'Oral Administration of Fluoxetine Incorporated Liposomal Nanoparticles coated with PEG in Treatment of Chronic Mild stress (CMS)'

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DEPARTMENT OF BIOMEDICAL ENGINEERING & SCIENCES SCHOOL OF MECHANICAL & MANUFACTURING ENGINEERING NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY, ISLAMABAD

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MASTER THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by : **Nadia Sadiq**

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Abstract:

Depression is one of the most increasing psychological mood disorders, depression does not have some specific physical symptoms, but it negatively affects the person mood, way of living and thinking chronic mild stress (CMS) is one of its type, depression is affecting more than 300million people in the world. Shortness of breath, headache, being miserable, stomach disturbance and physical tensions are some of the common symptoms of depression that is reported according to National Alliance of Mental Health, Anti-depressant are used for altering the neurotransmitters those are serotonin, dopamine and norepinephrine that are primarily involved in regulating and mood alleviating mood. Despite the availability of large number of drugs, many of patients are resistant to the current mode of treatment that are available now adays. The obstacle that is significant for the transportation of beneficial therapeutic entities to the nervous system is BBB that is the blood brain barrier. There are junctions that are present in the endothelial cells of the blood brain barrier that stop the drugs passage. Now nanoparticles are receiving significant limelight owing to their small size and efficient brain targeting activity, making them likely to cross the blood brain barrier while carrying the intact drug molecule otherwise incapable of permeation. In this study the fluoxetine loaded liposomal nanoparticle coated with PEG were developed to transport the drug across the BBB to the central nervous system having much greater efficiency. For testing its delivery, the animal that is mice model of depression is used with the specific name that is designed in order to induce depression like symptoms in mice. Before and After treatment great differences between the physical behaviors like Elevated maze, open field test, force swim test etc and sucrose consumption test were identified and plotted, as liposomes that having capabilities to carry the anti-depressant drug molecule that is administered through the Oral route of administration or the better and improved method of treatment.

Abbreviations:

CMS – chronic mild stress

DPPC – Dipalmitoyl phosphatidylcholine

CNS – Central Nervous system

SST – Sucrose splash Test

OFT – Open field test

Chapter 1

1. INTRODUCTION:

1.1 Chronic Mild stress:

Chronic Mild stress is a type of depression that is a mental disorder that primarily impairs a person's ability to think and results in inappropriate behavior. It can be predicted when a person starts showing some symptoms like hopelessness. Low and gloomy mood, poor sleep, disesteem, and hallucinatory suicidal thoughts or mood swings. When a person starts feelings like helplessness, worthlessness, and it becomes a way of life that could proceed one condition towards clinical depression. It is a significant disorder that has an impact on both physical and mental health. (Kurlowicz et al., 2007) .

Depression affects various people in different ways since it cannot be defined just by a set of symptoms. It can interfere with daily work, resulting in low productivity and laziness, as well as a general lack of enthusiasm for normal tasks.

It can also disrupt relationships and have an impact on physical and, especially, mental health.The repeated fluctuations in mood are a prominent symptom of depression.(Gaynes *et al.*, 2019)
Many people may not feel these emotional highs and lows and may appear to live a normal life on the surface, but they may develop anxiety and depression.
Because anxiety and depression are linked, anxiety can lead to despair and suicidal thoughts.
Depression is more than just a bad mood; it is neither a weakness nor an emotion, and a sufferer cannot just snap out of it or feel better about themselves.
Depression may need long-term therapy. Most depressed people feel better after attempting medication, treatment, or both.
(Davis *et al.*, 2008).

According to the FDA,52% of new antidepressants failed to work because the patients failed the symptoms necessary for prescription of that drug (Kobak *et al.*, 2007)

1.2. Treatment of Depression

Depression medication therapy advancements have been aggressively sought.

Antidepressants, which are classified into several classes of drugs with slightly different mechani sms of action, are widely used as a treatment option (Harmer *et al., 2017*).

Monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), serotonin reuptake inhibitors (SSRIs), and norepinephrine (NE)serotonin (5HT) reuptake inhibitors are the four major groups of antidepressant medications (Gundersenet *et al.,2013*)

SSRIs are the most often used therapy medicines for serious depressive disorders. They are considered to alleviate depression symptoms through a variety of methods, including increased serotonin levels in the brain. SSRIs suppress serotonin reuptake transporters in presynaptic terminals, allowing serotonergic neurotransmitter levels at postsynaptic clefts to remain stable. (Benfield et al., 1986; Murdoch and McTavish,1992)

Antidepressants, regardless of class, must pass through the BBB to reach their site of action within the brain. Despite several benefits, antidepressant medication is frequently limited by diminished therapeutic agent penetration into the central nervous system (CNS). Because of the presence of the blood-brain barrier (BBB) and the related P-glycoprotein efflux transporter, most bioactive substances do not readily penetrate brain tissues (O'Brien, 2011).

Aside from that, increased P-gp expression on intestinal epithelium may reduce the pace and concentration of medication diffusing through the basolateral membrane and its entry into general circulation from the colon. *(Nasar 2009*)

Because the oral route is the preferred method for antidepressant medication delivery, it is critical to overcome the absorption barrier provided by the P-gp efflux transporter. (Nasar2009; Hoosain 2015).

1.3. Polymer coated Liposomal nanoparticles and depression

Crossing the blood brain barrier (BBB) to treat depression is a complicated system made up of cl osed connected cells, tight junctions, and endothelial cells.

All these connections prevent the admission of any medicine in large quantities; only water and a few gases pass through.

To treat depression, a drug must overcome the BBB barrier; nanoparticles give a better answer in this regard because of their small size; they can cross the BBB with drug trapped inside them and effectively target the location where the drug is needed to be delivered.

For their unique effects, several antidepressants mix with various nanomaterials (Dimitrijevic and Pantic, 2014)

Lipid based nanoparticles, solid lipid nanoparticles, cationic nanoparticles, nano-emulsions, and polymeric nanoparticles are commonly used to deliver medications to CNS system.

These nanoparticles may pass the blood brain barrier via positive charge to negative charge attraction and deliver drugs directly to the target without drug loss. Proteins, peptides, and other molecules may also be bonded to the surface of nanoparticles, giving them a great lot of flexibility. Polymers like poly D, polylactide-co-glycolide (PLGA), L-glycolide , Polymers like polylactic acid, poly D, polylactide-co-glycolide (PLGA), L-glycolide (PLG)poly cyano-acrylate (PCA) these polymeric nanoparticles may provide better results. Triglycerides, water, and oil are also transported to Central Nervous region by the nano-emulsions. (Deore, Shahi and Dabir, 2016)

This dissertation's Different antidepressant nanoparticles produce huge outcomes against depression. All these antidepressant nanoparticles have been tested on models' depression-like symptoms.

The research consists of two section one Chronic mild stress (CMS) induction as it is type of depression that is common as well as a serious medical illness that negatively effects how you feel, the way we think and act. Chronic mild stress (CMS) symptoms are different and could be mild to severe research effort is divided into two sections. The primarily focusses on the behavioral changes of the animals suggesting that the mice have been successfully depressed and that their therapy is effective. Liposomal nanoparticles might aid in the treatment of Chronic mild stress. Then making and characterization of the nanoparticles those are lipid base and coated with PEG 2000.Scanning Electron Microscopy (SEM) were then used to analyze the liposomal formulation. These nanoparticles were given to reverse the Chronic mild stress analysis. Weight comparisons and behavioral assessments were used to track depressive symptoms. The focus of this research is to use oral administration of the drug with nanoparticles for depression more effectively and with minimum side effects.

1.4 Aims and Objectives:

Fluoxetine loaded liposomal nanoparticle development to transport the drug across the BBB to check its efficiency Because breaching the blood-brain barrier provides focused medication administration with a rapid onset of impact.

Following was the primarily goals:

- Fluoxetine loaded liposomal nanoparticle preparation and its characterization to transport the drug across the BBB to check its efficiency.
- PEG coating used to avoid the dissolution of drug in stomach.
- Developing mice model of Chronic Mild stress (CMS)
- Developing mice model of Chronic Mild stress (CMS) its confirmation
- Oral administration of the prepared drug to treat the disease
- Behavioral tests for confirmation of treatment.

Chapter 2

LITERATURE REVIEW:

2.1. Fluoxetine

Fluoxetine is a highly effective antidepressant. It is a selective serotonin reuptake inhibitor (SSRI) that has been authorized by the Food and Drug Administration. Fluoxetine has a better efficacy and fewer adverse effects than the other antidepressant classes, tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs), and it can be used to treat a variety of conditions, including panic disorder, bulimia, and binge eating disorders. (Haque et al., 2014) The primary reason fluoxetine was and continues to be favored above other antidepressants is its safety profile. It is appropriate for children above the age of eight, adults, and even pregnant women. Fluoxetine, sold under the brand name 'Prozac,' is well-known around the world and has done much to increase awareness of depression (Wenthur, Bennett and Lindsley, 2014).

The primary reason fluoxetine was and continues to be favored above other antidepressants is its safety profile. It is appropriate for children above the age of eight, adults, and even pregnant women. Fluoxetine, sold under the brand name 'Prozac,' is well-known around the world and has done much to increase awareness of depression (Abouhussein et al., 2018)

Fluoxetine has been extensively researched in animal models of depression and has been shown to treat depression-like symptoms in rats and other animals.

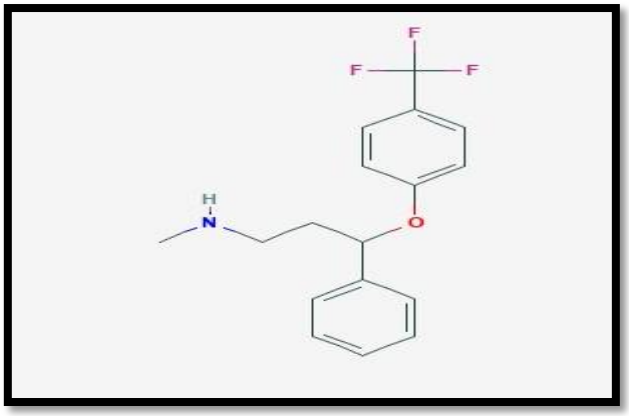


Figure 1: Chemical structure of Fluoxetine

2.2. Mechanism of action

Norepinephrine and amines serotonin play a big role in development of depression. People suffering from depression have low serotonin level in the cerebrospinal fluid and even low serotonin uptake sites in patients of depression.

