IJLSR (2017), Vol. 3, Issue 5 **Research Article**

International Journal of Life Sciences and Review (IJLSR)

Received on 07 March, 2017; received in revised form, 11 May, 2017; accepted, 16 May, 2017; published 31 May, 2017

Document heading doi: 10.13040/IJPSR.0975-8232.IJLSR.3(5).60-66

FORMULATION AND PERFORMANCE EVALUATION OF ESCITALOPRAM LOADED BIO-NANO SUSPENSION USING A NOVEL BIO-RETARDENT FROM *PIPER NIGRUM*

Deepika Raina and N. V. Satheesh Madhav*

DIT University, Makkawala, P. O. Bhagwantpura, Dehradun - 248 009, Uttrakhand, India.

ABSTRACT: Nanoparticles represent a promising drug delivery system of controlled and targeted release. The current research work was to explore novelistic route for targeting to brain through ear by formulating nanosuspension using escitalopram as a model drug permitting better control over depression. Depression is the second most prevailing disease after cardio-vascular diseases. The delivery has overcome the dose dumping problem in case of oral system. In this research work significant effort was made to explore novelistic platform for ear to brain. Bio-nano suspensions were prepared by using a biopolymer which was isolated from berries of *Piper nigrum*. Eight formulations were prepared of different ratios *i.e.* 1:0.5, 1:1, 1:2, 1:3, 1:4, 1:5, 1:7, 1:10,. The formulations were subjected to various evaluations, including pH, % transmittance, Content uniformity, *ex-vivo*, stability, release for over 36 hours. Different formulations of Escitalopram out of which F1 (1:0.5) was found to be the best formulation having r2 value of 0.9905 tT80: 22 hrs and best fit model was found to be higuchi matrix, and mechanism of transport was anomalous transport which was calculated by bits software. According to the *in-vitro* results obtained it can be concluded that significant amount of drug reaches to the brain *via* external acoustic meatus. The demand for curbing depression and other mental health conditions is on the rise globally. Nano suspensions are distinctive and commercially feasible approach to solve the problems of hydrophobic drug such as poor solubility and poor bioavailability.

Keywords: Acoustic meatus, Nano-suspension, Higuchi matrix, Anomalous transport.

Correspondence to Author:
Prof. (Dr.) N. V. Satheesh Madhav

DIT University, Makkawala, P. O. Bhagwantpura, Dehradun - 248 009, Uttrakhand, India.

E-mail: Satheesh_madhav@yahoo.com

INTRODUCTION: Escitalopram is the most selective serotonin reuptake inhibitor (SRI) antidepressant available [1]. Although pharmaco-logical and psychological interventions are both effective for major depression, antidepressant drugs remain the mainstay of treatment.

QUICK RESPONSE CODE

DOI:

10.13040/IJPSR.0975-8232.IJLSR.3(5).60-66

Article can be accessed online on:
www.ijlsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJLSR.3(5).60-66

During the last 20 years, selective serotonin reuptake inhibitors qqqq (SSRIs) have progressively become the most commonly prescribed antidepressants. Escitalopram, the last SSRI introduced in the market, is the pure S-enantiomer of the racemic citalopram. Escitalopram has no or very low affinity for other receptors (alpha- and beta-adrenergic, dopamine (D1-5), histamine (H1-3), muscarinic (M1-5), and benzodiazepine receptors) [2]. Depression is a significant contributor to the global burden of disease and affects people in all communities across the world. Today, depression is estimated to affect 350 million people.

Depression is a common mental disorder that presents with depressed mood, loss of interest or pleasure, decreased energy, feelings of guilt or low self-worth, disturbed sleep or appetite, and poor concentration. Moreover, depression often comes with symptoms of anxiety. At its worst, depression can lead to suicide. Almost 1 million lives are lost yearly due to suicide, which translates to 3000 suicide deaths every day [3]. External Acoustic Meatus: has length 24mm along posterior wall it is not a straight tube (S shaped) it has 2 parts, outer part-directed upwards, backwards and medially inner part-directed downward, forward and medially. The cartilaginous canal, the skin contains sebaceous and apocrine glands with many hair follicles. Together, the hair follicle, sebaceous gland, and apocrine gland are termed the apopilosebaceous unit.

