used were Analytical Reagent Grade [20].

SCREENING OF LIPIDS:

Solid lipids are one of the major components of NLCs. Glyceryl monostearate, stearic acid, Compritol 888 ATO, Gelucire 43/01, and Precirol ATO 5 were among the solid lipids used for screening. The lipids were heated to +5°C above the melting point of the respective lipid and the drugs were slowly added and shaken gently to solubilize the drugs [21]. Confirmation of drug solubility was performed by visual inspection to ensure that no visible drug particles remained in the molten lipid phase. Drugs with maximum solubility in solid lipids were finalized for subsequent studies [22].

SCREENING OF SURFACTANT:

NLC formulations should contain two surfactants that promote steric and electrostatic stabilization, avoid nanoparticle aggregation, and ensure long-term stability. Surfactants should be selected for formulation according to their charge, molecular weight, and suitability for the desired route of administration [23]. The phase diagram was essential for optimizing the Smix ratio and was constructed using the water titration method. This heated the selected oil phase and maintained it at a temperature of 75 °C, to which the selected Smix was added in various ratios to form a homogeneous mixture [24].

DRUG TO TOTAL LIPID RATIO:

NLC formulations consist of various Drug: Total lipid ratio depending upon nature of lipids used. According to literature various ratios was taken like 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:10, 1:15, 1:20, 1:25. The most stable and clear physical mixture was selected.

ANTI-OXIDANT ACTIVITY OF NIGELLA SATIVA OIL:

The radical scavenging activity of Nigella sativa oil was determined by using DPPH assay. Diphenyl 2-Picryl Hydrazyl is a stable (in powder form) free radical with dark purple colour which turns yellow or colourless when scavenged. The DPPH assay uses this character to show radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (HA) can be written as,

(DPPH)+ (H-A) DPPH-H+ (A)

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the colour changes to yellow or colourless and also the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability [25].

Briefly, 5 ml of an oil was dissolved in 25 ml of toluene. To obtain various test concentrations, different volumes of these oils were adjusted to 2.7 ml. with toluene. Then 0.3 mL of a freshly prepared toluenic solution of DPPH (1mmol/l) was added, and the mixture was vortexed for 1 min at ambient temperature. The samples were allowed to stand for 1 hour in the dark area avoiding sunlight. The colour of the test sample was compared with standard and observed for any colour changes.

DESIGN OF EXPERIMENT:

By performing a preliminary study, you will provide data to explore different formulation variables and processes to generate optimized batches [26]. Thus, we found that the ratio of drug to total lipids, solid to liquid lipids, and surfactant concentration significantly affected the particle size, zeta potential, and entrapment efficiency (% EE) of nanoparticle systems. Therefore, these three variables were selected as independent variables and particle size, zeta potential and % EE as dependent variables in the optimization process using statistical methods in the design of experiments [27]. 23 full factorial designs (Design Expert® software, version 12) were applied to examine the effects of the independent variables on the dependent variables at two levels of His, low and high, respectively (Table). Design batches (F1-F8) representing independent variables with coded values ​​and their levels are shown in Table 1.

DNZ- NLC PREPARATION:

The desired NLC formulation were prepared using Hot High-Pressure Homogenization followed by Ultra-sonication. GMS and Nigella sativa oil were used as the solid and liquid lipid, respectively. The lipid phase contains solid lipid and liquid lipid which were melted +5℃ above the melting point of solid lipid, to which the drug was added under continuous stirring for 5min [28]. Aqueous phase contain surfactant at desired concentration. Both the phases were heated at same temperature. The aqueous phase was added dropwise to lipid phase with mechanical stirring. This pre-emulsion was then pass-through High-Pressure Homogenization at 750bar pressure with 5-6 homogenization cycle [29]. Further, the solution was sonicated by using probe sonicator for 15mins and 50% amplitude. A clear microemulsion was formed after undergoing cooling in ice bath. The liquid nanodroplets of melted lipid transformed into solid nanoparticles at low temperature and produced NLC dispersion. Sodium lauryl sulphate was added as a stabilizer in final formulation. The basic rule for the formulation of NLC is to maintain process temperature at least 5℃ above the melting point of the solid lipid [30] [31].