Pre-synaptic terminal of serotonin that is 5HT1A is in prefrontal cortex of brain area.

Fluoxetine drug function is blocking of reuptake transported protein in the pre-synaptic terminal which result in blocking of reuptake of serotonin in pre-synaptic and increase the action of serotonin on a location $5HT_{1A}$. (Cao *et al.*, 2019)

Fluoxetine shows quicker result than the other antidepressant because it is having activating occurs because reuptake of serotonin, half-life of this drug is 2-4 days so, its result can be noticed in 2-4 weeks.

Nor Fluoxetine is a metabolite of this drug that is an active one with half-life 4-9 days, produced when cytochrome P450 enzyme CYP2D6 acts on it. (Robertson and Dodd, 2019)

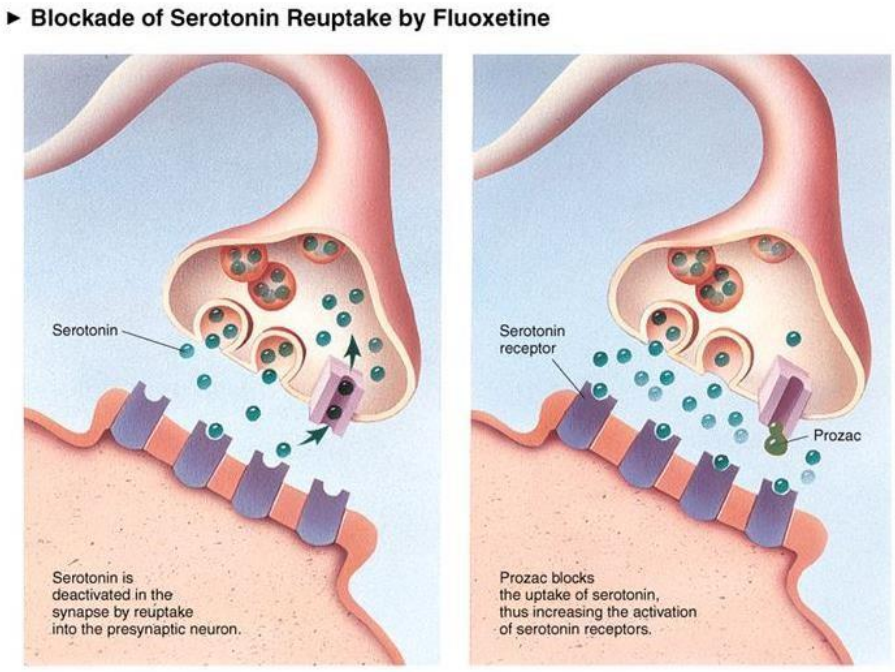


Figure 2: Mechanism of fluoxetine (Wenthur, Bennett and Lindsley, 2014)

2.3. Application of nanoparticles in biomedicine

Nanoparticles are small units whose dimensions almost resemble the building blocks of biological macromolecules such as proteins and DNA, this feature give a benefit to nanoparticles of being used for therapeutic purpose. Surface functionalization of nanoparticles can be done by various functional groups, signaling molecules, targeted molecules to make it target specific. (Mittal *et al.*, 2014) It can also be made biocompatible by binding with various functional groups and also it is conjugated with drug to be used as drug delivery vehicle. (Timbie, Mead and Price, 2015) The surfaces of nanoparticles can be modified in such a manner so as it can bind to various functional groups that defines the fate of the nanoparticles that where should it be targeted. Nanoparticles are of fundamental importance in nanofabrication because of their great scientific impact, their intense life changing effects on biomedical, optical, electronic fields and many other fields. Nanoparticles have unique properties such as a large specific surface area and consequently greater reactivity than macro-sized particles. They encompass a wide variety of products under one dimension and at least less than 1100nm in size. They have different physiochemical properties and colors depending on the material they are synthesized with.

(Khan, Saeed, and Khan, 2017) With these nanostructures drug delivery has been changed for the better more focused and controlled drug release drug delivery to a targeted area. Due to their small size penetration between and into the cell, blood brain barrier makes them feasible for brain targeting as well.

2.4. Nanomedicine and treatment of depression

In the last two decades, the success of nanotechnology has had a considerable influence on clinical therapies. (Rajesh et al., 2016) Very significant progress has been made in creating the discipline of Nano medicine in brain disease research to identify, diagnose, and efficiently treat brain-related disorders (Bozzuto, 2015) According to the National Institute of Health, nanomedicine is a medication formulation whose final product size is less than a micron. (Dimitrijevic and Pantic, 2014) Nanomedicine offers a significant advantage because of its capacity to bypass biological barriers, improve medication bioavailability, and even breach the blood-brain barrier. (Dong, 2018) It delivers hydrophobic medicines efficiently and selectively to illness locations. (Dailly *et al.*, 2004)

Nanoparticles are microscopic units with dimensions that are similar to the building blocks of biological macromolecules such as proteins and DNA; this property allows nanoparticles to be employed for therapeutic purposes. Surface functionalization of nanoparticles may be accomplished by the use of different functional groups, signaling molecules, and targeted compounds in order to make them target specific. (Mittal *et al.*, 2014)

It may also be made biocompatible by attaching to different functional groups, and it can be conjugated with drugs to be utilized as a drug delivery vehicle. (Timbie, Mead and Price, 2015)

The surfaces of nanoparticles can be changed such that they can attach to various functional groups, defining the fate of the nanoparticles and where they should be targeted.

One of the most important uses of nanomaterials in biomedicine is that they have an interior core or void in which the medication or substance to be targeted is encased.

As a result, not only is the drug's toxicity reduced, but also its prolonged release is accomplished.

To be employed in imaging methods, nanoparticles contain radiolabeled molecules and other small molecules in their interior core or void. Because of its target selectivity and nanoparticle encapsulation, this chemical has no adverse effects on the rest of the body and is also biocompatible. (Kaur *et al.*, 2014)

2.5. Liposomes as an efficient drug delivery system

Liposomes are specialized delivery vehicles that are tiny closed spherical vehicles with an interior aqueous portion enclosed by one or more lipid bilayers. The lipid bilayer mimics biological membranes and protects the medicine from being attacked until it reaches its target. These lipid bilayers surround the aqueous phase, which can contain medicines. Liposomes with diameters ranging from 400 nm to 2.5 mm and nanoparticles (NPs) with sizes ranging from 1 to 100 nm have outstanding chemical and physical features that are favorable for drug delivery by conjugation with pharmaceuticals. (Mura *et al.*, 2018) Liposomes have been extensively researched and are now utilized to treat a variety of ailments. Liposome membranes have unique bilayer-structure-like properties, they can sort out and solubilize both hydrophilic and hydrophobic materials, and they can be used as transferors for both water-soluble, lipophilic molecules, and they are composed of moderately biocompatible and biodegradable, non-immunogenic lipids. (De Jong and Borm, 2008)

Lipophilic medications, by definition, conjugate inside lipid bilayers. This important component aids in the drug's biodistribution and pharmacokinetics. Liposomes are highly recognised for their biological and technical benefits and are regarded as the most effective medication carrier to date. (Bozzuto, 2015) Liposomes improve pharmacokinetics and pharmacodynamics by decreasing fast disintegration, regulating numerous adverse effects, lowering toxicity, and extending the half-life of encapsulated medication. (Mura *et al.*, 2018)

Along with these liposome features and surface modification, it is the most effective drug delivery mechanism.High interstitial pressure produces limited drug solubility, and quick clearance of intravenously delivered medications from the blood circulation impedes adequate drug absorption in sick areas.

Because of the flexibility of their chemical composition and structure, liposomes provide a superior medication delivery method (Lamichhane *et al.*, 2018)

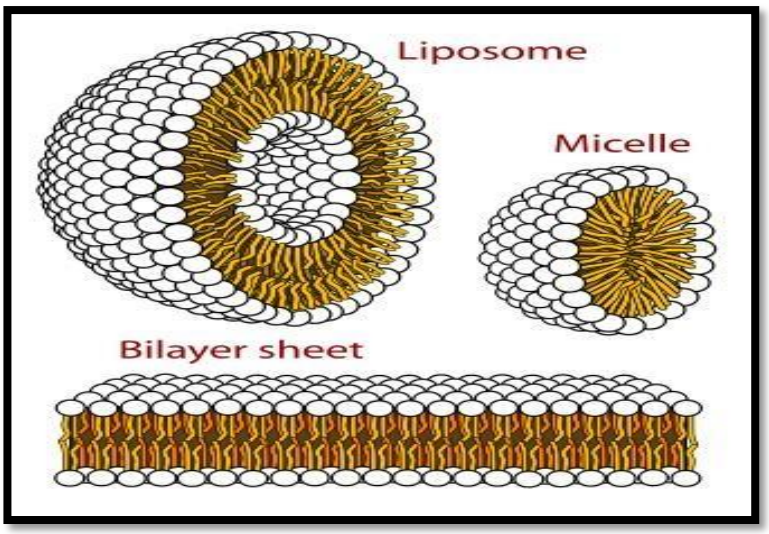


Figure 3: Different type of liposomes

2.6. Conventional and non-conventional liposomes

Liposomes and lipid nanoparticles (LNPs) are nanofabricated similarly but differ somewhat in function and composition. Both are lipid nano formulations that excel in drug delivery, transporting the medication/gene/protein of interest within a protective, outer layer of lipids that mimics a membrane bilayer for the safety of the material inside. LNPs can be used in a variety of ways. (Journal *et al.*, 2017)

LNPs are liposome-like structures that encapsulate a wide range of nucleic acids, including DNA and RNA, and are thus the most widely used non-viral gene delivery mechanism. Conventional liposomes include one or more layers of lipid bilayer enclosing an aqueous area, although not all LNPs have an associated bilayer, allowing them to be classified as lipid vesicles or liposomes. Some LNPs feature a micelle-like structure in which drug molecules are encapsulated. (Malam, Loizidou and Seifalian, 2009) Liposomes have many advantages that include high efficiency encapsulation efficiency regardless of drug solubility, low toxicity, drug protection against degradation factors like pH and light and the reduction of tissue irritation. (Laouini *et al.*, 2012)

Liposomes' biodegradability and biocompatibility, paired with their nano size, improve efficiency in nanomedicine, the food industry, and cosmetics. Liposomal nanoparticles may transport both hydrous phases and lipid in the liposome structure, allowing for a wide range of modifications in drug administration, encapsulation, and release of lipid-soluble, amphiphilic components, medicines, and biological molecules such as peptides or genes. As a result, they have a wide range of uses, from cosmetics to medicine. (Journal *et al.*, 2017).