Auricle: The auricle, a part of the external ear, consists of elastic cartilage covered by skin. The lobule consists merely of fibrous tissue and fat. The auricle is connected with the fascia on the side of the skull by unimportant anterior, superior, and posterior auricular muscles, which are supplied by the facial nerve. The auricle is supplied by both cranial (auriculotemporal nerve from cranial nerve V; probably also twigs from cranial nerves VII, IX, and X) and spinal (lesser occipital and great auricular) nerves [4].

Blood Supply of External Canal: Laterally supplied by the post auricular and superficial temporal arteries. Medially it is supplied by deep auricular artery which is a branch of first portion of the internal maxillary artery. This deep auricular artery supplies the tympanic vascular ring. Veins from the external canal drain into the superficial temporal and post auricular veins. The post auricular vein connects to the sigmoid sinus *via* the mastoid emissary vein, this anastomosis provide a route for infections of the external ear to spread to the intra cranial cavity [5].

Ear canal is having a unique histology, blood supply, nerve supply like mandibular (auriculo-temporal branch), vagus nerve (auricular branch), internal maxillary (tympanic branch), glosso-pharyngeal nerve connections present in auditory canal. The unique platform can be used for targeting brain by various Active Pharmaceutical ingredients used for brain diseases having various drawbacks of more adverse reactions and withdrawal symptoms. As on date the oral and parenteral dosage form exist for the antidepressant drug in the market but these molecule upon administration in long term therapy produces short term ADR's and Long term ADR's. Delivery of API molecule to the brain for the management of depressive disorder is significant, minimizes the ADR and side effects of therapeutic molecule and offer good patient compliance through this novelistic approach. The unique anatomical arrangement of blood vessels and sinuses in the human skull and the brain, the prevalence of a high density of skin appendages in the scalp, extra cranial vessels of the scalp communicating with the brain *via* emissary veins and most importantly, the way that the scalp is used in Ayurvedic medical system in treating diseases associated with the brain show that a drug could be trans cranially delivered and targeted to the brain through the scalp.

The emissary veins draining blood from extra cranial sites into the intracranial sinuses pierce a series of foramina present in the cranial bones. Scalp veins communicate with the sinuses of the brain *via* emissary veins. There are thirteen emissary veins connecting extra cranial sites of the head with intracranial sinuses. Seven major sinuses within the skull are inter connected by a number of anastomosing veins, which finally drain intra cranially into jugular veins giving ample scope for the diffusion of the drug molecules into the nerve tissue of the brain. These anatomical arrangements of the vascular system of the brain are made use of in the investigations to establish the brain targeted trans cranial route (TCR) of drug delivery [6].

In recent years, there has been a considerable interest in the development of novel drug delivery systems using particulate delivery systems like nanoparticles. Nanoparticles represent a promising drug delivery system of controlled and targeted release. In this context, nano suspensions will be effective in increasing the solubility, bioavailability of poorly soluble drugs. A large proportion of new chemical entities coming from drug discovery are water insoluble, and therefore poorly bio-available, leading to hurdles in formulation development efforts.

There are number of formulation approaches like micronisation, solubilization using co-solvents, precipitation techniques etc., to resolve the problems of low solubility and low bio-availability. Each of them has their own limitations. Other techniques like micro emulsions, solid dispersions and inclusion complexes using cyclodextrins even though showed increased solubility, but not applicable for drugs which are insoluble in both aqueous and organic media. The next development step is transformation of the micronized drug to drug nanoparticles and nano suspensions.

Nanoparticulate drug delivery system may offer plenty of advantages over conventional dosage forms which include improved efficacy, reduced toxicity, enhanced bio distribution and improved patient compliance. Nanosuspension technology offers novel solution for these poorly soluble drugs. Nanosuspension consists of pure poorly water soluble drugs with or without any matrix material suspended in dispersion. Nano-suspensions are distinctive and commercially feasible approach to solve the problems of hydrophobic drug such as poor solubility and poor bioavailability [7].

MATERIALS AND METHOD:

Isolation of Bio-Material Fruit of *Piper nigrum*: Piper comes in two types black and white piper obtained from the ripen and after the separation of pericarp of fruits of *piper nigrum*, family piperaceae. It constis of phenolic esters, ethers, pyrrolidone, volatile oils and ligands [8]. Isolation of bio-polymer from white pepper (*Piper nigrum*), which is a flowering vine in the family Piperaceae [9]. White pepper corns were powdered and soaked in Methanol: Glacial acetic acid: Concentrated Sulplhuric acid (85:10:5). Solution was kept on the magnetic stirrer for continuous stirring for 30 minis then filtered and 10 ml of sodium hydroxide was added.

