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

IJLSR INTERNATIONAL JOURNAL OF LIFE SCIENCES AND REVIEW

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

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

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ABSTRACT: Nanoparticles represent a promising drug delivery system of controlled and targeted release. The current research work was to explore a novelistic route for targeting to the brain through the ear by formulating nanosuspension using Escitalopram as a model drug permitting better control over depression. Depression is the second most prevailing disease after cardiovascular diseases. The delivery has overcome the dose dumping problem in case of the oral system. In this research work, significant effort was made to explore the novelistic platform for the ear to the 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 h. Different formulations of Escitalopram out of which F1 (1:0.5) was found to be the best formulation having an r^2 value of 0.9905 t 80: 22 h 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 a significant amount of drug reaches the brain *via* external acoustic meatus. The demand for curbing depression and other mental health conditions is on the rise globally. Nanosuspensions are a distinctive and commercially feasible approach to solve the problems of a hydrophobic drug such as poor solubility and poor bioavailability.

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

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INTRODUCTION: Escitalopram is the most selective serotonin reuptake inhibitor (SRI) anti-depressant available [1]. Although pharmacological and psychological interventions are both effective for major depression, antidepressant drugs remain the mainstay of treatment. During the last 20 years, selective serotonin reuptake inhibitors qqqq (SSRIs) have progressively become the most commonly prescribed antidepressants.

QUICK RESPONSE CODE

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

The 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

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 24 mm along the posterior wall it is not a straight tube (S-shaped) it has 2 parts, outer part-directed upwards, backward 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 postauricular and superficial temporal arteries. Medially it is supplied by a deep auricular artery which is a branch of the 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 postauricular vein connects to the sigmoid sinus *via* the mastoid emissary vein; this anastomosis provides a route for infections of the external ear to spread to the intracranial cavity [5].

The ear canal is having unique histology, blood supply, nerve supply like mandibular (auriculo-temporal branch), vagus nerve (auricular branch), internal maxillary (tympanic branch), glossopharyngeal nerve connections present in the auditory canal. The unique platform can be used for targeting the 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 exists for the antidepressant drug in the market, but these molecules upon administration in long term therapy produce 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 the 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, extracranial vessels of the scalp communicating with the brain *via* emissary veins and most importantly, the way that the scalp is used in the 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 extracranial 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's veins. There are thirteen emissary veins connecting extracranial sites of the head with intracranial sinuses. Seven major sinuses within the skull are interconnected by several anastomosing veins, which finally drain intracranially 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 transcranial route (TCR) of drug delivery [6].

In recent years, there has been 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, 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 bio-available, leading to hurdles in formulation development efforts.

There is several formulation approaches like micronization, solubilization using co-solvents, precipitation techniques, *etc.*, to resolve the problems of low solubility and low bio-availability.

Each of them has its limitations. Other techniques like microemulsions, 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 a transformation of the micronized drug to drug nanoparticles and nanosuspensions.

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 a 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 a distinctive and commercially feasible approach to solve the problems of a hydrophobic drug such as poor solubility and poor bioavailability [7].

MATERIALS AND METHODS:

Isolation of Bio-Material Fruit of *Piper nigrum*: Piper comes in two types black and white piper obtained from the ripening and after the separation of the pericarp of fruits of *piper nigrum*, family Piperaceae. It consists 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 peppercorns were powdered and soaked in methanol: glacial acetic acid: concentrated sulphuric acid (85:10:5). The solution was kept on the magnetic stirrer for continuous stirring for 30 min then filtered, and 10 ml of sodium hydroxide was added.

To the above solution, cold water was added, and the precipitate was obtained, kept in the refrigerator for 24 h centrifuged at 3000 rpm for 15 min, dried and stored. The biopolymer was subjected to various spectral analyses, including UV, IR, SEM. Nanosuspensions were prepared by sonication method using *Piper nigrum* as a retardant and with another 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 h.

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

Drug Excipient 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 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 1 mg/ml was prepared, and 1 ml drug solution poured in the 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 biological ear membrane

Formulation of Bio Nano Suspension: Nanosuspensions were prepared by sonication method using *Piper nigrum* as a retardant and with another co-processing agent like glycerin and dextrose as a nanosized. A weighed amount of drug, dextrose, and the polymer was triturated together in mortar and pestle and kept on sonicator.

Glycerin was added to the 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, odor, taste, solubility, color 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 biopolymer. 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 min.

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, sulfates, and starch was observed **Table 1**.

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 is above 400 nm, and the data was correlated with the SEM analysis **Fig. 2**.

TABLE 1: CHARACTERIZATION OF BIOPOLYMER

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

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 Excipient Study: The drug interaction study revealed that there was no interaction between the drug and the excipients, including the biopolymers. 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 λ_{max} by UV method.

The value which was observed to be 289 nm before 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 biological ear membrane. A permeation graph was plotted between concentration *vs.* time, depicting the amount of drug permeated **Fig. 1**.