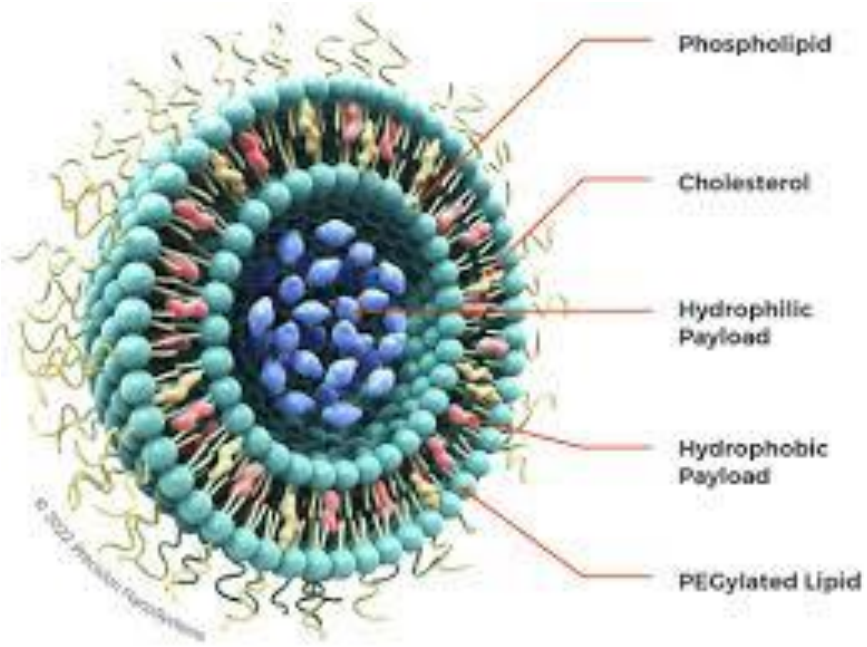


Figure 4: Liposomal nanoparticle (Bozzuto, 2015)

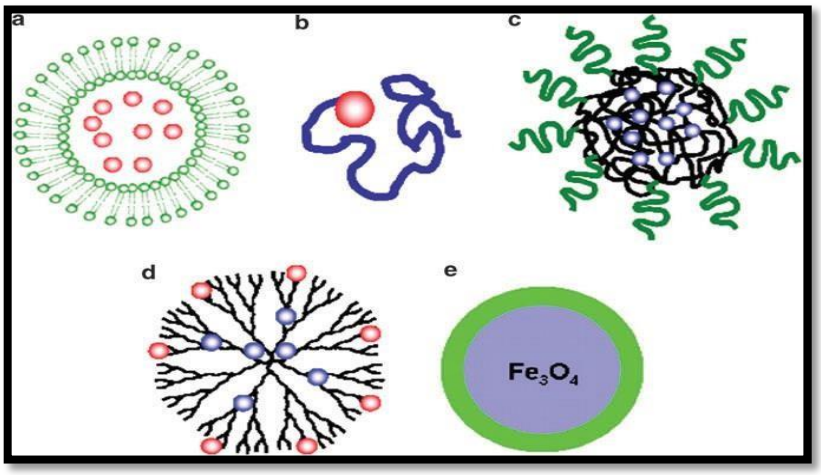


Figure 5: liposomal particles example (Bozzuto, 2015)

(a) liposome, (b) polymer–drug conjugate, (c) polymeric nanoparticle, (d) dendrimer, and (e) iron oxide nanoparticle. Red dots are hydrophilic drugs, and the blue dots are hydrophobic drugs. (Zhang *et al.*, 2008)

The most important aspect of utilizing nanoparticles in medicine and diagnostics is that they are biocompatible. (Couvreur,2013) The outer surface of nanoparticles is changed by attaching a tiny functional group molecule or encapsulating it with polyethylene glycol (PEG) to make it biocompatible, so that it does not elicit immunological responses or other inflammatory processes and is called a self-molecule.

Surface functionalization of nanoparticles with tiny functional group molecules or other ligands makes nanoparticles extremely targeted; also, the surface group connected to the nanoparticle allows for regulated and sustained drug release (Tong, Qin and Sun, 2017)

Furthermore, functionalization influences drug biodistribution and plays a crucial role in its pharmacokinetic behavior. The surface of nanoparticles modified by tiny functional groups has an essential influence in the mechanism of excretion of nanoparticles from the body as well as their bio-distribution. offers an indication of the sort of clearing that the nanoparticle uses (Swati Deore, S. R. Shahi, 2018).

2.7. Mode of treatment of depression

Antidepressants are commonly used to treat depression because they can pass the blood-brain barrier, but they are occasionally removed by PGP in the brain, leaving very little antidepressant at the intended location. (Lamichhane *et al.*, 2018).

Chapter 3

3. MATERIALS AND METHODS

3.1. Materials:

Fluoxetine hydrochloride, Milli Q water, Ethanol, Deionized water, cholesterol, 1,2-dipalmitoyl-sn-glycero-3-phospocholine powder and PEG 2000. All these chemicals were purchased from a Sigma-Aldrich (USA).

3.2. Methods:

Synthesis and characterization of PEG coated Fluoxetine loaded Liposomal nanoparticles

Modified ethanol Injection method, Blank liposomes, Fluoxetine loading, PEG coating

3.2.1. Synthesis and characterization of Drug loaded liposomal nanoparticles

At first lipid and Fluoxetine were dissolved in ethanol at 0.1mg/ml concentration separately. The lipid phase was made at a concentration of 100 *u*mol/ml by dissolving DPPC and cholesterol in ethanol in a ratio of 4:1 in 10ml ethanol. In the lipid phase, 500 ul of fluoxetine from the prior combination was added and sonicated for 45 minutes. 10ml of Milli Q water and sonicated suspension were put in a water bath to obtain a temperature of 60ºC. After reaching a temperature of 60 ºC, both phases were mixed and agitated in a water bath for another 15 minutes. The mixture was placed into a round bottom flask and a rotatory apparatus. To remove the ethanol, the liquid was placed into a round bottom flask and rotatory evaporated. After this evaporation, the nanoparticles were ready and kept at 4 ºC in a glass vial.

3.2.2. Preparation of liposomal nanoparticles coated with PEG2000

PEG-coated FLX-liposomal nanoparticles were also created utilizing a modified ethanol injection technique (Chorachoo *et al.*, 2013). Fluoxetine hydrochloride was dissolved in 100% ethanol in sufficient quantities to achieve a final concentration of 0.1mg/ml. To prepare the lipid phase at a concentration of 100 mol/ml, DPPC and cholesterol were dissolved in 10ml ethanol in a 4:0.75 ratio, and 500l of fluoxetine from the prior mixture was added. 2.5 mg/ml PEG 2000 was added to the solution dropwise, and the solution was magnetically agitated for 1 hour. The combination was then sonicated for 45 minutes (Branson Sonifier® SFX250, Danbury, USA). 10ml of Milli-Q water was put in a previously set temperature water bath previously preheated to 60 ºC, together with sonicated lipid and medication suspension The water phase was emulsified with the lipid phase after warming to 60 ºC, and the resultant suspension was agitated in a water bath for 15 minutes to allow for equal mixing and phase inversion. The resulting mixture was then transferred to a round bottle flask, which was then linked to an ethanol-evaporating rotary evaporator (Eyela Rotary Vacuum Evaporator N-100 series, Jeol, Japan). Following that, the turbid solution of fluoxetine with liposomal nanoparticles was poured into a glass vial, sealed, and stored until future usage.

3.2.3. Characterization of Fluoxetine-Incorporated Liposomal Nanoparticles:

Uncoated and PEG-coated Fluoxetine-liposomal nanoparticles were characterized in order to evaluate and analyze their particle size and surface charge, drug encapsulation efficiency and release efficiency, and to conduct further nanoparticle characterization tests.

3.2.4 Area Distribution and Size of Particles:

Scanning electron microscopy (SEM) was performed to determine the practical size. The particle size was further investigated using scanning electron microscopy, and the area distribution of the nanoparticles was computed using image j software. An analysis was carried out on a specific location. The 'Analyze Particles' command counts and measures items in binary and threshold pictures. It operates by scanning an image or selection until it discovers an object's edge. It then outlines the thing with the Wand Tool before measuring it with the Measure [m] command, and then repeats scanning until the end of the picture or selection is reached. By defining appropriate Size and Circularity ranges, as well as whether particles should be tracked by their outer edge or by flood filling, features of threshold pictures may be retrieved. For particle

size, values were assigned between 0 and 'Infinity.' Particles having sizes (areas) that fall outside of the range given in this field are disregarded. Circularity was assigned a value ranging from 0 (infinitely extended polygon) to 1. (Perfect circle). Particles with size circularity values beyond the prescribed range are likewise disregarded. 8-bit binary image of the best-fit ellipse (cf. Edit.Selection. Fit Ellipse) of each particle.

3.2.5. Drug efficiency:

Drug efficiency provides information regarding drug trapping inside liposomal vesicles. After preparing the nanoparticles, a UV spectrum usually for these nano particles shows absorbance at 263 nm. They are plot in the y=mx+c equation to obtain the concentration value that is for the original drug concentration to determine the amount of free drug in the medium.

3.3 Development of Chronic Mild Stress (CMS) model of Depression

Chronic Mild stress is a mental disorder that develop when a person or animal is subjected to stressful conditions in intervals in the beginning they try to escape from the environmental condition or the stresses but with the passage of time they become aggressive and then they become diseased when they still try to do the act changing conditions didn’t allow them to do so. So, they are more likely to develop the disease

3.3.1 Subjects:

Female 6 -8 weeks old mice Adolescents Blab/c mice were bought from the NIH in Islamabad. acclimatized for roughly a week with a steady supply of clean water and food. These mice were randomly divided into 4 groups. Mice were placed in 30, 15, 14 cm standard home cage. Home cages were filled with new bedding, and a 9:15 h light/dark cycle was maintained. The temperature was kept at 27°C ± 2°C, while the humidity was kept at 50% ± 5%.

