CHAPTER 3
OBJECTIVES, RATIONAL, DRUG PROFILE & PLAN OF WORK

2012
JAMIA HAMDARD
3.1. OBJECTIVES OF THE STUDY

The nervous system in man is protected with blood-brain-barriers (BBBs) which are recognized as the major obstacle to the treatment of most brain disorders. The delivery of neurotherapeutics to the brain needs not only novel delivery system (e.g., lipidic nanocarriers) but also a defined route that may improve the bioavailability and bypass the blood brain barrier (BBB) as well. Thus, the study has been designed to investigate the intranasal route for brain targeting using nanostructured lipid carrier (NLC) as drug delivery carrier. Since the intranasal route is a direct route of drug delivery to the brain and NLC is a drug carrier which is lipidic in nature so, it is expected that better outcome will result for the treatment of depression. With this background the main objectives of the present study were to:

1. Target duloxetine (DLX) to the brain by administering through intranasal route.
2. Bypass the blood brain barrier (BBB) by employing the nose to brain route drug administration.
3. Enhance CNS availability of DLX.
5. Reduce systemic availability and side effects related to DLX.
6. Reduce dosing frequency by sustaining the release of DLX from NLC.
7. Eliminate the problems associated with DLX on oral administration like extensive hepatic first-pass metabolism, acidic degradation (DLX is acid labile at gastric pH) and plasma protein binding (DLX has > 90% affinity to bind plasma proteins).
8. Develop and evaluate intranasal NLC formulations.
   1) Optimize and characterize the NLC formulations for nasal delivery.
   2) Perform in vitro release studies for the developed NLC formulations.
   3) Evaluate NLC formulations using in vivo animal models.
   4) Evaluate the stability of the NLC formulations and determine their shelf life.
3.2. RATIONALE OF THE STUDY

The rational for the present study was to target duloxetine, an antidepressant drug for the treatment of depression to the brain using NLC as the formulation approach and intranasal route as the drug delivery approach.

3.2.1. Depression

Depression is the major cause of absenteeism and loss of productivity at the work place. According to WHO estimates, depression will become the second-largest cause of the global health burden by 2020. Yet, depression remains one of the most under diagnosed conditions. WHO says that 181 out of 1000 Indians suffer from one or the other mental disorders. Around 3.4 percent of people with major depression commit suicide. Up to 60 percent of all people who commit suicide have a mood disorder, such as depression, and their risk may be especially high if they feel a marked sense of hopelessness or have both depression and borderline personality disorder.

3.2.2. Formulation approach for brain targeting

The nervous system in man is outfitted with protective blood-brain-barrier (BBB) that restricts the delivery of many potentially therapeutic and diagnostic compounds to the brain. These brain barriers are now recognized as a major obstacle to the treatment of most brain disorders. The inability to treat most CNS disorders is not due to the lack of effective CNS drug discovery. Rather, it is due to the ineffective CNS delivery. Novel strategies are being researched for transport existing drugs into CNS for treatment of CNS disorders. It is an appealing idea to use small particles such as nanoparticles to shield and disguise the drug entity in the systemic circulation and to direct this drug-bearing package to the specific target. Nanoparticles are taken up by cells more efficiently than larger micromolecules and therefore, could be used as effective transport and delivery systems. The effective transport and delivery of drugs into the brain by nanoparticulate carriers can be described by different mechanisms. The nanoparticles make possible an opening of the tight junctions between the endothelial cells. This leads to increased drug paracellular permeation in the free and/or nanoparticulate-bound form. Another mechanism reported is the transeptosis through the endothelial cell layer which leads to direct delivery of nanoparticles bound drug into the brain parenchyma. This causes an increase in retention of the
nanoparticles in the brain-blood capillaries, and/or their adsorption to the capillary walls. This creates a higher concentration gradient enhancing the transport across the BBB and leading to drug delivery to the brain.