**CHARACTERIZATION OF NLC:**

**1] DETERMINATION OF PARTICLE SIZE, POLYDISPERSITY INDEX AND ZETA POTENTIAL:**

HORIBA SZ-100 for Windows [Z Type] Ver2.20 was used to measure the size and zeta potential (ZP) of NLCs of all drug loaded samples. All samples were diluted with distilled water to make up a suitable concentration. The Z-average particle size, polydispersity index (PI), And Zeta potential (ZP) values were determined.

**2] DETERMINATION OF TOTAL DRUG CONTENT:**

The total amount of drug in the formulation was determined by dissolving 0.1 ml of the suspension in 10 ml of PBS 6.4. The amount of DNZ in each sample was determined using UV spectrophotometer (1800, Shimadzu, Japan) by measuring the absorbance at a λmax value of 231nm [32]. Each experiment was performed in triplicate. The total drug content was calculated using the following equation:

Total Drug Content = Actual drug added ×100

Theoretical drug added

**3] DETERMINATION OF %EE AND % DRUG LOADING:**

Percent entrapment efficiency & drug loading of NLC were determined by taking 3ml of NLC formulation and centrifuging with the help of ultracentrifuge for 30mins at 60000rpm. The supernatant was collected and further diluted suitably. After dilution the absorbance was taken for all the batches and %EE was calculated by following formula [33].

Entrapment Efficiency= (Amount of drug added − Amount of drug in supernatant) × 100

Amount of drug added

Drug loading Capacity= Amount of drug added − Amount of drug in supernatant × 100

Weight of NLCs

**4]THERMAL ANALYSIS BY DSC:**

Thermal analysis was performed for DNZ HCI, GMS, Nigella sativa oil and DNZ-NLC using DSC (Mettler Toledo, SW STARe, USA). Samples were weighed (5-6mg), the bottom pans were used and hermetically covered with lead. The heating range was 30-300 °C for all samples with constant increasing rate of temperature at 10°C/min under the nitrogen atmosphere (50-60ml/min).The resulted thermogram of drug was compared with thermography obtained for physical mixture and confirm any changes that occurs in the principle peaks.

**5]FTIR**

The conformation for structure of Donepezil HCl was determined using FTIR study. IR affinity 8400 Shimadzu instrument was used. The samples were selected i.e., physical mixture of GMS & Drug, Drug & Nigella sativa oil, P407, Tween 80 & optimized formulation. The sample compartment was cleaned with ethanol & set for background clearance then sample was placed on sample compartment & swivel pressure tower was fitted & samples was analysed. pH of liquid samples was measured prior to IR study because of sensor sensitivity.

**6] pH DETERMINATION:**

pH determination of the optimized formulation is most important parameter I order to assure that it should not interrupt to nasal tissue inflammation, pH of optimized DNZ-NLC was determined by taking 10 ml of the formulation in beaker, then pH was measured at room temperature using digital pH meter, (pH tester 20, Eutech instruments USA).Measurements were done in triplicates.

**7] IN-VITRO RELEASE STUDY:**

An invitro release study of the DNZ from NLCs solution was performed in simulated nasal fluid (SNF) pH 6.4 using Franz Diffusion cell with Dialysis membrane. For saturating the membrane, they were initially soaked in phosphate buffer solution pH6.4 for 24h before the experiment [34]. Diffusion cell was filled with PBS pH 6.4 and dialysis membrane was mounted on cell. The temperature was maintained at 37℃. After a pre-incubation time of 20minutes, DNZ NLC formulation was placed in donor chamber. At predetermined time points, 3ml sample from each batch were withdrawn from the receptor compartment, replacing the sampled volume with PBS pH 6.4 after each sampling, for a period of 360 minutes (6h). The amount of permeated drug was determined using a UV- spectrophotometer at 231nm [35].