3.3.2. Groups

These mice were divided into 4 groups after letting them acclimatize for 1 weeks with supply of water and food. Mice were divided into 4 groups. Control Normal(group C), simple fluoxetine), (peg coated nanoparticles), CMS/Depressed group

Groups of mice	Treatment	Treatment dosage
CMS 1	PEG coated Flx drug loaded nanoparticles	5mg/kg
CMS 2	Simple Flx Drug	10mg/kg
CMS 3	No treatment	No treatment
Normal Group	No treatment	No treatment

Table :1 Mice categorization based on treatment

3.3.3. Cages

Cages used was having segregation. Boxes of different measurement were used for the experiments. As during different behavior test separated room was used.

3.3.4. Protocol:

Mice were divided into four Groups that is Normal/Control group, Simple fluoxetine Group, depressed (CMS) Group and PEG coated flx loaded nanoparticles Group.As marking was done on the tail for identification purpose. The temperature of the room was maintained at 32-37 ^{o}C. as mice were placed for the different types of stressors 5 days a week.

All stressors duration was 3-4 hours and after every stressor animal was put back int to new clean bedding, Stressors were as following:

Monday: Damped bedding for 04 hours Food and water deprivation and also a weight measuring day.

Figure no 6: Wet Bedding

Tuesday: Falcon tubes, Shallow bath.

Figure no 7: Falcon Tube Stressor Test

Wednesday: Solid cages, Tilted cages

Cages were filled with mud to stress the mice for 4 hours.

The bedding was removed from the cages and the cages were tilted for 3 hours.

Thursday: Dark cycle, Sucrose test

Friday: Lights illumination, Light cycle

3.3.5 Chronic Mild stress

Prior to the start of the mice all were showing activeness, like running in cages, eating properly, roaming around like phenotype. After starting the protocol, mice developed some different behaviors, at the start of week they shown aggressive behavior and then they became depressed/Chronic mild stress (CMS)

Mice were exposed to the beam of light they tried moving from their compartment but after the development of the disease they stopped showing any resistance because of the chronic mild stress, After the development of the disease that is Chronic mild stress mice started showing Symptoms like.

Aggressiveness, rearing behavior, hiding under the bedding, huddling in the corner, Jumping out of cages, Sore coat\ fur shedding, Tail biting, Excessive grooming

As a type of depression is common as well as a serious medical illness that negatively effects mood and thinking.

Figure 8: **Rearing of mice**

Figure 9: Mice huddling in the corner

Figure 10: Mice feed measurement

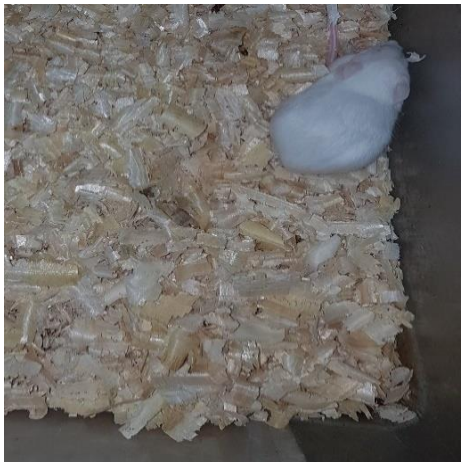


Figure 11: Depressed mice

Average food consumption of a normal mouse per day is 3 to 4 grams but the depressed mice consumed less than 2 grams, some consumed 0.5 grams of feed 44g of feed was put to each group, after the consumption the weight of the feed was more than expected weight.

As in figure 11 we can see mice biting their tail showing CMS symptoms.

Chapter 4

4. Results:

The result consists of two parts one is before treatment and the second is after treatment.

4.1 Results Before treatment:

Mean of weights before treatment

Weights for different mice groups during disease induction	PEG coated Flx nano particles	simple fluoxetine	depressed group	Normal group
no of weeks				
week1	31.6	33.9	33.6	33.6
week2	30.8	33	32.8	33.4
week3	30	32.4	32.5	34.2
week4	28.8	31.9	31	34.8
week5	27.6	30.73	30.6	36.5
week6	26.5	29.8	30	36.8
week7	26	28	29.8	37
week8	25.8	27.5	28.8	37.6
week9	25	27	28	38.2
week10	24	26	27.2	39.5

Table no :2 weight of mice of ten weeks

This table shows the result that mean of weights of the four group of mice that the mice show a drastic change in their weight during the induction and after the development of the disease that is chronic mild stress as they stopped the consumption of feed, so the weight of the diseased group was declined and that is visible in the graph of the data in table.

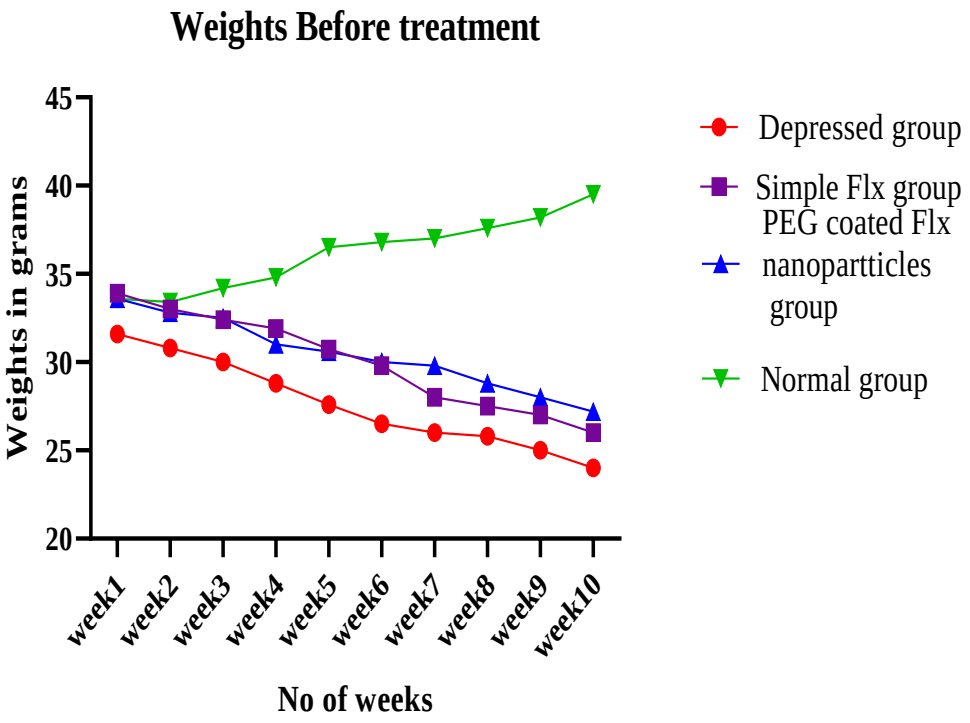


Figure 12: weights of mice before treatment.

4.1.2 Mean of sucrose consumption before Treatment:

2%sucrose solution was given to all groups at every Saturday and Sunday to evaluate the consumption and found that normal mice group consumption was same or sometimes more than the previous week while the (CMS) group was decreasing their consumption.as in the beginning both groups were consuming equal amount almost 70-75 ml while after 2nd week of the induction mice those were normal consumed more quantity that was 70-75ml while the (CMS)group consumed less than 68ml.

Similarly, at the 10th week of induction (CMS) group consumed 40-45ml of sucrose solution while the normal consumed more than 75ml of sucrose solution.

That was also one confirmation test for the chronic mild stress identification.

The Graph no: shows the decline curve in the consumption. statistical analysis (t-test" was used for it and gave the 0.0002 that is significant value.

4.1.3 Mean of the Elevated maze Plus Test:

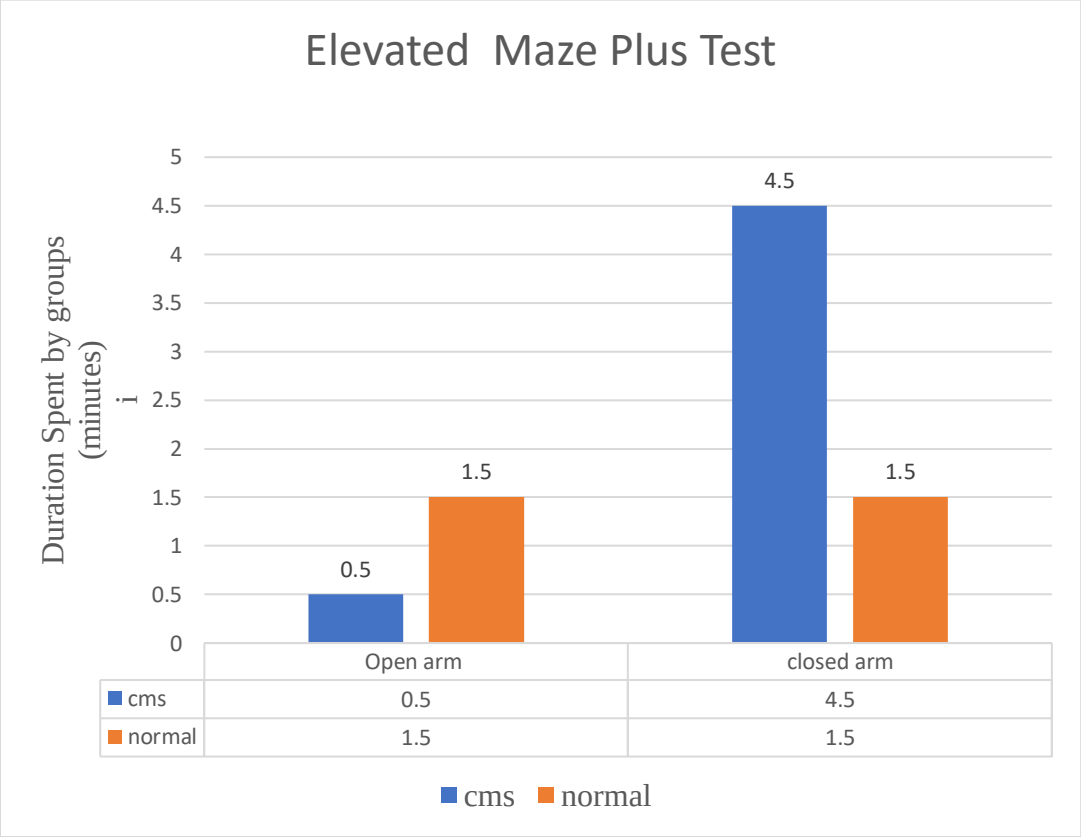


Figure 13: Elevated maze plus test

The elevated maze consists of open and closed arms all the groups were placed for same duration of 5mintues to notice their movements.