3.2.2.1. Nanostructured lipid carriers (NLC)

Brain is protected with BBB which is a lipophilic membrane. Therefore lipophilic carriers like SLN, NLC, microemulsion, nanoemulsion has been extensively studied and investigated for the transport of therapeutic substances to the brain. NLC as a lipid carrier is better than SLN as NLC possesses many imperfections increasing drug loading capacity and minimizing or avoiding drug expulsion during storage (Muchow et al., 2008). NLCs have many features that are advantageous for brain targeting. They are colloidal carriers providing controlled release profiles for many substances. They are composed of physiological and biodegradable lipids exhibiting low toxicity and low cytotoxicity that means an excellent tolerability. The small size ensures a close contact to the BBB and can increase the amount of drug penetrated into the brain. Furthermore, lipid nanoparticles are able to enhance the chemical stability of compounds sensitive to light, oxidation and hydrolysis (Pardeike et al., 2009).

3.2.3. Intranasal route as drug delivery approach

Intranasal administration offers a non-invasive alternative route to the central nervous system (CNS) for drug delivery, effectively bypassing the BBB. The nasal route is one of the most permeable and highly vascularized site for drug administration ensuring rapid absorption and onset of therapeutic action. It has been potentially explored as an alternative route for drugs with poor bioavailability (first-pass hepatic metabolism) and for the delivery of biosensitive (susceptible to enzymatic or acidic degradation) and high molecular weight (MW) compounds such as proteins, peptides, steroids, vaccines, and so on.

3.2.4. Choice and selection of drug candidate

Mood disorders are among the most prevalent forms of mental illness. Severe forms of depression affect several persons worldwide. The use of medications in the treatment of depression began in the late 1950s with the successful introduction of tricyclic antidepressants and monoamine oxidase (MAO) inhibitors. Treatment of depression with medications has greatly
increased since the advent of selective serotonin reuptake inhibitors (SSRIs). Tricyclic antidepressants (e.g., hydrazines) have more side effects than SSRIs and are usually reserved for the treatment of inpatients, for which the tricyclic antidepressant amitriptyline, in particular, appears to be more effective. The MAO inhibitors have historically been plagued by questionable efficacy and life-threatening adverse effects. They are still used only rarely, although newer agents of this class, with a better side effect profile, have been developed. Selective serotonin reuptake inhibitors (SSRIs), such as sertraline, escitalopram, fluoxetine, paroxetine, and citalopram are the primary medications considered, due to their relatively mild side effects and broad effect on the symptoms of depression and anxiety. Venlafaxine, a serotonin-norepinephrine reuptake inhibitor (SNRI) may be moderately more effective than SSRIs; however, it is not recommended as a first-line treatment because of the higher rate of side effects, and its use is specifically discouraged in children and adolescents.

Duloxetine, an SNRI is the first in the class of anti-depressants that ensures rapid and sustained efficacy in the treatment of both emotional and physical symptoms of depression. Till now, the available anti-depressants addressed only the emotional aspects of depression. Duloxetine promises treatment of physical symptoms that accompany major depressive disorder (MDD) such as aches, pains, and gastrointestinal disturbance as well. It also possesses a safety profile as per selective serotonin re-uptake inhibitor (SSRI) medications. On oral administration DLX undergoes hepatic first pass metabolism and has a systemic bioavailability of 50% (Lantz et al., 2003). Moreover the drug is acid labile at gastric pH. Oral administration of the drug also causes side effects including nausea, dry mouth, headache, dizziness, orthostatic hypotension fatigue etc. Furthermore due to the presence of BBB, the drug is not able to cross it and reach the brain. Therefore the brain bioavailability of DLX is less.

3.3. DRUG PROFILE OF DULOXETINE

Duloxetine was created by Lilly researchers. David Robertson, David Wong, a co-discoverer of fluoxetine (Prozac), and Joseph Krushinski are listed as inventors on the patent application filed in 1986 and granted in 1990 (Wong et al., 1988). The (+)-enantiomer of was more effective than (-)-enantiomer as it inhibited serotonin reuptake in rat synaptosomes two times more potently (Bymaster et al., 2003).
Generic name: Duloxetine Hydrochloride

Brand names: Cymbalta (Eli Lilly), Duloxyl (Cipla).