To the above solution cold water was added and precipitate was obtained, Kept in refrigerator for 24 hrs centrifuged at 3000rpm for a period of 15 minutes, dried and stored. The bio polymer was subjected to various spectral analyses including UV, IR, SEM. Nanosuspensions were prepared by sonication method using *Piper nigrum* as retardant and with other co-processing agent. Eight formulations were prepared *viz*. 1:0.5, 1:1, 1:2, 1:3, 1:4, 1:5, 1:7, 1:10. The formulations were subjected to various evaluations, including pH, % transmittance, Content uniformity, *Ex-vivo*, stability release for over 36 hours.

Nano-Sizing of Escitalopram: To 100mg of Escitalopram, 5ml methanol was mixed and triturated. 5ml distilled water was added slowly and sonicated for 5 cycles (1 cycle for 3 min.). After each sonication cycle absorbance and %T were measured. It was then micro centrifuged. Supernatant and residue were collected. Residue was dried and nanoparticles were recovered [10].

Drug Excepient study: The pure drug along with the formulation excipients were subjected to interaction study by U.V Spectroscopy. The study was carried out by dry and wet mixing of the drug and excipient in ratios of 1:1, 1:3, 3:1. Both the mixture was stored at room temperature and at 55°C for three days. The dilution was made by the solvent and the sample was scanned at λ_{max} using UV spectroscopy.

Permeability: Drug solution of 1mg/ml was prepared and 1ml drug solution poured in donor compartment. pH 7.2 buffer was prepared and was kept in the receptor compartment. The sample was replaced completely every time. Egg membrane was used as a biological membrane as it mimics the action of the ear biological membrane

Formulation of Bio Nano Suspension: Nanosuspensions were prepared by sonication method using *Piper nigrum* as retardant and with other co-processing agent like glycerin and dextrose as a nanosizent. Weighed amount of drug, dextrose and polymer was triturated together in mortar and pestle and kept on sonicator. Glycerin was added to above mixture in sonication mode. Eight formulations were prepared viz. 1:0.5, 1:1, 1:2, 1:3, 1:4, 1:5, 1:7, 1:10, 1:15, and 1:20. The formulations were subjected to various evaluations parameters

Physicochemical Characterization of the Bio-Polymer: The isolated bio-material was check for color, odour, taste, solubility, colour changing point, and viscosity. The biopolymer was also tested for the presence of carbohydrates and proteins.

SEM Analysis: The SEM analysis of the bio-polymer revealed that it has a smooth surface with no rough edges. It shows the smooth, amorphous nature of the bio-polymer. The bio-polymer showed a morphological structure similar to the polymers and hence it confirms the polymeric nature of the bio-polymer (**Fig. 4**).

***In-vitro* Adhesive Study using the Shear Stress Method:** The adhesive property of the isolated biomaterial was determined by *in-vitro* shear stress method. Three different concentration of the biomaterial (1%, 3%, 5%) were placed between two glass plates and subjected to shear stress for assessment of *in-vitro* adhesive strength in terms of weight required for breaking adhesive bonds between the material and the glass plate after a specified contact time of 5, 10, 15 and 30 minutes.

RESULTS AND DISCUSSION:

Isolation of Bio-Material from the Fruit of *Piper nigrum*: The % yield for *piper nigrum* was found to be 15.2 ± 2.33% with a color changing point of 215 °C ± 5 °C. The bio-materials were purified and no presence of chlorides, sulphates and starch was observed (**Table 1**).

TABLE 1: CHARACTERIZATION OF BIOPOLYMER

1.	Colour	Light brown
2.	Odour	Odourless
3.	Taste	Characteristic
4.	Solubility	Partially soluble in water
5.	Melting point	215-220
6.	Proteins	Present
7.	Carbohydrates	Absent

Nano-Sizing of Escitalopram: When a sample is subjected for measurement of % T at different wavelengths the percentage of transmittance reflects the percentage of the particles which are present in the mixture below 400 nm. Whereas the % blockade indicates the % particle which are above 400 nm and the data was correlated with the SEM analysis (**Fig. 2**).