As per result and the statistics, the mice that were diseased, when they were placed on the Elevated maze plus table showed less movement in the open arm of the maze while the normal group more time in the open area.

The normal group kept on roaming around as they were healthy and active as their exploration indicates that they are healthy and active, while the other kept on showing symptoms of depression/Chronic mild stress by staying at closed arm and keep on rearing that is also a trait of depression. All the disease group was showing less activness.

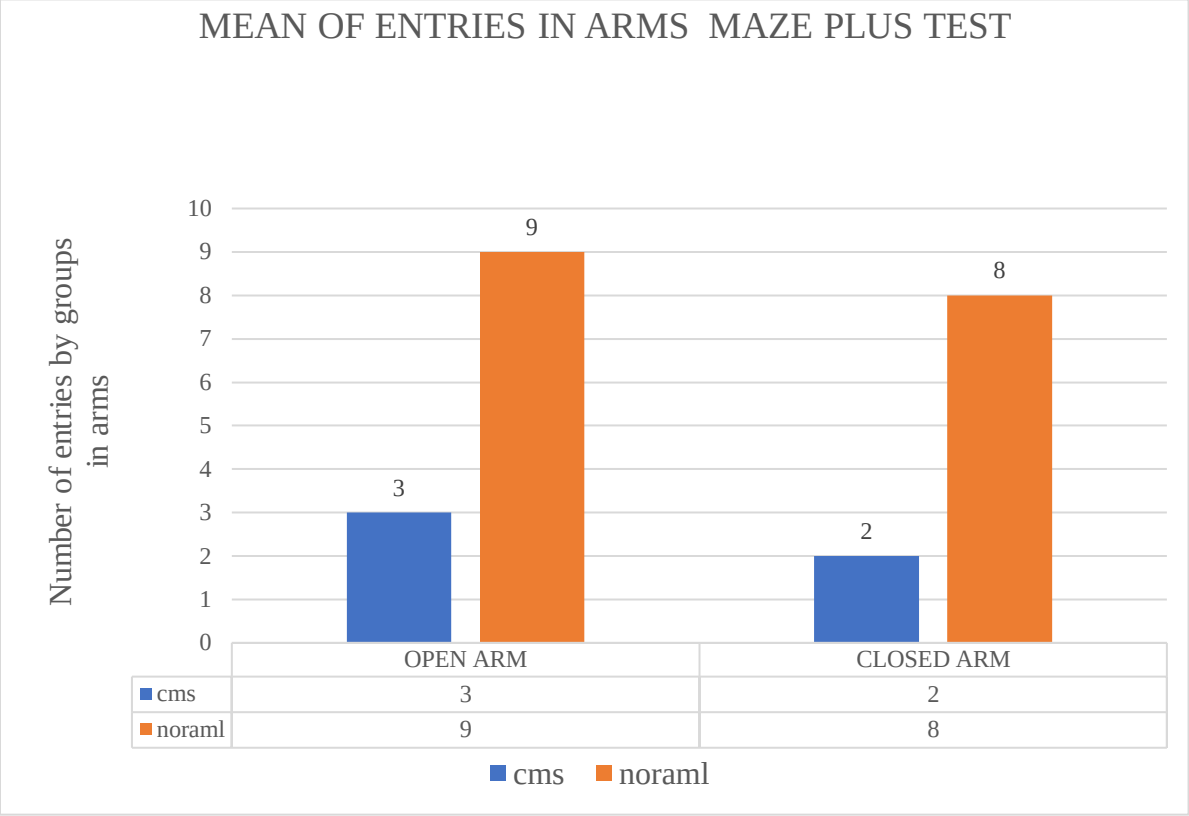
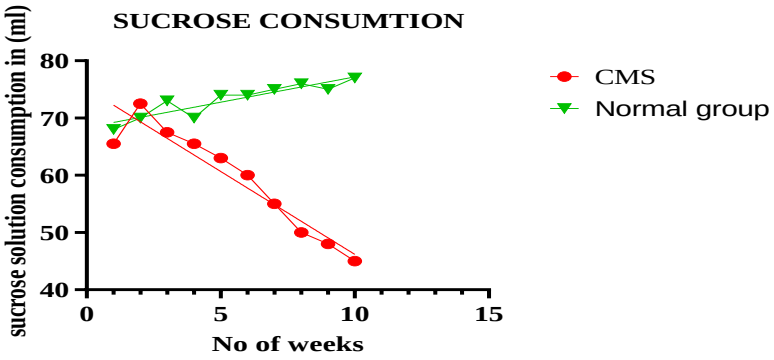


Figure 14: Mean no of Entries in Elevated maze plus test

The mean no of entries in open and closed arm was noticed as the CMS group made maximum 2 times entry in closed arm and remained there while made almost 3 times entry in the open arm.

Similarly, Normal/Control group was active kept on exploring and made 8 entries in close while 9 in open arm.

Sometimes normal group showed entries by moving completely like with four limbs in the open arm of elevated maze while sometimes just two limbs while the depressed groups hardly moved their four limbs out in the open arm . they kept on rearing in the closed arms . that was the darker so they preferred to stay there.



	CMS	Normal group
P value	<0.0001	0.0002

"t test" for data showed a significant P value (<0.05)

P value in Normal group vs Depressed (CMS) Group is **0.0002**

Figure 15: 2%sucorose solution consumptions

2% Sucrose solution consumption was regularly monitored in the duration of 10 weeks as it was for the verification of disease induction as well. As the normal/control group kept on utilizing the sucrose solution more than 70ml from 1st to 10th week but in case of the diseased groups 1st and 2nd weeks they consumed the sucrose solution but with the passing weeks they reduced the consumption and the decline can be seen from 65ml in 1st week , then almost 70 ml in the 2nd week but after that declined was started as that reduced from 65ml to almost 40 to 45ml.

4.1.4. Coat analysis:

The coat of the mice can also give some idea about its disease. When the animal is diseased it shows some of the shedding of fur like symptoms and here is one of the example in Figure 16 this animal having shredded fur from the upper back body . similarly some of animals were having baldness on their other parts of body like head and lower body parts.

Figure 16: Coat Analysis

4.2 Scanning Electron Microscopy and Particle size

The mean size of Blank Liposomes, drug loaded and Pegylated measured by SEM (standard electron Microscopy), were 23.5 nm and 117.8respectively.

There was a significant increase in nanoparticle size while using PEG-2000, And this size verification was performed by the image J software. The analysis was performed on the specific selected area.

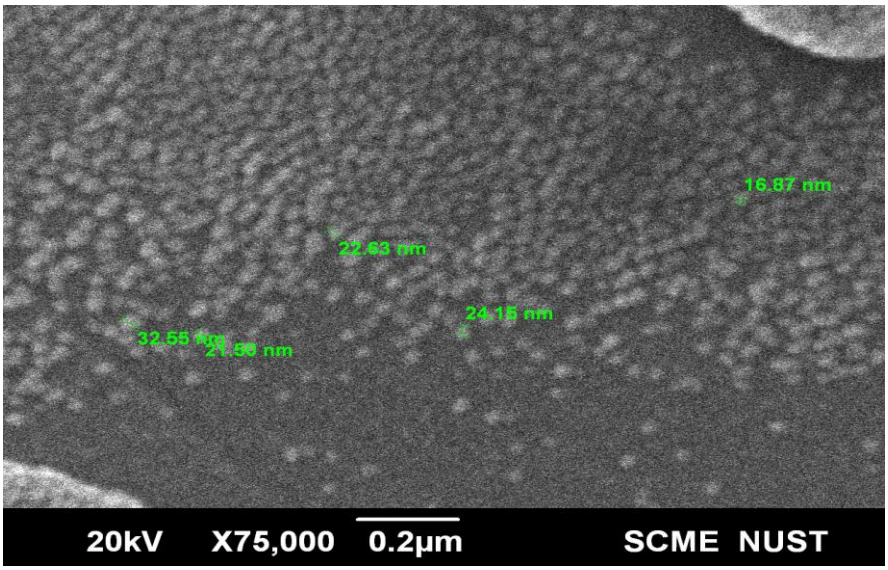


Figure 17 (a): Nano particles those containing simple fluoxetine

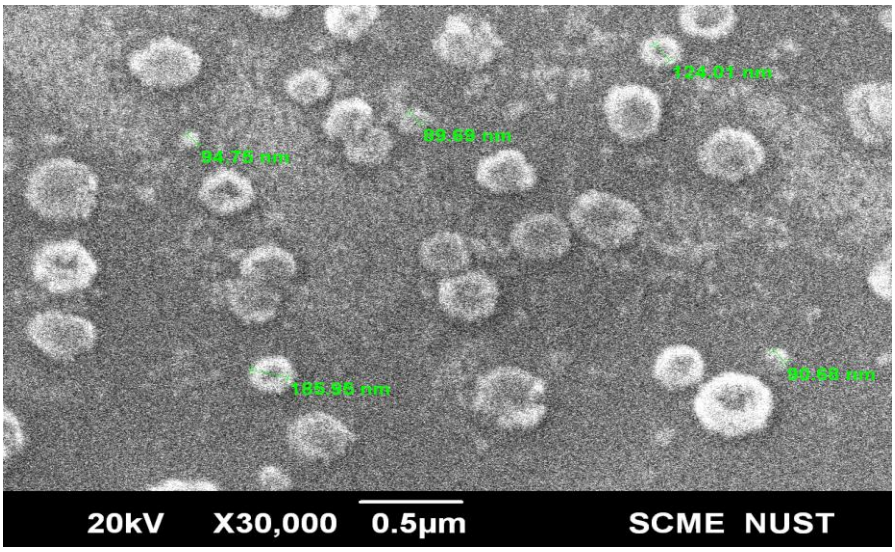


Figure:17 (b):Figure no and these are particles which were coated with PEG 2000,

Are the SEM of the Liposomal Nanoparticles. They were properly coated with PEG2000 and loaded with Fluoxetine Drug. There is a visible change in the size of the particles.