3.3.1 Physicochemical properties

Description: Duloxetine is a white to slightly brownish white solid.

State: Solid

Drug Category: serotonin-norepinephrine reuptake inhibitor (SNRI)

Chemical Name: (+)-(S)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)propan-1-amine

Molecular formula: C_{19}H_{19}NOS

Molecular weight: 297.41456 g/mol

Partition coefficient: log P = 4.72 (1-octanol/water)

Melting point: 206.72 °C; 223.5°C (decomposition)

Solubility: Slightly soluble in water (0.00296 mg/mL), Freely soluble in methanol, and sparingly soluble in acetonitrile.

3.3.2. Pharmacology

3.3.2.1. Mechanism of action

Duloxetine is a potent inhibitor of neuronal serotonin and norepinephrine reuptake and a less potent inhibitor of dopamine reuptake. Duloxetine has no significant affinity for dopaminergic, adrenergic, cholinergic, histaminergic, opioid, glutamate, and GABA receptors. The
antidepressant and pain inhibitory actions of duloxetine are believed to be related to its potentiation of serotonergic and noradrenergic activity in the CNS.

3.3.2.2. Pharmacokinetics
Duloxetine has an elimination half-life of about 12 hours (range 8 to 17 hours) and its pharmacokinetics is dose proportional over the therapeutic range. Steady-state plasma concentrations are typically achieved after 3 days of dosing. Elimination of duloxetine is mainly through hepatic metabolism involving two P450 isozymes, CYP2D6 and CYP1A2. Its bioavailability was found to be ~ 50% (32 – 80%).

Absorption and Distribution
Orally administered duloxetine hydrochloride is well absorbed. There is a median 2-hour lag until absorption begins (Tlag), with maximal plasma concentrations (Cmax) of duloxetine occurring 6 hours post dose. Food does not affect the Cmax of duloxetine, but delays the time to reach peak concentration from 6 to 10 hours and it marginally decreases the extent of absorption (AUC) by about 10%. There is a 3-hour delay in absorption and a one-third increase in apparent clearance of duloxetine after an evening dose as compared to a morning dose.

The apparent volume of distribution averages about 1640 L. Duloxetine is highly bound (> 90%) to proteins in human plasma, binding primarily to albumin and α1-acid glycoprotein. The interaction between duloxetine and other highly protein bound drugs has not been fully evaluated. Plasma protein binding of duloxetine is not affected by renal or hepatic impairment.

Metabolism and Elimination
Biotransformation and disposition of duloxetine in humans have been determined following oral administration of 14C-labeled duloxetine. Duloxetine comprises about 3% of the total radiolabeled material in the plasma, indicating that it undergoes extensive metabolism to numerous metabolites. The major biotransformation pathways for duloxetine involve oxidation of the naphthyl ring followed by conjugation and further oxidation. Both CYP2D6 and CYP1A2 catalyze the oxidation of the naphthyl ring in vitro. Metabolites found in plasma include 4-hydroxy duloxetine glucuronide and 5-hydroxy, 6-methoxy duloxetine sulfate. Many additional metabolites have been identified in urine, some representing only minor pathways of elimination. Only trace (< 1% of the dose) amounts of unchanged duloxetine are present in the urine. Most
(about 70%) of the duloxetine dose appears in the urine as metabolites of duloxetine; about 20% is excreted in the feces.

3.3.2.3. Indications

The main uses of duloxetine are in major depressive disorder, general anxiety disorder, stress urinary incontinence and painful peripheral neuropathy. In addition, it has been studied for various other indications including chronic fatigue syndrome.