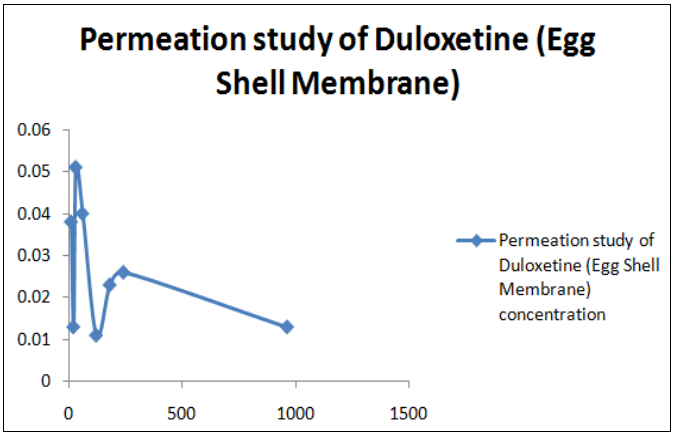

FIG. 1: PERMEATION STUDY OF DULOXETINE

Physico-Chemical Characterization of the Bio-Polymer: The isolated bio-material was light brown in color, odorless, characteristic taste, partially soluble in water, color 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. 3**. These groups, like the nitro groups, indicate the mucoadhesive activity of the biopolymer 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.

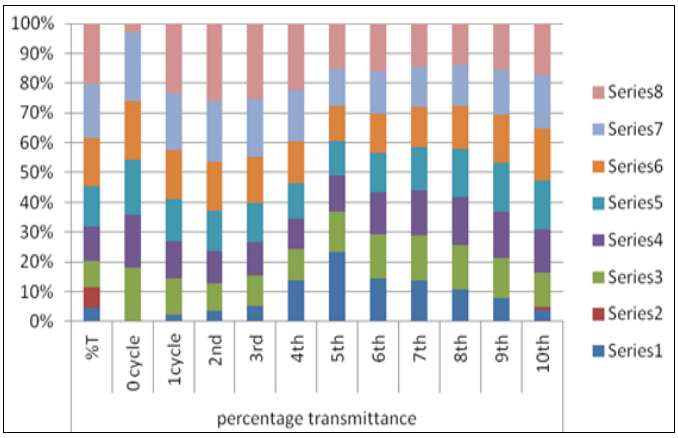

FIG. 2: NANOSIZING

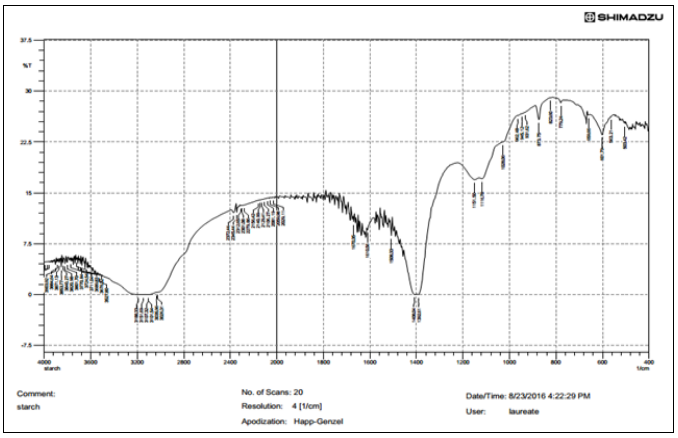

FIG. 3: IR SPECTRA OF *PIPER NIGRUM*

Characterization of Drug Loaded Nano Suspension:

pH Studies: The value of pH was noted from a digital pH meter. The method was performed in triplicate, and the 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 handshaking method. 10 mg of accurately weighed nanoparticles were taken in a test tube and dispersed in 10 ml of double distilled water. After the dispersion of the nanoparticles, time taken for the settling of particles to the bottom of the test tube was noticed, and redispersion of nanoparticles on shaking of the test tube was noticed. Visual observation was done to investigate the 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 5 mg of formulated nanoparticles and dissolving them in 5 ml of methanol. The solution was sonicated for 10 min and kept for 24 h as such. After 24 h, each solution was diluted to 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 the following formula:

Entrapment efficacy = The 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 specifies that when the light of specified wavelength passes through the media containing particles less than or greater than specified particle range, the % blockage represents particle beyond size range at particular range whereas % Transmittance is considered that the particles lie above the size range at particular range. The 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 an the average diameter range and intensity of the particles in particular size range was studied.

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**.