Dose calculations, Encapsulation Efficiency and its formulas for the treatment were following

5mg/kg

1micro liter in solution.

Encapsulation Efficiency:

E.E(%) = (Total Drug added-free none trapped) ×100

Total Drug added

By this formula encapsulation efficiency as calculated was 70%

Loading capacity was calculated as 34%.

$$LD(\%) = \frac{entrapped\ Drug}{Nano\ particle\ weight} \times 100$$

The above formula tells us that the 70% EE value is the amount of drug that is successfully being incorporated inside both coated and uncoated liposomal nanoparticles which will release in the body over the duration of 8 and 4.5 hour respectively.

All these characterization data demonstrated that nanoparticles were effectively created, and that due to their appropriate size and charge, they were able to pass the blood brain barrier and transp ort targeted nanoparticles to the brain to treat depression.3000 cm1 that were distinguished by the presence of asymmetric and symmetric stretching vibrations CH2and CH3 groups (Liu et al., 2002; Zhou et al 1997.Theygive strong peak at 2899 cm1 is caused by CH2 symmetric stretching

Vibrations Cholesterol contains one double band (CC) in the second ring.

This was detected at 1674 cm -1 (Gupta, Singh, Kumar & Khajuria, 2014).

The DPPC spectra revealed the DPPC characteristic vibration for methylene (CH2) stretching (2915 cm-1 for asymmetrical resonance at 2850 cm-1 for symmetrical vibration). Carbonyl group (C=O) was discovered at 1750 cm.

1, whereas symmetric PO2-stretching vibration was recorded at 1095 cm-1 (Mahato et al., 2015). The fluoxetine spectrum presents characteristic secondary amine groups at 3340 cm1 and to chains and rings C–H vibrations together with vibrational modes detected at 2900–2975 cm1 of CH, CH2 groups. Similarly, the band at 1635cm-1 can be used to show the bending of Nitrogen and Hydrogen single bond and Carbon and Florine single sigma bond at 1045cm-1 (González et al., 2011).

Conformational changes within the lipid structure were observed in PEG coated. FLX loaded liposomal nanoparticles, that were clarified by the CH2 stretching frequency regions of the acyl chain (2800-3000 percm), the C=O stretching regions (1700to 1760 cm) PO2 stretching bands of lipid head group (1000-1300 cm -1).

The detected shift in IR frequencies verifis lipid conformational changes in the structure of the lipids with the inclusion of fluoxetine and PEG coating. (Pham et al,2018)

4.3. Oral Administration of Drug:

Previously the drug was used to insert by Intra venous route while this research was performed on Oral administration route

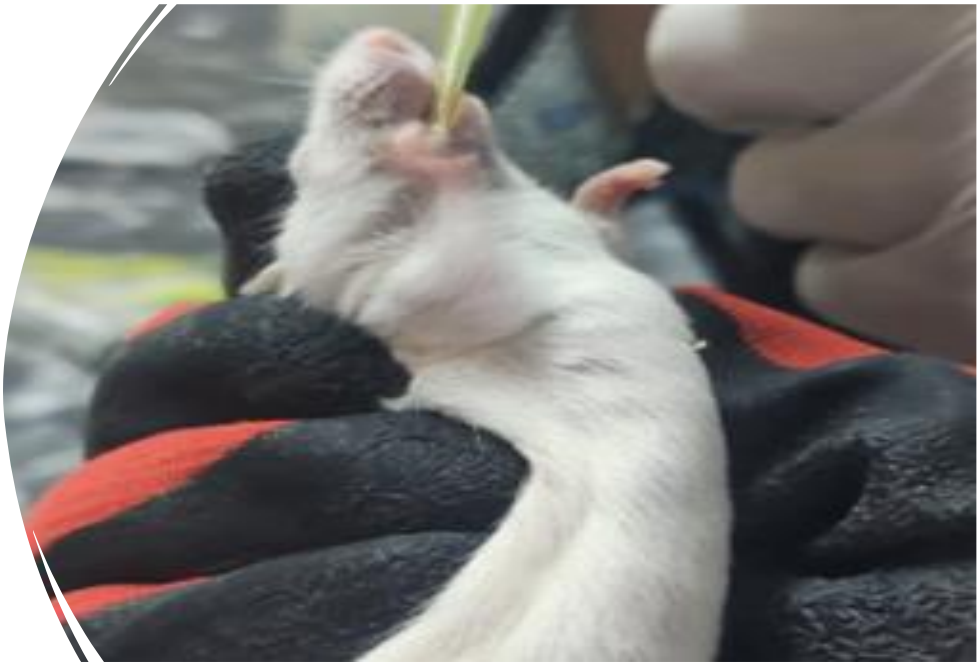


Figure 18: Oral Administration of Drug

Weight of mice was monitored during treatment and results were significant as the difference between the treated and untreated group is visible in the graph.

After treatment includes Open Filed test, Grooming Test, and multiple behavior tests along with weight measurement. As the consumption of food get increased and they started gaining weight.

4.3.1: Weights during and after treatment:

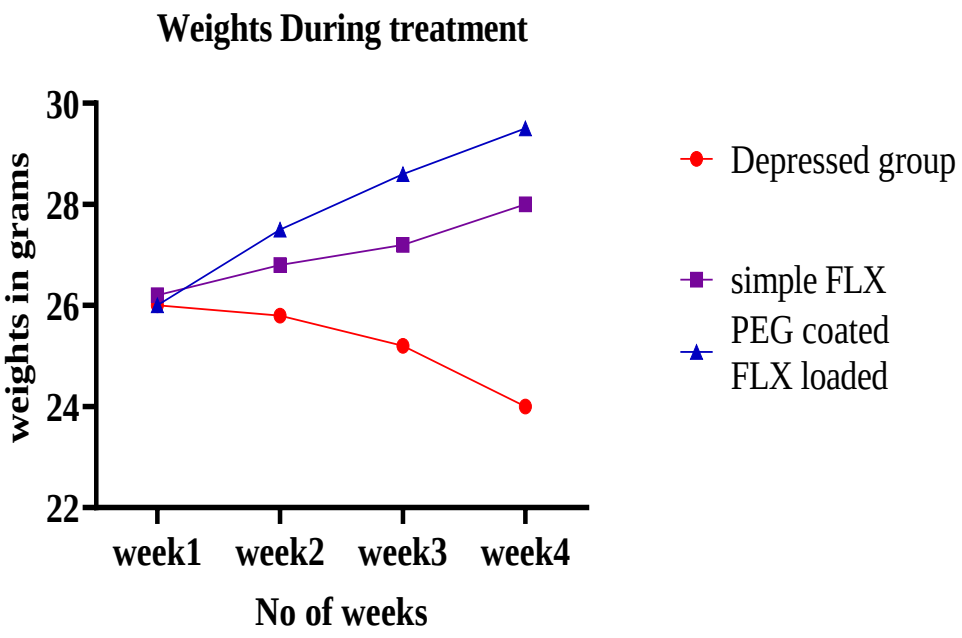


Figure 19: Graphical representation of weight.

As 1st week the average weight of PEG coated Flx started elevation and was significantly improved at the end of treatment, while the depressed group was continuously in the decline phase. simple Flx treated group also show some elevation in the weights.

4.3.2: Grooming test after treatment:

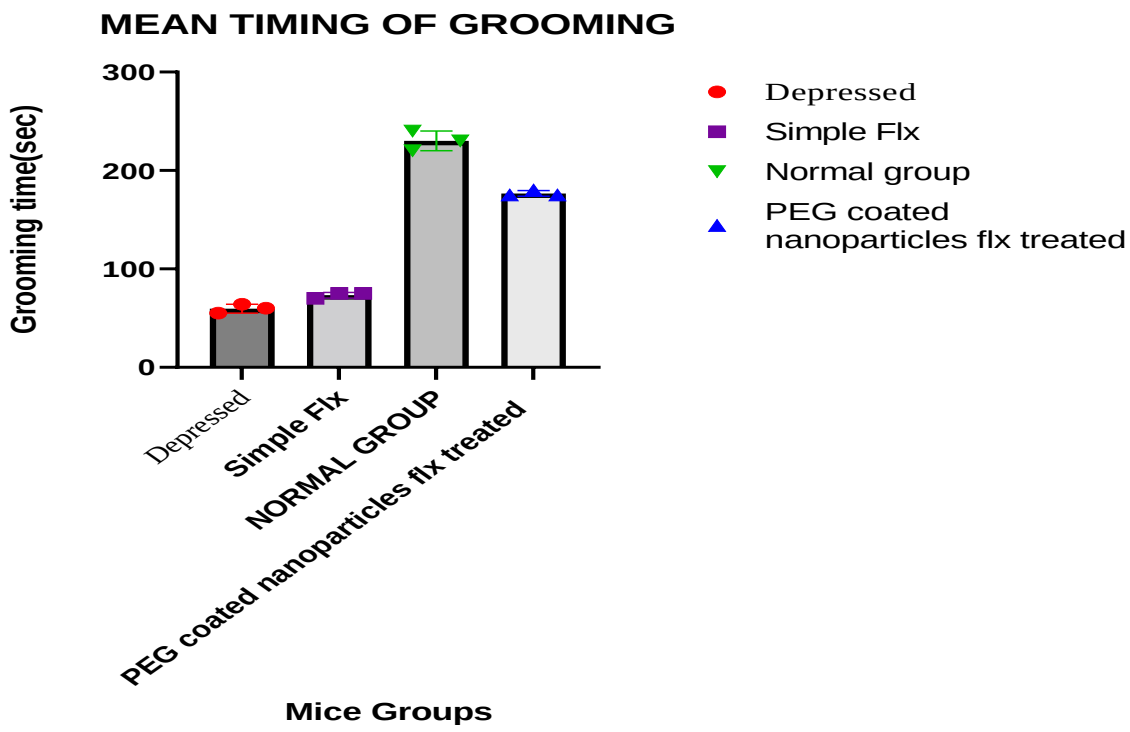


Figure 20: Graphical representation of mean Grooming Time.

The above graph tells us the mean duration of the grooming of four groups of mice as they were sprayed with sucrose solution, and they were put into observation to notice their grooming time in 5 minutes that is 300sec.

In duration of 300 sec as per graph the Depressed group of mice spent less than 50 sec in grooming while simple fluoxetine treated group spent (80-90) sec, Normal group spent most of duration in licking its fur that was 250 seconds while the PEG coated, and Drug loaded nanoparticles grooming duration was apars proximately (185-195) sec.