3.3.2.4. Contraindications

The following contraindications are listed by the manufacturer:

- Hypersensitivity: Duloxetine is contraindicated in patients with a known hypersensitivity to duloxetine or any of the inactive ingredients.
- Monoamine oxidase inhibitors: Its concomitant use in patients taking monoamine oxidase inhibitors is contraindicated.
- Uncontrolled narrow-angle glaucoma: It is associated with an increased risk of mydriasis (dilation of the iris); therefore, its use should be avoided in patients with uncontrolled narrow-angle glaucoma, in which mydriasis can cause sudden worsening.
- CNS acting drugs: It should be used with caution when it is taken in combination with or substituted for other centrally acting drugs, including those with a similar mechanism of action.
- Duloxetine and thioridazine should not be co-administered.

3.3.2.5. Warnings and precautions

General

- Use of selective serotonin reuptake inhibitors (SSRIs) and other newer anti-depressants in patients under the age of 18 may be associated with behavioural and emotional changes, including an increased risk of suicidal ideation.
- SSRIs and other newer antidepressants are reported to cause severe agitation-type adverse events in both pediatrics and adults coupled with self-harm or harm to others. The agitation-type events include: akathisia, agitation, disinhibition, emotional lability, hostility, aggression, and depersonalization.
Chapter 3

Objectives, Rational, Drug profile & Plan

- Patients currently taking SSRIs or newer anti-depressants should not be discontinued abruptly, due to risk of discontinuation symptoms. At the time that a medical decision is made to discontinue an SSRI or other newer anti-depressant drug, a gradual reduction in the dose rather than an abrupt cessation is recommended.

Hepatic Impairment
- Patients with clinically evident hepatic impairment have decreased duloxetine metabolism and elimination.

Cardiovascular
- Duloxetine has been associated with an increase in blood pressure and clinically significant hypertension in some patients. This may be due to the noradrenergic effect of duloxetine.

Dependence
- In drug dependence studies, duloxetine did not demonstrate any dependence-producing potential in monkeys or rats.
- Discontinuation symptoms have been occurred at a significantly higher rate in duloxetine-treated patients compared with those discontinuing from placebo. A gradual reduction in the dose rather than abrupt cessation is recommended whenever possible.

Endocrine
- Duloxetine was associated with a small increase in mean fasting blood glucose as compared to placebo.

Hematologic
- There have been reports of bleeding abnormalities with selective serotonin reuptake inhibitors (SSRIs) and serotonin/norepinephrine reuptake inhibitors (SNRIs), including very rare cases of ecchymoses and gastrointestinal bleeding reported with duloxetine. Skin and other mucous membrane bleedings have been reported following treatment with duloxetine. Thus, caution is advised in patients taking anticoagulants (e.g. warfarin) and/or medicinal products known to affect platelet function (e.g. nonsteroidal anti-inflammatories and ASA), and in patients with known tendency for bleeding or those with predisposing conditions.
Neurologic

- Anticonvulsant effects of duloxetine have been observed in animal studies therefore, it should be used with caution in patients with a history of a seizure disorder.
- On rare occasions serotonin syndrome or neuroleptic malignant syndrome-like events have occurred in association with treatment with SSRIs, particularly when given in combination with other serotonergic and/or neuroleptic drugs. Duloxetine should not be used in combination with MAOIs and should be used with caution in combination with other serotonergic drugs (triptans, certain tricyclic antidepressants, lithium, tramadol, St. John’s Wort) due to the risk of serotonergic syndrome.

Psychiatric

- Suicidal ideation and suicidal behaviours have been reported during duloxetine therapy or early after treatment discontinuation.

Renal

- Increased plasma concentrations of duloxetine occur in patients with end-stage renal disease (requiring dialysis). For this reason duloxetine is not recommended for patients with endstage renal disease or severe renal impairment.

Pregnant Women:

- Safe use of duloxetine during pregnancy has not been established. Therefore, it should not be administered to pregnant women or those intending to become pregnant, unless, in the opinion of the treating physician, the expected benefits to the patient markedly outweigh the possible hazards to the fetus.