TABLE 2: FORMULATION OF ESCITALOPRAM BIO-NANOPARTICLES LOADED WITH *PIPER NIGRUM*

Formulations	FA1 (1:05)	FA2 (1:1)	FA3 (1:2)	FA4 (1:3)	FA5 (1:4)	FA6 1:5)	FA7 (1:7)	FA8 (1:10)
Drug:polymer ratio	1:0.5	1:1	1:2	1:3	1:4	1:5	1:7	1:10
Escitalopram (mg)	10	10	10	10	10	10	10	10
Piper nigrum Bio-polymer (mg)	0.5	10	20	30	40	50	70	100
Glycerin µl	10	10	10	10	10	10	10	10
Dextrose (mg)	100	100	100	100	100	100	100	100
Distilled water (ml)	30	30	30	30	30	30	30	30

Drug Excepient Study: The drug interaction study revealed that there was no interaction between the drug and the excipients including the bio-polymers. This was proved by the result of the thin layer chromatography in which no change was seen in the RF value in the TLC method. Also there was no change in the ƛ max by UV method.

Value which was observed to be 289 nm prior to the test and after the test it was 289 nm hence confirming that there was no interaction between the drug and excipients. No observable signs of drug interaction were seen. It was concluded that none of the excipients had a detrimental effect on the drug and could be safely used for the formulation of the suspension.

Permeability: Egg membrane was used as a biological membrane as it mimics the action of the ear biological membrane. A permeation graph was plotted between concentration *vs* time, depicting the amount of drug permeated (**Fig. 1**).

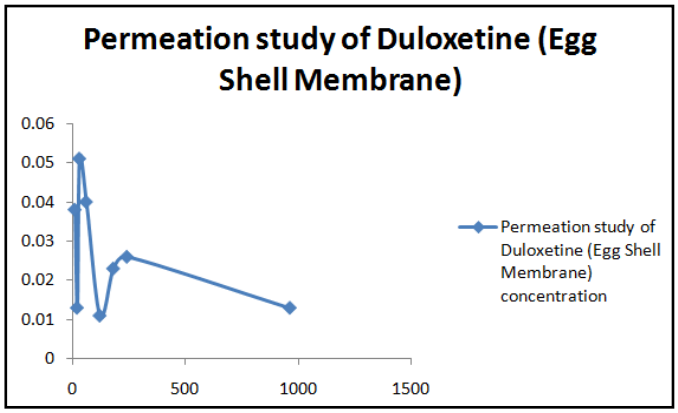

FIG. 1: PERMEATION STUDY OF DULOXETINE

Physico-Chemical Characterization of the Bio-Polymer: The isolated bio-material was light brown in colour, odourless, characteristic taste, partially soluble in water, colour changing point of

215 °C ± 5 °C. It had a viscosity of 1.44 cps, carbohydrates were absent while proteins were present. **(Table 1)** The IR spectra revealed the presence of amines, thiocarbinol (C=S), aromatic rings (1598.88 cm^{-1}) and the presence of alkanes, alkenes (2925.81 cm^{-1}) and nitro compounds (**Fig. 1**). These groups like the nitro groups indicate the mucoadhesive activity of the bio-polymer as these groups are observed in the mucoadhesive polymers like HPMC, polycarbophil (**Fig 3**). The isolated biomaterial was further evaluated for its adhesivity by using shear stress method.