4.3.3: Open Field Tests:

BOUNDERIES	outer square	Inner square	wall area
CMS group	5	3	22
simple Flx group	14	4	12
PEG coated nanoparticles	15	7	8
control group	15	12	3

Table : 3 Open Field test (duration spent in different zones)

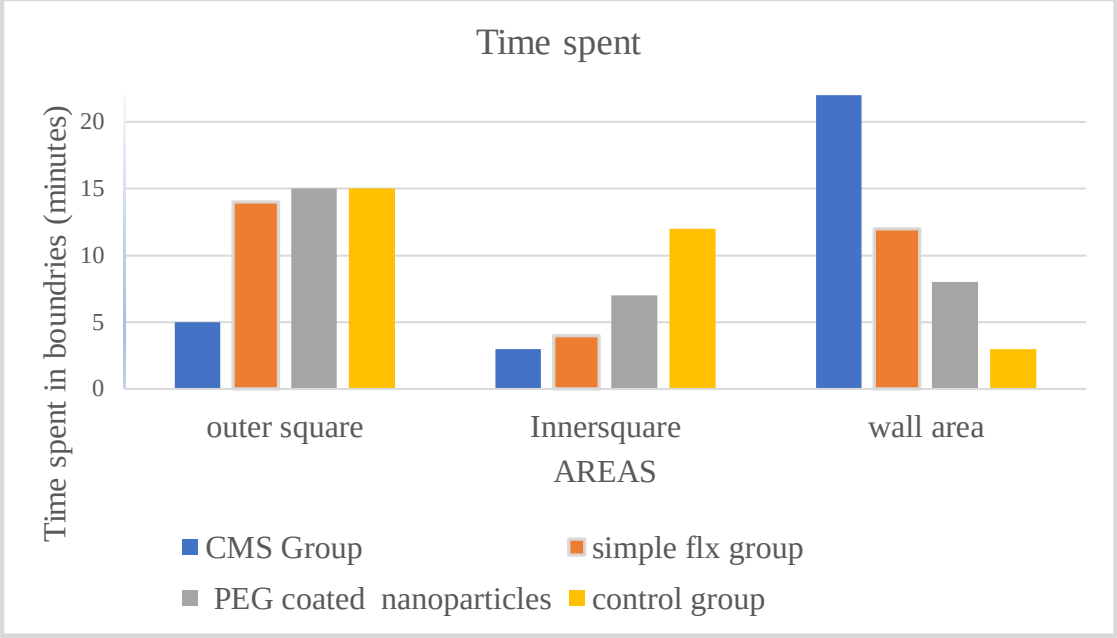


Figure 21: Graphical representation of Mean of duration spent by groups

The box is separated into three sections. Inner, outer, and wall zones and counted the time our groups entered these places. The graphs indicate the duration that the groups spent in the areas that is mentioned in the graph. peaks according to this graph Depressed mice preferred not to explore the inner zone of the box or the open field region. normal mice enjoyed exploring the inner and outer zones, while the depressed group remained in one spot. It can be observed that this group did not investigate the inner zone/open field, as the in the duration of 30 minutes CMS (depressed) group spent more the 20 minutes in the corner, less thAn 4 minutes in the inner and 4-5 minutes in the outer zone. The control group 3-4 minutes in the wall area, approximately 12 to 14 minutes in the inner while 15 minutes in the outer square in exploring. Similarly PEG coated nanoparticles spent approximately 15 minutes in the outer area while 6,7 minutes in the inner while 5,6 in the wall area. The simple fluoxetine group of mice spent 14-15 minutes in the outer area.

10-12 minutes in the inner square and 2-3 minutes in the wall area.

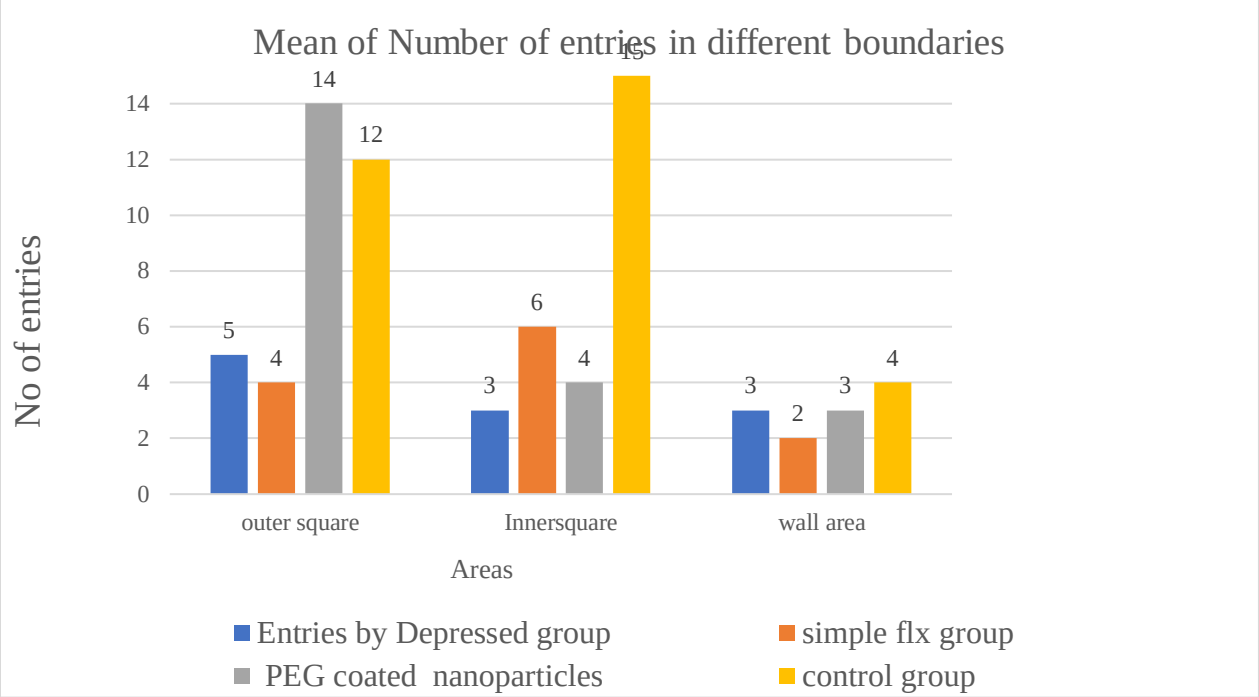


Figure 22: Graphical representation of Mean of numbers of entries in different boundaries

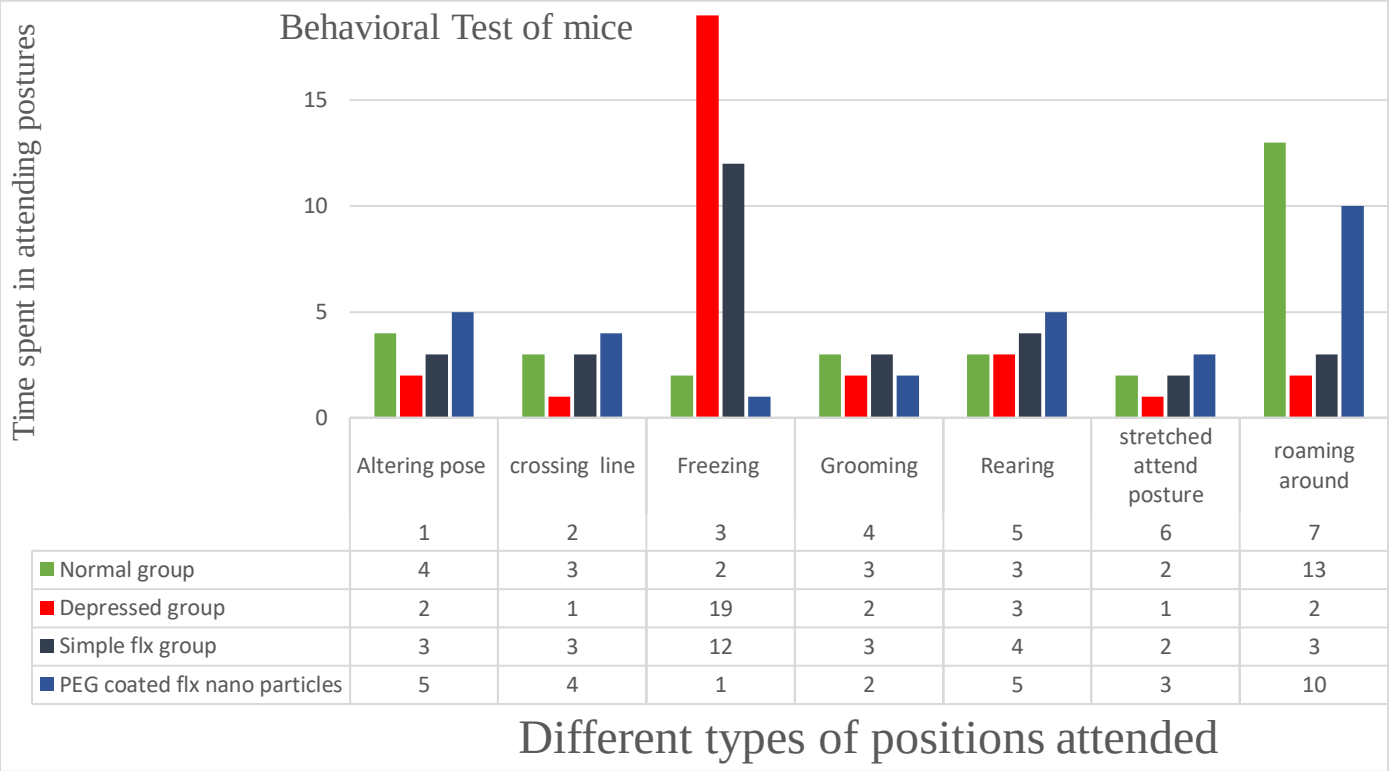
All mice were placed in the boxes for 30 minutes and observed. As the box is divided into three zones. Inner zone, outer and wall area. And noticed the number of entries made by our four groups of mice into these areas. Graphs shows that mean number of entries of the groups. according to this graphs peaks Depressed mice did not like to explore the inner zone of box or the open field area any normal mice liked to explore the inner zone and outer zone, so they remained still in the one location. Here it can be seen did not explore the inner zone/open field instead all the other groups were normal enough to explore the open field area.

