FORMULATION AND EVALUATIONOF DULOXETINE LOADED BIO NANO GELS FOR EFFECTIVE DELIVERY TO THE BRAIN VIA EXTERNAL ACOUSTIC MEATUS.

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ABSTRACT

Background: Depression is a significant contributor to the global burden of disease and affects people in all the communities worldwide. Depressive disorders often start at a young age; they reduce people's functioning and often are recurring. For these reasons, depression is the leading cause of disability world across. The external auditory canal is used as a delivery platform and has overcome the dose dumping problem in the case of oral system.Objective: The current research work was to explore a novelistic route for targeting to the brain through ear by formulating bio-nanogel using duloxetine as a model drug permitting better control over depression and reducing the dose of Duloxetine nearly 100 folds.Method: The concept was proved by preparing a nano-sized formulation of API i.e duloxetine and observed its pharmacological actions by sonication bath method. Bio-nano gels were prepared by using a biopolymer which was isolated from berries of ***Tagetes patula*.** Ten formulations were prepared viz 1:0.5, 1:1, 1:2, 1:3, 1:4, 1:5, 1:7, and 1:10,1:15,1:20.Results: The formulations were subjected to various evaluations, including pH, % transmittance, Content uniformity, *Ex-vivo*, stability, release for over 36 hours. Drug content was found to be 91.4±0.02 – 98.4±0.04, entrapment efficiency 73.67±0.08 – 78.38±0.02. According to the *in-vitro* release,best formulation was found to be FTP 2 (1:1) having r^2 value 0.9329 best fit model Higuchi matrix, t_{50} 4.5 and t_{80} 32hrsConclusion: on the basis of the *in-vitro* results and pharmacokinetic data obtained it can be concluded that significant amount of drug reaches the brain via external acoustic meatus and so it is feasible to deliver Duloxetine by this novelistic route.

Keywords: *Tagetes patula* ,Acoustic meatus, nano-gels, Higuchi matrix, anomalous transport.

No: of Tables: 3 **No: of Figures: 7** **No: of References: 11**

Introduction

Depression is one of the most common mental disorders; the need of curbing depression and other mental health conditions is on the rise globally. A recent World Health Assembly called on the World Health Organization and its member states to take action in same direction [1,2]

The external ear is enriched with neuronal nerve endings which belong to mixed motor and sensory in nature. The ear canal is having a unique histology, blood supply, nerve supply like mandibular (auriculotemporal 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 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 this 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 the therapeutic molecule and offer good patient compliance through this novelistic approach. The external auditory canal is a tube like structure that extends from concha of Pinna laterally to the tympanic membrane medially. It is 24mm in length. It is tortuous from meatus to the tympanic membrane. The auricle skin is unique of about 0.8-1.2 mm in thickness that is securely seized to the perichondrium and also consists of convoluted elastic cartilage lacking blood vessels of 1.0-3.0 mm in thickness. Blood supply to the external auditory canal is by: anteriorly supplied by an auricular branch of superficial temporal artery and deep auricular branch of the maxillary artery, posteriorly by the auricular branch of the posterior auricular artery, and nerve Supply by anteriorly by the auriculao temporal nerve, posteriorly by the auricular branch of the vagus.

The current objective of the research work was to develop bio nano gels using a novel bio retardant isolated from *Tagetes patula* flower and its *in-vitro* performance parameter as per the standard published method. Nanogels can serve as a suitable dosage form for the management of depression upon administration from EAM.

Materials and Method

Isolation of bio-material fruit of *Tagetes patula*

Tagetes patula flower was procured from market. The thalamus part of the flower was separated. About 50 gm of spongy portion were taken & mashed with the help of pestle mortar. To the mashed portion 250 ml of double distilled water was added & was stirred and subjected for stirring using a mechanical stirrer at 2000 rpm for the period of one hour. Solution was strained with the help of muslin cloth. About 200 ml of the supernatant liquid was taken & treated with acetone in the ratio of 1:1. Solution was refrigerated for 12 hour, & mixture was centrifuged at 4000 rpm. The bio-material was taken out by discarding the supernatant liquid & was dried in

dessicator for the period of 12 hrs. Finally bio – material was passed through sieve # 120 to obtain the free flowing polymer. The process of bio-material extraction was repeated 6 times & practical yield was calculated. Each time 10 gm of fruit part was used for extraction & yield was compared. The bio polymer was subjected to various spectral analysis including UV, IR, SEM.