Nursing Women:

- Duloxetine is excreted into the milk of lactating women. The estimated daily infant dose on a mg/kg basis is approximately 0.14% of the maternal dose. Because the safety of duloxetine in infants is not known, nursing while on duloxetine is not recommended.

Pediatrics (<18 years of age):

- The safety and efficacy of duloxetine in paediatric patients (<18 years of age) have not been established and its use in this patient population is not indicated.

Use in Patients with Substantial Alcohol Use:

- Use of duloxetine in patients who consume substantial amounts of alcohol may be associated with severe liver injury. Isolated cases of liver failure, including fatal cases,
have been reported. Duloxetine should only be used in exceptional circumstances and with extreme caution in these patients.

3.3.2.6. Drug interactions

Duloxetine exhibit serious drug interactions with monoamine oxidase inhibitors, linezolid, fluvoxamine, ciprofloxacin, enoxacin, thioridazine.

Potential for other Drugs to Affect Duloxetine

Both CYP1A2 and CYP2D6 are responsible for duloxetine metabolism.

Inhibitors of CYP1A2:

Duloxetine AUC was increased by approximately 6-fold, the Cmax was increased about 2.5-fold, and duloxetine t½ was increased by approximately 3-fold when co-administered with fluvoxamine (100 mg), a potent CYP1A2 inhibitor. Therefore, duloxetine should not be used concomitantly with potent CYP1A2 inhibitors (e.g., fluvoxamine) and some quinolone antibiotics (e.g., ciprofloxacin and enoxacin).

Inhibitors of CYP2D6:

Because CYP2D6 is involved in duloxetine metabolism, concomitant use of duloxetine with potent inhibitors of CYP2D6 would be expected to, and does, result in higher concentrations (on average 60%) of duloxetine. Paroxetine (20 mg QD) increased duloxetine (40 mg QD) AUC and Cmax by 60%. Caution is advised if administering duloxetine with inhibitors of CYP2D6 (e.g., SSRIs).

Dual Inhibition of CYP1A2 and CYP2D6:

Concomitant administration of duloxetine 40 mg BID with fluvoxamine 100 mg, a potent CYP1A2 inhibitor, to CYP2D6 poor metabolizer subjects (n=14) resulted in a 6-fold increase in duloxetine AUC and Cmax.

Potential for Duloxetine to Affect Other Drugs

Caution is advised when duloxetine is taken in combination with other centrally acting drugs and substances, especially those with a similar mechanism of action, including...
alcohol. Concomitant use of other drugs with serotonergic activity (e.g., SNRIs, SSRIs, triptans, or tramadol) may result in serotonin syndrome.

- Duloxetine is highly bound to plasma proteins (>90%). Therefore, administration of duloxetine to a patient taking another drug that is highly protein bound may cause increased free concentrations of either drug.

- The combination of duloxetine and lorazepam resulted in increased sedation compared with lorazepam alone.

- Cases of life-threatening serotonin syndrome have been reported during combined use of selective serotonin reuptake inhibitors (SSRIs)/serotonin norepinephrine reuptake inhibitors (SNRIs) and triptans.

- Duloxetine is a moderate inhibitor of CYP2D6. When duloxetine (60 mg BID) was coadministered with a single 50-mg dose of desipramine, a CYP2D6 substrate, the AUC of desipramine increased 3-fold. The co-administration of duloxetine (40 mg BID) increased steady-state AUC of tolterodine (2 mg BID) by 71% but did not affect the pharmacokinetics of the 5-hydroxyl metabolite. Therefore, caution should be used if duloxetine is co-administered with medications that are predominately metabolized by the CYP2D6 system and which have a narrow therapeutic index such as antiarrhythmics (e.g. flecainide and encainide).

**Drug-Food Interactions**

- Food delays the time for duloxetine to reach peak concentration from 6 to 10 hours and it marginally decrease the extent of absorption (approximately 11%). However food does not affect the Cmax of duloxetine. Duloxetine may be taken with or without food.

**Drug-Herb Interactions**

- In common with other SSRIs and SNRIs, pharmacodynamic interactions between duloxetine and the herbal remedy St. John’s Wort may occur and may result in an increase in undesirable effects.