8] HPLC ANALYSIS:

HPLC method was developed for determining the drug concentration in rat brain and blood using Agilent RP-HPLC (Waters, USA).This bio-analytical method was developed for the determination of donepezil HCl in plasma/tissue homogenate. Agilent C-18 column (150 4.6 mm, Agilent 1260 infinity) with a mobile phase of methanol, 0.02 M phosphate buffer and tri-ethyl amine (50:50:0.5 v/v) was used for analysis [43]. The pH of the mobile phase was adjusted to 3.2 by phosphoric acid in isocratic mode at a flow rate of 1 ml/min. Detection wavelength was set at 231 nm and 20µl injection volume was used. Calibration curve was prepared in the concentration range of 0.01 to 0.1 mg/ml by spiking known amount of donepezil HCl and 0.02 ml of fresh plasma obtained from rat. All data reported as mean ± SD and the difference between the groups was tested.

**9]IN-VIVO STUDY:**

All animal experiments were approved and performed in accordance with the guidelines of Institutional Animal Ethics Committee of Marathwada Mitra Mandal’s College of Pharmacy, Thergaon, Pune[1379/PO/Re/S/10/CPCSEA].

Pharmacokinetic & biodistribution study was performed on Male Sprague Dawley Rats weighing 200-250gms. The rats will be maintained on a 12-hour light and dark cycle at a temperature of 23-25°C and relative humidity of 30-70%. The animals were given food and water ad libitum and will be closely monitored for any kind of behavioural changes during the experiment. Before the start of the pharmacokinetic study, the animals will be fasted overnight. As per the protocol, the rats were divided into two groups. The animals in group I were administered 4.0 mg/kg body weight Donepezil Hydrochloride (DNZ) solution via intravenous route (1 ml/kg) & group II Donepezil Hydrochloride loaded NLC formulation (equivalent to 4.0mg of DNZ/kg body weight) was administered via intranasal route in each nostril in the group with the help of micropipette (10–100 µL) with 0.1mm internal diameter. The rats were anaesthetized prior to nasal administration by isoflurane and held firmly from the back in a slanted position during nasal administration. The blood samples of 0.5ml will be collected by retro-orbital method at 15,60,120,180,300mins. Blood was centrifuged to separate plasma. Animals was sacrificed by CO 2 Asphyxiation at 15,60,120,180,300mins. The brain tissues will be collected and will be washed thrice with saline, wiped with the soft fabric, weighed, and stored at -20°C until analysis. The brain tissues were homogenized in phosphate buffer for extraction and analysed through HPLC for DNZ level. The homogenate was centrifuged at 4000 rpm for 20 min with the temperature of 4º C, and aliquots of the supernatant was separated and stored at -20ºC until drug analysis by HPLC.Pharmacokinetic parameters for formulations were calculated by pharmacokinetic software (Kinetica). The maximum plasma concentration of DNZ (C max) and the time required to reach the Maximum concentration (T max) was obtained directly from the actual plasma profiles. The degree of DNZ targeting to brain after I.N., administration can be evaluated by the drug targeting index (DTI) which can be described as the ratio of the value of AUC brain/ AUC blood following I.N. administration to that of I.V. administration. The higher the DTI is, more the degree of DNZ targeting to brain can be expected after I.N. administration. Brain targeting efficiency i.e., Drug targeting efficiency (DTE %) that represents time average partitioning ratio was calculated [36] [37].

10] STABILITY STUDY:

The stability study was performed on Optimized NLC formulation under at 4℃. Various parameters were evaluated to check the stability of NLC for 0, 15, 30, 60, 90 days. Changes in pH, particle size, zeta potential & drug content was measured.