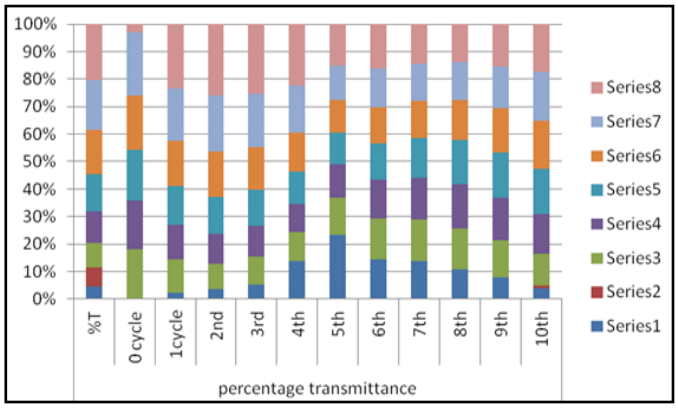

FIG. 2: NANOSIZING

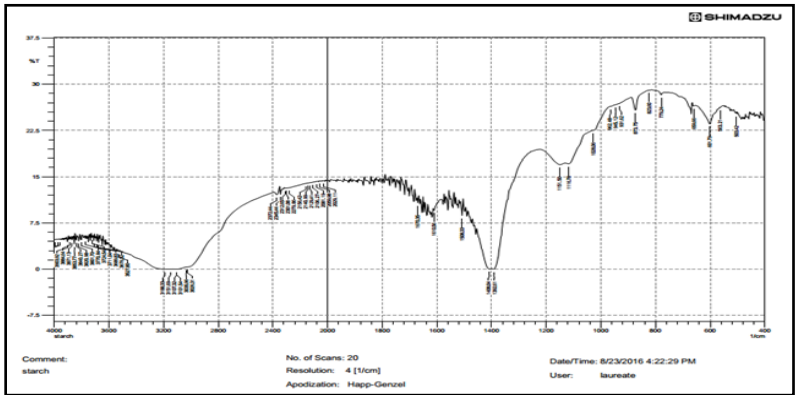

FIG. 3: IR SPECTRA OF *PIPER NIGRUM*

Characterization of Drug Loaded Nano Suspension (Table 2):

pH Studies: value of pH was noted from digital pH meter. The method was performed in triplicate and mean value of pH was calculated and was found between 7.2-7.8 (**Table 3**).

TABLE 3: pH STUDIES

FA1	7.2
FA2	7.3
FA3	7.4
FA4	7.4
FA5	7.3
FA6	7.5
FA7	7.5
FA8	7.2

Dispersibility: Evaluation of dispersibility of nanoparticles was done by manual hand shaking method. 10 mg of accurately weighed nanoparticles were taken in test tube and dispersed in 10 ml of double distilled water. After dispersion of the nanoparticles time taken for settling of particles to the bottom of the test tube was noticed and redispersion of nanoparticles on shaking of test tube was noticed. Visual observation was done to investigate formation of any aggregates or precipitates after shaking.

Entrapment Efficacy: Entrapment efficacy was calculated to find out the amount of entrapped drug inside the nanoparticles. It was calculated by accurately weighing 5mg of formulated nanoparticles and dissolving them in 5ml of methanol. The solution was sonicated for 10 mins and kept for 24 hrs as such. After 24 hrs each solution was diluted upto 10µg/ml and was analyzed under UV at 289 nm against the blank methanol solution and drug content was calculated. Entrapment efficacy was calculated by following formula:

Entrapment efficacy- amount of drug in nanoparticles / drug added in nanoparticles × 100

Preliminary Method to Screen the Nano Particle Size Range by UV Method: Transmittance of the nanosuspensions was studied as preliminary study for the particle size analysis. It gave an idea regarding the particle size of the nanosuspensions formulation. Transmittance is based on the concept of Tindal effect which specify that when light of specified wavelength passes through the media containing particles less than or greater than specified particle range, the % blockage represent

particle beyond size range at particular range whereas % Transmittance is considered that the particles lies above the size range at particular range. Transmittance of the formulation was studied by UV spectroscopy between 400-600 nm ranges keeping plain double distilled water as the blank. The reading showed the number of particles that allow the UV light to pass through it and rest of the particles showed the range of particles that blocked the light thus providing an idea of the range of particles in the nanosuspension (**Fig. 2**).

Particle Size (Size Distribution by Intensity): Preliminary study for particle size study by % transmittance was followed by Particle size range and size distribution study of the nanosuspension. Nanoparticle size was studied and average diameter range and intensity of the particles in particular size range was studied.

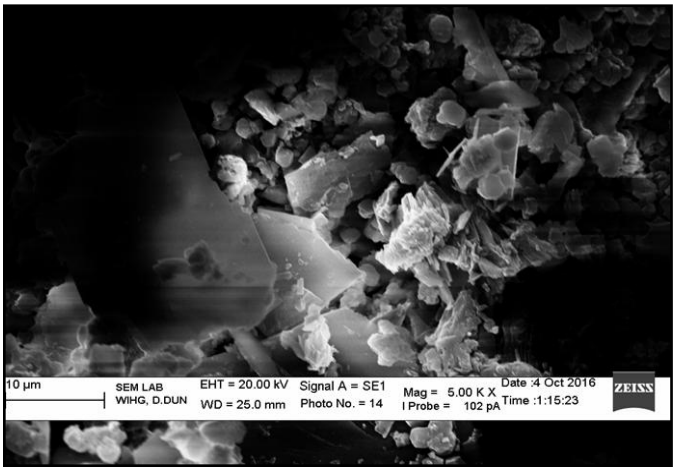

FIG. 4: SEM IMAGE OF *PIPER NIGRUM*

***In-vitro* Studies:** The *in-vitro* release pattern of FA1-FA8 were studied by dynamic method and a graph is plotted between % drug release and time, r^2 value t50 and t80 were calculated from the graph, which showed drug release ranging from 85-89% (**Fig. 5** and **6**).