**Drug-Lifestyle Interactions**

- Duloxetine bioavailability appears to be about 34% lower in smokers than in nonsmokers, although dosage modifications are not routinely recommended.
Although duloxetine does not increase the impairment of mental and motor skills caused by alcohol, the concomitant use of duloxetine and substantial amounts of alcohol is not recommended.

3.3.2.7. Adverse effects
Nausea, somnolence, insomnia, and dizziness are the main side effects, reported by about 10% to 20% of patients. In a trial for mild major depressive disorder (MDD), the most commonly reported treatment-emergent adverse events among duloxetine-treated patients were nausea (34.7%), dry mouth (22.7%), headache (20.0%) and dizziness (18.7%), and except for headache, these were reported significantly more often than in the placebo group (Peralia et al., 2006). Other side-effects include: orthostatic hypotension, fatigue, vivid nightmares, increased sweating, decreased appetite and weight loss etc. Duloxetine and SSRIs have been shown to cause sexual side effects in some patients, both males and females. Although usually reversible, these sexual side effects can sometimes last for months, years, or longer, even after the drug has been completely withdrawn. This disorder is known as post-SSRI sexual dysfunction.

3.3.2.8. Dosage and administration
- Duloxetine is not indicated for use in children less than 18 years of age.
- Duloxetine should be swallowed whole and should not be chewed or crushed, nor should the contents be sprinkled on food or mixed with liquids. All of these might affect the enteric coating.
- The recommended dose is 60 mg once daily with or without food. A lower starting dose of 30 mg may be considered for tolerability reasons in some patients, with a target dose of 60 mg/day within 1-2 weeks. Therapeutic response is usually seen after 1-4 weeks of treatment. There was no evidence that doses greater than 60 mg/day conferred any additional benefits.

General Considerations for Dosing in Special Populations

Dosage for Patients with Renal Impairment:
- Duloxetine is not recommended for patients with end-stage renal disease (requiring dialysis) or in severe renal impairment (estimated creatinine clearance <30 mL/min)
Dosage for Patients with Hepatic Impairment:
- Duloxetine should not be used in patients with any liver disease resulting in hepatic impairment.

Dosage for Elderly Patients:
- No dose adjustment is recommended for elderly patients on the basis of age. Caution should be exercised in treating the elderly. Pharmacokinetic results suggest no overall differences between these subjects and younger subjects. Other reported clinical experience has not identified differences in responses between the elderly and younger patients, but greater sensitivity of some older individuals cannot be ruled out. When individualizing the dosage, extra care should be taken when increasing the dose.

Dosage for Pediatric Patients:
- The safety and efficacy of duloxetine in paediatric patients (<18 years of age) have not been established and its use in this patient population is not indicated.

Treatment of Pregnant Women during the Third Trimester:
- Post-marketing reports indicate that some neonates exposed to SSRIs or other newer antidepressants late in the third trimester have developed complications requiring prolonged hospitalization, respiratory support, and tube feeding. When treating pregnant women with duloxetine during the third trimester, the physician should carefully consider the potential risks and benefits of treatment. The physician may consider tapering duloxetine in the third trimester.

3.3.2.9. Overdosage
In clinical trials, cases of acute ingestions above 3000 mg, alone or in combination with other drugs, were reported, with none being fatal. However, in post marketing experience fatal outcomes have been reported for acute overdoses, primarily with mixed overdoses, but also with duloxetine only, at doses as low as approximately 1000 mg. Signs and symptoms of overdose (duloxetine alone or with mixed drugs) included somnolence, serotonin syndrome, seizures, vomiting, and tachycardia.
Management of Overdose:
No specific antidote is known, but if serotonin syndrome ensues, specific treatment (such as with cyproheptadine and/or temperature control) may be considered. An airway should be established. Monitoring of cardiac and vital signs is recommended, along with appropriate symptomatic and supportive measures. Gastric lavage may be indicated if performed soon after ingestion or in symptomatic patients. Activated charcoal may be useful in limiting absorption. Duloxetine has a large volume of distribution and forced diuresis, hemoperfusion, and exchange perfusion are unlikely to be beneficial.