**RESULTS & DISCUSSIONS**

**SCREENING OF LIPIDS:**

Among the five solid lipids i.e., GMS, stearic acid, compritol 888ATO, precirol ATO 5 and Gelucire 43/01 screened for solubility study with DNZ, the GMS showed maximum solubility of DNZ. Hence GMS was finalized for further formulation of NLC. Nigella sativa oil was used as liquid lipid [38].

**SELECTION OF SURFACTANT:**

Surfactant and Co-surfactant are used to stabilize the formulation and helps in solubilisation of the drugs in lipid. Smaller particle sizes have been observed when a higher surfactant : lipid ratio was used. Accordingly, polysorbate 80(Tween 80) a non-ionic surfactant containing a polyoxyethylene chain tetrahydrofuran ring that provides stearic stabilization and hydrophobic tail that prevents particle aggregation was selected based on previous work that showed its compatibility with lipids used. Poloxamer 407, was selected on its emulsification capacity for selected lipid mixture, it’s non-irritating effect on the nasal mucosa, and its ability to minimize the polymorphic state transitions of lipids.

**ANTI-OXIDANT ACTIVITY:**

The radical scavenging activity of Nigella sativa oil was determined by using DPPH assay. The DPPH free radical with dark purple colour which turns yellow or colourless when scavenged. This indicated that Nigella sativa oil has Antioxidant activity.

**PARTICLE SIZE, PDI & ZETA POTENTIAL:**

The particle size was found to be significantly affected with increase in total lipid amount at lower surfactant concentration. This may be attributed to inability of the surfactant solution to stabilize the emulsion at very low concentrations. Further higher concentrations of surfactant are sufficient enough to stabilize the emulsion with consistent particle size. Different concentrations of liquid lipid showed significant effect on particle size. As concentration of liquid lipid increases there observed decrease in particle size. The zeta potential value is a crucial parameter that influences the stability of nanocarriers, since it is related to the surface charge of the nanoparticles and indicates the degree of repulsion between closely positioned and similarly charged particles in dispersion. This repulsive force prevents the aggregation of particles. As presented in Table 1. the NLCs exhibited a negative zeta potential value. The particle size, PDI & Zeta potential was found to be between 85.3 to 331.1nm, 0.238 to 0.365 & -23.4 to -41.7 respectively.

**ENTRAPMENT EFFICIENCY AND DRUG LOADING:**

EE of drug is depended upon its solubility in lipid melt which is generally more than in the solidified lipid. Long chain triglycerides with higher melting point exhibit higher entrapment efficiency due to their lipophilic nature. Percent EE and % DL was found to be 64.81 to 70% & 10 to 17% respectively.

**DRUG CONTENT:**

The Drug content was used to determine the actual drug content present in formulation. Average drug content was found to be in range **75 to 90%.**

**FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR):**

The FTIR spectrum of DNZ HCL was taken and the observed peaks are shown in figure. The conformation for structure of Donepezil HCl was determined using FTIR study, IR affinity 8400 Shimadzu instrument was used. TheFTIR spectra of donepezil exhibited strong intense peaks at 1681cm-1(due to C=O carbonyl stretching vibrations), 1593cm-1 (due to C=C aromatic ring stretching and 1313 & 1265 cm-1 (due to aromatic amine C-N stretching), 700cm-1 ( due to presence of halogen atom i.e. chlorine). These peaks represent main functional groups in the chemical structure of Donepezil HCl.