Nano-sizing of Duloxetine:

To 100mg of Duloxetine, 5ml methanol was mixed and triturated. 5ml distilled water was added slowly & solicited for 5 cycles (1 cycle for 3 min.). After each sonication cycle absorbance & %, T was measured. It was then micro centrifuged. Supernatant and residue were collected. Residue was dried & nanoparticles were recovered

Drug Excipient study:

The pure drug along with the formulation excipients was 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} 289 using UV spectroscopy. There was no shift in the λ_{max} the drug which confirmed the integrity of the drug with various excipients in different ratio.

Permeability:

Drug solution of 1mg/ml was prepared and 1ml drug solution poured in donor compartment. pH7.2 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 gels:

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

Physico-chemical 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 bio-polymer. The bio-polymer showed a morphological structure similar to the polymers and hence it confirms the polymeric nature of the bio-polymer

An *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 *Tagetes patula*:

The percentage yield was found to be 12.18±0.02. The biopolymer was light brown in colour, odourless and characteristic in taste. Its melting point was found to be 220-225°C. It was tested positive for proteins, carbohydrates.

Nano-sizing of Duloxetine:

When a sample is subjected to 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)

Drug Excipient 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. The 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 external ear membrane. A permeation graph was plotted between concentration vs. time, depicting the amount of drug permeated. (Fig.1)

Physico-chemical characterization of the bio-polymer:

The percentage yield was found to be 12.18±0.02. The biopolymer was light brown in color, odourless and characteristic in taste. Its melting point was found to be 220-225°C. It was tested positive for proteins, carbohydrates.It had a viscosity of 1.44 cpsThe IR spectra revealed the presence of amines, thiocarbonyl (C=S), aromatic rings(1598.88 cm-1) and the presence of alkanes, alkenes(2925.81 cm-1) and nitro compounds(fig. no I). 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.

Characterization of drug loaded nano gels:

pH studies: the value of pH was noted from digital pH meter. The method was performed in triplicate & mean value of pH was calculated and was found between 7.2-7.8 (Table III)

Dispersibility:

Evaluation of dispersibility of nanoparticles was done by manual hand shaking method. 10 mg of accurately weighed nanoparticles were taken in the test tube & dispersed in 10 ml of double distilled water. After dispersion of the nanoparticles, the time taken for the settling of particles to the bottom of the test tube was noticed & re-dispersion 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 & dissolving them in 5 ml of methanol. The solution was sonicated for 10 minutes & kept for 24 hrs as such. After 24 hrs each solution was diluted up to 10 µg/ml & was analyzed under UV at 289 nm against the blank methanol solution & drug content was calculated. Entrapment efficacy was calculated by following formula-

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

To identify the concentration of active medicament in prepared nanosuspension.

Preliminary method to screen the nano particle size range by UV method:

The transmittance of the nanosuspensions was studied as the 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 represent 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 & 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 II).

Particle size (Size Distribution by Intensity):

Preliminary study for particle size study by % transmittance was followed by Particle size range & size distribution study of the

nanosuspension. Nanoparticle size was studied & average diameter range & intensity of the particles in particular size range was studied. It was also confirmed by zeta sizing by Malvern zeta sizer. (Fig VI)

***In-Vitro* studies:**

Samples were analyzed by UV at 289 nm. To evaluate the *in vitro* release egg shell membrane was used at ph 7.4. Using egg shell membrane made IVIVC easy to predict as egg shell membrane cannot mimic the mucous membrane of the ear skin. Also a concentration gradient is established as nanoparticles are thought to attach to the skin and diffuse the drug in a controlled manner this phenomenon can also be clearly depicted by egg shell membrane. The *in-vitro* release pattern of FA1-FA10 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 91-98%.(Fig V)

a *In- vivo* studies:

Bio – availability studies & in-*vivo* studies were done to calculate drug content in blood was in µg/ml. Graph was plotted between the time (hrs) & drug content (µg/ml) & a trapezoidal curve was plotted using a graph paper & AUC was calculated using the appropriate formula. Cmax & Tmax were also calculated.