In managing overdose, consider the possibility of multiple drug involvement. A specific caution involves patients who are taking or have recently taken duloxetine and might ingest excessive quantities of a tricyclic antidepressant. In such a case, accumulation of the parent tricyclic and/or its active metabolite may increase the possibility of clinically significant sequelae and extend the time needed for close medical observation.

3.3.2.10. Storage and stability
Duloxetine hydrochloride (CYMBALTA™) has been advised to be stored between 15°C and 30°C.

3.3.2.11. Dosage forms, composition and packaging
Availability of Dosage Forms:
CYMBALTA™ (duloxetine hydrochloride) delayed-release capsules are available in 30 mg and 60 mg strengths.

Composition:
Each capsule contains enteric-coated pellets of duloxetine hydrochloride equivalent to 30 mg or 60 mg of duloxetine that are designed to prevent degradation of the drug in the acidic environment of the stomach.
Nonmedicinal ingredients include FD&C Blue No.2, gelatin, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose acetate succinate, sodium lauryl sulfate, sucrose, sugar spheres, talc, titanium dioxide, and triethyl citrate. The 60 mg capsules also contain yellow iron oxide.
3.4. PLAN OF WORK

1. Literature review and search for patents
2. Selection and procurement of drug
3. Selection and procurement of lipids, chemicals and solvents
4. Physicochemical characterization
   - Organoleptic properties
   - Solubility
   - Partition coefficient
   - Loss on drying
   - Melting point
5. Identification tests
   - UV spectral analysis
   - FTIR spectral analysis
   - Differential scanning calorimetry (DSC) analysis
   - X-ray diffraction analysis
6. Drug excipients interaction study (Compatibility studies)
7. Analytical methodology
   a) Determination of $\lambda_{\text{max}}$ (maximum wavelength) in different media
   b) Preparation of calibration curves in different solvents for determining drug entrapment efficiency and drug loading:
      1. In distilled water
      2. In phosphate buffer (pH 6.0)
      3. In methanol
      4. In chloroform & ethanol (1:1 v/v)
   c) HPLC method development and validation for in vitro studies
   d) HPLC method development and validation for quantification in biological samples
8. Formulation, optimization and evaluation of NLC formulation.
   a) Formulation and optimization of DLX loaded NLC (DLX-NLC).
      i. Optimization of lipids (fat and oil) and drug concentration
      ii. Optimization of surfactant and co-surfactant
      iii. Optimization of duration and speed of homogenization
iv. Optimization of duration of sonication
v. Optimization of cryoprotectant

(b) Characterization of the optimized DLX-NLC formulation

- Particle size, particle size distribution and Zeta potential
  - Photon correlation spectroscopy (PCS) with Malvern Zetasizer
- Surface morphology and shape
  - Transmission electron microscopy (TEM)
  - Scanning electron microscopy (SEM)
- Entrapment efficiency (EE)
- Drug loading (DL)
- Crystallinity
  - Differential scanning calorimetry (DSC)
  - X-ray diffraction (XRD)

9. In vitro studies of DLX-NLC

- In vitro release studies
- Permeation studies
- Adhesion properties
- Surface hydrophilicity/hydrophobicity

10. In vivo studies for intranasal drug delivery

- Pharmacodynamic studies
  - Forced swimming test (FST)
  - Locomotor activity test (LAT)
- In vivo nasal permeation studies
  - In vivo nasal absorption studies (Whole animal or in vivo model)
  - Ex vivo nasal perfusion studies (Isolated organ perfusion or ex vivo model)
- Pharmacoscintigraphic studies
  - Biodistribution studies
  - Pharmacokinetic studies
  - Gamma scintigraphic studies

11. Stability studies of optimized DLX-NLC formulation