Stability Studies: Stability studies were performed according to ICH guidelines. The formulations were stored in hot air oven at 37 ± 2 °C, 45 ± 2 °C , 4± 2 °C and 60 ± 2 °C for a period of 3 months. The samples were analyzed for drug content every two weeks by UV-Visible Spectrophotometer at 289 nm. Stability study was also carried out by measuring the change in pH of nano-suspension weekly in terms of change in color, odour, taste, its entrapment efficiency, and *in-vitro* drug released.

SEM of Formulation: The SEM analysis of the formulation containing bio-polymer revealed that it has a smooth surface with no rough edges. It shows the smooth, amorphous nature of the formulation (**Fig. 5**).

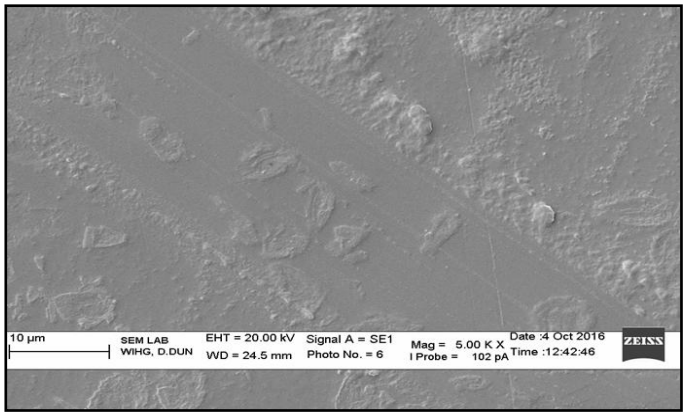

FIG. 5: SEM IMAGE OF THE BEST FORMULATION

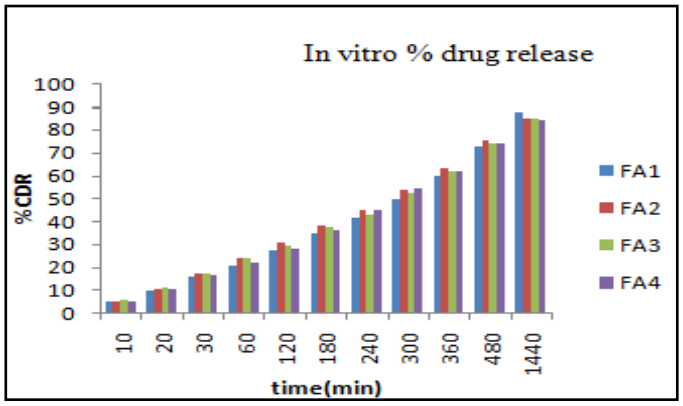

FIG. 6: *IN-VITRO* % DRUG RELEASE

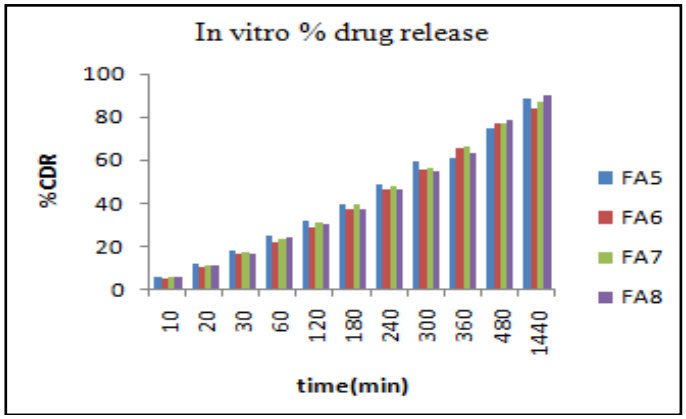

FIG. 7: *IN-VITRO* % DRUG RELEASE

DISCUSSION: This research work is focused to explore a novelistic platform for brain specificity *via* external ear canal by suitably designing an antidepressant loaded nano-suspension. As natural bio- polymers possess novel in-built properties like filmability, retardebility emulsifibility, suspendi-bililty and flow ability.