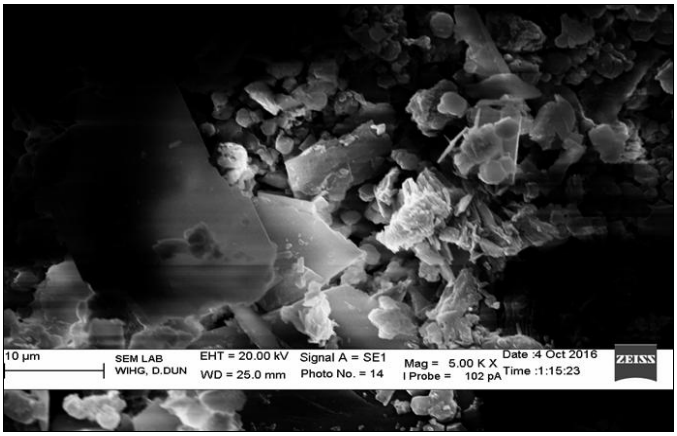

FIG. 4: SEM IMAGE OF *PIPER NIGRUM*

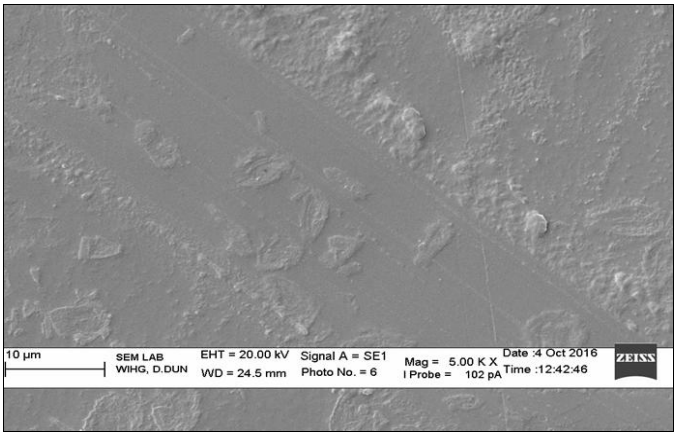

FIG. 5: SEM IMAGE OF THE BEST FORMULATION

***In-vitro* Studies:** The *in-vitro* release pattern of FA1-FA8 were studied by the 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. 6** and **7**.

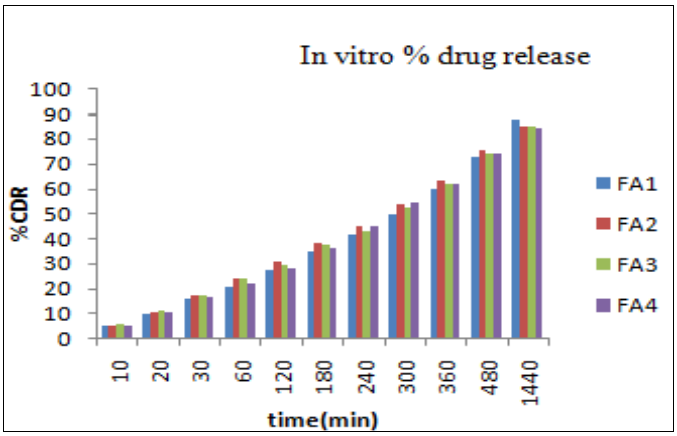

FIG. 6: *IN-VITRO* % DRUG RELEASE FA1-FA4

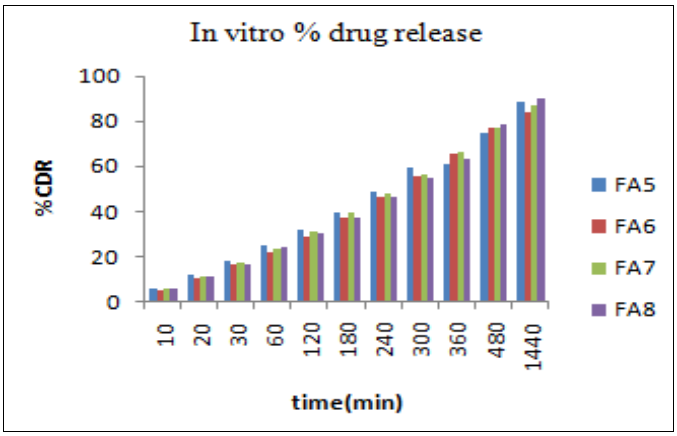

FIG. 7: *IN-VITRO* % DRUG RELEASE FA5-FA8

Stability Studies: Stability studies were performed according to ICH guidelines. The formulations were stored in a hot air oven at 37 ± 2 °C, 45 ± 2 ° C, 4± 2 °C, and 60 ± 2 °C for 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, odor, taste, its entrapment efficiency, and *in-vitro* drug released.

DISCUSSION: This research work is focused on exploring a novelistic platform for brain specificity *via* external ear canal by suitably designing an antidepressant loaded nano-suspension. As natural biopolymers possess novel in-built properties like filmability, readability emulsifibility, suspendi-bililty, and flowability. 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 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 consists of unique histology and blood supply. It is highly enriched with a dense neural network, which in turn connects with the cranial nerve in the medulla oblongata.

Our *in-vitro* release patterns reveal that over an extended period of a significant amount of drug reaches to the brain. There are no pharmaceuticals designed specifically for brain targeting to treat the depression *via* the 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 a busy schedule and long term therapy course thus prevents the precipitation of the disease from the chronic stage. The long term therapy and multiple dosing is the main reason for the discomfort of the patient. All the above-mentioned problem can be overcome by the instilling of Escitalopram loaded nano-suspension into the ear, which is targeted directly to the brain *via* inter and intraneural pathway.

Ethical Approval: NA.

Clinical Trial Registration: NA.

ACKNOWLEDGEMENT: 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.

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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.