**DIFFERENTIAL SCANNING CALORIMETRY :**

DSC is a basic method to investigate crystallinity or amorphous state of drug, lipids and formulation by determining the variation of temperature. Fig shows DSC curves of Donepezil hydrochloride, GMS, DNZ+GMS, DNZ+ NS oil and optimized NLC formulation. The DSC thermogram of DNZ showed endothermic peak at around at 231.27℃ indicating crystalline nature of drug. GMS showed sharp endothermic peak at 60.36℃. The physical mixture showed the drug melting peak at 231.27℃ & whereas GMS exhibited endothermic peak at 60.36℃ is sharp and not much shifted indicating compatibility of the drug and GMS. Thermogram of physical mixture showed the drug melting peak at 224.73℃ & whereas NS oil doesn’t show any peak and parent peak of drug is not much shifted indicating compatibility of the drug and oil. In case of formulation , one endothermic peak is observed there is conversion of crystalline DNZ to amorphous form.

**pH OF NLC FORMULATION:**

As this is intranasal formulation therefore pH of NLC is very important to travel a drug around nasal mucosa. The pH of Nasal mucosa lies in between 4.5 to 6.5. The pH of formulation batches was found to be in range of 6.4 to 6.5 .

**OPTIMIZATION:**

23 factorial designs have generated 8 experimental runs for the prepared formulations. Responses obtained from these runs were shown in table 8. The values of 3 independent variables are Y1: particle size (nm), Y2: Zeta potential(mV), Y3: Entrapment Efficiency(%). The main effect process order was suitably fitted. The values of R2, SD, % coefficient of variation of each of the 3 responses are shown in table 8. The effect of independent variables on drug: total lipid, solid lipid: liquid lipid & surfactant concentration is presented on a #D surface and contour plots. Moreover, a quantitative comparison that is resulted from experimental values of the responses with that of the predicted values can be analysed from [39]

**Response 1(Y1): Effect of Independent variables on particle size:**

On applying factorial design, the linear model was suggested by design expert software and found to be significant model with F value of 347.87 and R2  value of 0.9962 which implies model was significant (P<0.0001). And there was only chance that 0.01% a “model F value” this large occur due to noise. Values of probe P-Value less than 0.0500 indicate model terms are significant. Inthis case X1, X2 were significant model terms.

**Response 2(Y2): Effect of Independent variables on Zeta potential:**

On applying factorial design, the linear model was suggested by design expert software and found to be significant model with F value of 16.34 and R2  value of 0.9246 which implies model was significant (P<0.03182). And there was only chance that 0.052% a “model F value” this large occur due to noise. Values of probe P-Value less than 0.0500 indicate model terms are significant. Inthis case X1, X2 were significant model terms.

**Response 3(Y3): Effect of Independent variables on EE:**

On applying factorial design, the linear model was suggested by design expert software and found to be significant model with F value of 1.62 and R2  value of 0.5489 which implies model was significant (P<0.0104). And there was only chance that 0.01% a “model F value” this large occur due to noise. Values of probe P-Value less than 0.0500 indicate model terms are significant. Inthis case X1, X2 were significant model terms.

**INVITRO DRUG RELEASE:**

The in-vitro drug release studies were carried out on Franz diffusion cell apparatus using dialysis membrane using PBS 6.4. Optimized NLC showed the significant release from the prepared NLC, the initial burst release was observed at 60mins, followed by sustained release. This biphasic release pattern may be related to drug diffusion from the lipid matrix of NLCs, as has been evidenced for NLCs, related to the drug location in this lipid matrix. The rapid cooling of lipids during NLC preparation favours enrichment of the drug in the outer layers of the particles, leading to superficial entrapment, and consequently the initial burst release. The NLCs can also undergo partial erosion of the outer phospholipids layer, which results in fast diffusion of the hydrophobic drug [40]. The release kinetic model that best fit the release data was evaluated by the correlation coefficient (r) value. The release data from DNZ-NLC followed the Korsmeyer-Peppas release kinetics model having r = 0.9818 with n =0.7257 suggesting anomalous or non-Fickian diffusion, which is related to both the diffusion of the drug and dissolution of the NLC matrix. The goodness of models was evaluated with Akaike information criteria (AIC)=40.89 & Model Selection Criteria (MSC)=3.32. So, the sustained release pattern of DNZ over a period of 6h could be attributed to drug diffusion through the lipid matrix of NLCs as well to the slow degradation of NLCs in the release medium [41].