Following is mean of Number of entries

Zones	Depressed	simple	PEG	control group
outer square	5	4	14	12
Inner square	5	6	4	15
wall area	3	2	3	4

Table : 4 No of Entries of made by Groups

4.3.5 Behaviors test of Mice:



	Altering pose	crossing line	Freezing	Grooming	Rearing	stretched attend posture	roaming around
	1	2	3	4	5	6	7
Normal group	4	3	2	3	3	2	13
Depressed group	2	1	19	2	3	1	2
Simple flx group	3	3	12	3	4	2	3
PEG coated flx nano particles	5	4	1	2	5	3	10

Figure 23 : Behavioral Test of Mice

This Figure 23 includes the Table containing the values that shows how different the behaviors of the mice of different groups were after the treatment. According to this figure the normal group of mice is showing activeness. More roaming around in the box in the duration of 30 minutes normal group mean was 13 minutes in roaming around ,4 minutes in altering poses ,3 minutes in crossing lines that is into different zones of the box 3 minutes in grooming ,3 minutes in rearing and only 2 minutes in the Freezing posture.

Depressed Group

This group spent 2 minutes in roaming around ,2 minutes in altering poses ,1 minute in crossing lines that is into different zones of the box 2 minutes in grooming ,4 minutes in rearing and 19 minutes in the Freezing posture.

Simple fluoxetine group

Simple fluoxetine group was one of the treated groups. They showed some improvement but were not as good as those group of mice which were treated with the Fluoxetine encapsulated with liposomal coated with PEG. as their duration was

As following spent 3minutes in roaming around, 2mintues in stretched attending posture, 4miutes rearing, 3 minutes in Grooming, 12minutes in Freezing, 3 minutes in crossing line and 3 minutes in altering poses.

The last and the treated group of mice that is PEG coated fluoxetine nanoparticles showed a very drastically changed behaviors as respect to the depressed on as it spent 10minutes in roaming around, 3mintues in stretched attending posture, 5miutes rearing, 2minutes in Grooming, 1minute in Freezing, 4minutes in crossing line and 5 minutes in altering poses.

Conclusion:

As the aim was induction of CMS and its treatment with PEG coated liposomal nanoparticles with fluoxetine drug.

Oral route administration of drug, Behavioural test for confirmation of treatment results. After 10 weeks period of chronic mild stress protocol animals monitored for unusual symptoms like sleeping alone remain at the corner zones. Coat(fur) condition, increase in some activities like grooming, rearing and exploratory activity reflection anxiety and depression like behaviour in mice. The difference in the sucrose splash test, different postures and movement of CMS induced and treated has been noticed. Polyethylene glycol (PEG) on the liposomal surface is known to be effective as it increases formulation stability, prolonging blood circulation time, controlling release rate and preventing carrier uptake by the reticuloendothelial system.

These properties made PEGylated liposomes an attractively increased the therapeutic index of the drug. Depression is a sort of serious psychological condition that effect almost every person at least once in their lifetime, that is characterized by different symptoms like sad feelings, low self-esteem, lack of focus and interest, loss of appetite leads to weight loss, sleep lacking and many more. the main goal of the study was to prepare a nano formulation that can serve in a more effective and efficient way. Though many other anti-depressants drugs are available, but the blood brain barrier selective permeability is a real hurdle that limit the advantages offer by these medications. So, this is made up of continuous fenestrated vessels that regulate the chemicals, ions and cells to words and from membrane in order to maintain homeostasis to protect the CNS from

the pathogens, toxins, injury and disease. Nano technology has revolutionized the world of medicine and sciences so that enabled the development of fine small nano sized structure can pass out through any minute system for the beneficial service.

References:

Abouhussein, D. M. N. *et al.* (2018) 'Journal of Drug Delivery Science and Technology Brain targeted rivastigmine mucoadhesive thermosensitive In situ gel : Optimization , in vitro evaluation , radiolabeling , in vivo pharmacokinetics and biodistribution', *Journal of Drug Delivery Science and Technology*. Elsevier, 43(July 2017), pp. 129–140. doi: 10.1016/j.jddst.2017.09.021.

Bozzuto, G. (2015) 'Liposomes as nanomedical devices', pp. 975–999.

Cao, B. *et al.* (2019) 'Progress in Neuropsychopharmacology & Biological Psychiatry Pharmacological interventions targeting anhedonia in patients with major depressive disorder : A systematic review', *Progress in Neuropsychopharmacology & Biological Psychiatry*. Elsevier, 92(November 2018), pp. 109–117. doi: 10.1016/j.pnpbp.2019.01.002.

Couvreur, P. (2013) 'Nanoparticles in drug delivery : Past , present and future ☆', *Advanced Drug Delivery Reviews*. Elsevier B.V., 65(1), pp. 21–23. doi: 10.1016/j.addr.2012.04.010.

Dailly, E. *et al.* (2004) 'Dopamine , depression and antidepressants', 18, pp. 601–607. doi: 10.1111/j.1472-8206.2004.00287.x.

Davis, L. *et al.* (2008) 'Major depression and comorbid substance use disorders', pp. 14–18.

Deore, S., Shahi, S. R. and Dabir, P. (2016) 'NANOPARTICLE : AS TARGETED DRUG DELIVERY SYSTEM FOR DEPRESSION', 8(3).

Dimitrijevic, I. and Pantic, I. (2014) 'Application of nanoparticles in psychophysiology and psychiatry research', *Reviews on Advanced Materials Science*, 38(1), pp. 1–6.

Dong, X. (2018) 'Current strategies for brain drug delivery', *Theranostics*, 8(6), pp. 1481–1493. doi:
10.7150/thno.21254.

Gaynes, N. I. *et al.* (2019) 'Perinatal Depression : A Systematic Review of Prevalence and Incidence', (290), pp. 2–3. doi: 10.1097/01.AOG.0000183597.31630.db.

Haque, S. *et al.* (2014) 'Development and evaluation of brain targeted intranasal alginate nanoparticles for treatment of depression', *Journal of Psychiatric Research*. Elsevier Ltd, 48(1), pp. 1–12. doi:
10.1016/j.jpsychires.2013.10.011.

De Jong, W. H. and Borm, P. J. A. (2008) 'Drug delivery and nanoparticles: Applications and hazards', *International Journal of Nanomedicine*. doi: 10.2147/IJN.S596.

Journal, A. I. *et al.* (2017) 'Recent advances on liposomal nanoparticles : synthesis , characterization and biomedical applications', *Artificial Cells, Nanomedicine, and Biotechnology*. Informa UK Limited, trading as Taylor & Francis Group, 0(0), pp. 0–1. doi: 10.1080/21691401.2017.1282496.

Kaur, P. *et al.* (2014) 'Brain delivery of intranasal in situ gel of nanoparticulated polymeric carriers containing antidepressant drug : behavioral and biochemical assessment', *Journal of Drug Targeting*. Informa UK Ltd, 00(00), pp. 1–12. doi:
10.3109/1061186X.2014.994097.

Khan, Ibrahim, Saeed, K. and Khan, Idrees (2017) 'Nanoparticles : Properties , applications and toxicities', *Arabian Journal of Chemistry*. The Authors. doi:
10.1016/j.arabjc.2017.05.011.

Kobak, K. A. *et al.* (2007) 'Why Do Clinical Trials Fail ? The Problem of Measurement Error in Clinical Trials : Time to Test New Paradigms ?', 27(1), pp. 1–5. doi: 10.1097/JCP.0b013e31802eb4b7.

Kurlowicz, B. L. (2007) 'The Geriatric Depression Scale (GDS) Geriatric Depression Scale : Short Form', 107(4).

Lamichhane, N. *et al.* (2018) 'Liposomes : Clinical Applications and Potential for Image-Guided Drug Delivery', pp. 1–17. doi: 10.3390/molecules23020288.

Laouini, A. *et al.* (2012) 'Preparation , Characterization and Applications of Liposomes : State of the Art', 1(2), pp. 147–168. doi: 10.1166/jcsb.2012.1020.

Malam, Y., Loizidou, M. and Seifalian, A. M. (2009) 'Liposomes and nanoparticles : nanosized vehicles for drug delivery in cancer'. doi: 10.1016/j.tips.2009.08.004.

Mittal, D. *et al.* (2014) 'Insights into direct nose to brain delivery: Current status and future perspective', *Drug Delivery*, 21(2), pp. 75–86. doi: 10.3109/10717544.2013.838713.

Mura, P. *et al.* (2018) 'European Journal of Pharmaceutics and Biopharmaceutics In situ mucoadhesivethermosensitive liposomal gel as a novel vehicle for nasal extended delivery of opiorphin', *European Journal of Pharmaceutics and Biopharmaceutics*. Elsevier, 122(October 2017), pp. 54–61. doi: 10.1016/j.ejpb.2017.10.008.

Rajesh, E. *et al.* (2016) 'Harvesting of stem cell in dental tissue', *Biomedical and Pharmacology Journal*, 9(2), pp. 819–821. doi: 10.13005/bpj/1008.

Robertson, O. D. and Dodd, S. (2019) 'Putative neuroprotective pharmacotherapies to target the staged progression of mental illness', (December 2018), pp. 1–18. doi: 10.1111/eip.12775.

SWATI DEORE, S. R. SHAHI, P. D. (no date) 'nano'.

Timbie, K. F., Mead, B. P. and Price, R. J. (2015) 'Drug and gene delivery across the blood-brain barrier with focused ultrasound', *Journal of Controlled Release*. Elsevier B.V., 219, pp. 61–75. doi: 10.1016/j.jconrel.2015.08.059.

Tong, G. F., Qin, N. and Sun, L. W. (2017) 'Development and evaluation of Desvenlafaxine loaded PLGA-chitosan nanoparticles for brain delivery', *Saudi Pharmaceutical Journal*. King Saud University, 25(6), pp. 844–851. doi: 10.1016/j.jsps.2016.12.003.

Wenthur, C. J., Bennett, M. R. and Lindsley, C. W. (2014) 'Classics in Chemical Neuroscience :

Fluoxetine (Prozac)'.

Zhang, L. *et al.* (2008) 'Nanoparticles in Medicine : Therapeutic Applications and Developments', 83(5), pp. 761–769. doi: 10.1038/sj.clp.