Hence, these polymers can serve as a potential bio-carrier or bio-inactive pharmaceutical ingredients in designing various drug loaded dosage forms, liquid dosage form and semi-solid dosage form. Many existing pharmaceuticals are rendered ineffective in the treatment of cerebral diseases due to our inability to effectively deliver and sustain them within the brain.

CONCLUSION: In recent years, there has been a considerable interest in the development of novel drug delivery systems using particulate delivery systems like nanoparticles. In this context, nanosuspensions will be effective in increasing the solubility, bioavailability of poorly soluble drugs. A large proportion of new chemical entities coming from drug discovery are water insoluble, and therefore poorly bioavailable, leading to hurdles in formulation development efforts. External acoustic meatus consist of unique histology and blood supply. It is highly enriched with dense neural network which in turn connects with the cranial nerve in medulla oblongata.

Our *in-vitro* release patterns reveal that over an extended period of significant amount of drug reaches to the brain. There are no pharmaceuticals designed specifically for brain targeting to treat the depression *via* ear. We have designed a dosage form to combat the disease and increase patient compliance thereby minimizing the incidences of dose missing which are relatively quite high due to busy schedule and long term therapy course thus prevents he precipitation of the disease from chronic stage. The long term therapy and multiple dosing is the main reason for the discomfort of the patient. All above mentioned problem can be overcome by the instilling of Escitalopram loaded nano-suspension in to the ear which is targeted directly to the brain *via* inter and intra neural pathway.

Ethical Approval: NA.

Clinical Trial Registration: NA.

ACKNOWLEDGMENT: The authors are grateful to the Vice chancellor of DIT University Dehradun. For providing immense support in carrying out the research work.

CONFLICT OF INTEREST: There is no conflict of interest.

REFERENCES:

1. Bielski RJ, Ventura D and Chang CC : A double-blind comparison of escitalopram and venlafaxine extended release in the treatment of major depressive disorder. The Journal of clinical psychiatry 2004; 65(9): 1190-6.
2. Cipriani A, Santilli C, Furukawa TA, Signoretti A, Nakagawa A, McGuire H, Churchill R and Barbui C: Escitalopram versus other antidepressive agents for depression. The Cochrane Library 2009.
3. Marcus M, Yasamy MT, van Ommeren M, Chisholm D and Saxena S: Depression: A global public health concern. WHO Department of Mental Health and Substance Abuse 2012; 1: 6-8.
4. O 'Rahilly R and Müller F: Basic human anatomy: a regional study of human structure. WB Saunders Company 1983.
5. Thiagarajan B: Anatomy of external auditory canal a review from otolaryngologist's perspective. ENT SCHOLAR 2012.
6. Pathirana W, Abhayawardhana P, Kariyawasam H and Ratnasooriya WD: Transcranial route of brain targeted delivery of methadone in oil. Indian journal of pharmaceutical sciences 2009; 71(3): 264.
7. Debjit B, Harish G, Duraives S, Kumar K, Raghuvanshi V and Kumar KP: Nanosuspension-A novel approaches in drug delivery systems. The Pharma Innovations 2012; 1: 50-63.
8. Rai N, Yadav S, Verma AK, Tiwari L and Sharma RK: Quality specifications on *Piper nigrum* L.-A spice and herbal drug of Indian commerce. International Journal of Advanced Food Science and Technology 2012; 1(1): 1.
9. Khan IA and Abourashed EA: Leung's encyclopedia of common natural ingredients: used in food, drugs and cosmetics. John Wiley and Sons. Shah, C. S., and Qadry JS. A textbook of pharmacognosy. Messrs BS Shah 2011.
10. Madhav NVS and Raina D: Formulation and evaluation of Duloxetine loaded bio-nano suspension for brain specificity *via* acoustic meatus. SOJ Pharm Sci 2017; 3(4): 1-5.

How to cite this article:
Raina D and Madhav NVS: Formulation and performance evaluation of Escitalopram loaded bio-nano suspension using a novel bio-retardent from *Piper nigrum*. Int J Life Sci Rev. 2017; 3(5): 60-66:.doi:10.13040/IJPSR.0975-8232.IJLSR.3(5).60-66.