**IN-VIVO STUDY /PHARMACOKINETIC STUDY**

**Sample preparation**

Briefly, brain homogenate (1 ml) and plasma samples (0.5 ml) were mixed with 0.2 ml of acetonitrile to precipitate out plasma proteins. The mixture was vortex mixed for 5 min; and centrifuged for 5min. The supernatant was taken and the volume was made up to 10 ml with mobile phase. Then 20µl of aqueous phase was injected directly into the HPLC column for drug content analysis [42].

**Drug analysis in biological samples**

RP-HPLC (Waters, USA) bio-analytical method was developed for the determination of donepezil HCl in plasma/tissue homogenate. Agilent C-18 column (150 4.6 mm, Agilent 1260 infinity) with a mobile phase of methanol, 0.02 M phosphate buffer and tri-ethyl amine (50:50:0.5 v/v) was used for analysis [43]. The pH of the mobile phase was adjusted to 3.2 by phosphoric acid in isocratic mode at a flow rate of 1 ml/min. Detection wavelength was set at 231 nm and 20µl injection volume was used. Calibration curve was prepared in the concentration range of 0.01 to 0.1 mg/ml by spiking known amount of donepezil HCl and 0.02 ml of fresh plasma obtained from rat. All data reported as mean ± SD and the difference between the groups was tested using. [44] All data were dose and weight normalized. Pharmacokinetic parameters for donepezil HCl formulations were calculated using Kinetica 5.0 software. The Cmax and Tmax values after intranasal administration were read directly from the concentration–time profile. The AUC0–240 values obtained from curve were used to calculate the absolute bioavailability [45].These findings are for intranasal administration of DNZ load NLC formulation of nose to brain direct pathway. The highest concentration was observed in plasma after IV administration, where Cmax was 1.0258(ug/ml) and 5.83305(ug/ml) at Tmax 2H and 15min in Brain and blood respectively whereas Cmax was 4.597(ug/ml) and 2.2583(ug/ml) at Tmax 1hr in Brain and Blood respectively.In vivo study suggests that the hign intial plasma concentration after IV adminisrtration may have caused lower transport of DNZ crossing BBB by passive diffusion. A statistically significant difference [P<0.05] between the two formulation was found from ANOVA analysis [46].

**CONCLUSION:**

The Donepezil HCl loaded NLC were formulated for brain targeting through intranasal route where Nigella sativa oil was used as liquid lipid for its synergistic action & antioxidant activity. The GMS, tween 80 & P407 was used as solid lipid, surfactant & co-surfactant respectively. HPH followed by Ultrasonication method was used to prepare NLC dispersion. The 23 factorial design was used to formulation & optimization of variables, average particle size, PDI, Zeta potential was found to be 80-330nm, 0.2 to 0.4, -30 to -45mV respectively. The maximum Entrapment efficiency & drug content was found to be 70.20% & 90.07%. Invitro release drug profile showed significant increase in % drug release also showing a sustained release action. The release kinetic model that best fit the release data from DNZ-NLC followed the Korsmeyer-Peppas release kinetics model having r = 0.98with n =0.72 suggesting anomalous or non-Fickian diffusion, which is related to both the diffusion of the drug and dissolution of the NLC matrix. The highest concentration was observed in plasma after IV administration, where Cmax was 1.0258(ug/ml) and 5.83305(ug/ml) at Tmax 15min in Brain and blood respectively whereas Cmax was 4.597(ug/ml) and 2.2583(ug/ml) at Tmax 1hr in Brain and Blood respectively.

In conclusion, the DNZ loaded NLC could be a promising drug delivery system for treating Alzheimer’s disease. Based on investigation results investigators found a significant improvement in donepezil HCl permeation through nasal membrane and better distribution to the brain was achieved when compared to drug solution..

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