Formulation **FTP 2** showed the AUC of 78.093 & Cmax of 3.891 µg/ml & Tmax of 24 hrs for Duloxetine loaded bio nano gels. (Fig6a), CGDRCP/IAEC/15/17/07/PCL/AE-5RATS-F

IVIVC

Regression Statistics								
Multiple R	0.91161							
R Square	0.831032							
Adjusted R Squa	0.78879							
Standard Error	0.601129							
Observations	6							
ANOVA								
	df	*SS*	*MS*	*F*	*gnificance F*			
Regression	1	7.109029	7.109029	19.67318	0.011374			
Residual	4	1.445426	0.361356					
Total	5	8.554455						
	Coefficients	*tandard Err*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.0%*	*pper 95.0%*
Intercept	-0.30836	0.663774	-0.46455	0.666413	-2.15129	1.534576	-2.15129	1.534576
X Variable 1	0.048292	0.010888	4.435445	0.011374	0.018063	0.078522	0.018063	0.078522

IVIVC report of Duloxetine (FTP2) Bio nano gel

Stability Studies

Stability studies were performed according to ICH guidelines. (stability study chamber) maintained at 37±5°C and 75±5%R.H. for 6 months. The change in appearance, physical characteristics and release behavior of the stored films were investigated from 0-6 months (Ezhumalai et al. 2011).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.

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 V).

Discussion

Current work focused on exploring a novelistic platform for brain specificity via external ear canal by suitably designing an antidepressant loaded bio-nano-gels. As natural bio- polymers possess novel in-built properties like filmability, retardebility emulsifibility, suspendibiilty 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. Different formulations of duloxetine were prepared and evaluated out of which FTP 2 (1:1) was found to be the best formulation having r^2 value 0.9329 best fit model Higuchi matrix, t_{50} 4.5 and t_{80} 32hrs. According to the *in-vitro* release of the formulated nano gels, ratios showed the release pattern as FTP 2 > FTP 1 > FTP 3 > FTP 4 > FTP 5 > FTP 6 > FTP 7 > FTP 8 > FTP 9 > FTP 10, which was calculated by bits software. Experimental result reveals that the biopolymer posses promising retardibility cum stability and mucoadhesivity as IR spectra shows many peaks having amino groups, and hydroxyl groups which are same as in the natural polymer chitosan (fig:3) (5)

Table I:

1.	**Color**	Light brown
2.	**Odor**	Odorless
3.	**Taste**	Tasteless
4.	**Solubility**	Partially soluble in water
5.	**Melting point**	220-225
6.	**Proteins**	Present
7.	**Carbohydrates**	Present

Characterization of biopolymer

Table II:

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)**	**FA8 (1:15)**	**FA8 (1:20)**
Drug: polymer ratio	1:0.5	1:1	1:2	1:3	1:4	1:5	1:7	1:10	1:15	1:20
Duloxetine (mg)	10	10	10	10	10	10	10	10	15	20
Piper nigrum Bio-polymer (mg)	0.5	10	20	30	40	50	70	100	150	200
Glycerin µl	10	10	10	10	10	10	10	10	10	10
Dextrose (mg)	100	100	100	100	100	100	100	100	100	100
Distilled water(ml)	30	30	30	30	30	30	30	30	30	30

Formulation of Duloxetine bio-nanogels loaded with *piper nigrum* biopolymer.

Table III

FA1	**7.2**
FA2	**7.3**
FA3	**7.4**
FA4	**7.4**
FA5	**7.3**
FA6	**7.5**
FA7	**7.5**
FA8	**7.2**
FA9	**7.1**
FA10	**7.5**

pH studies

Fig:I

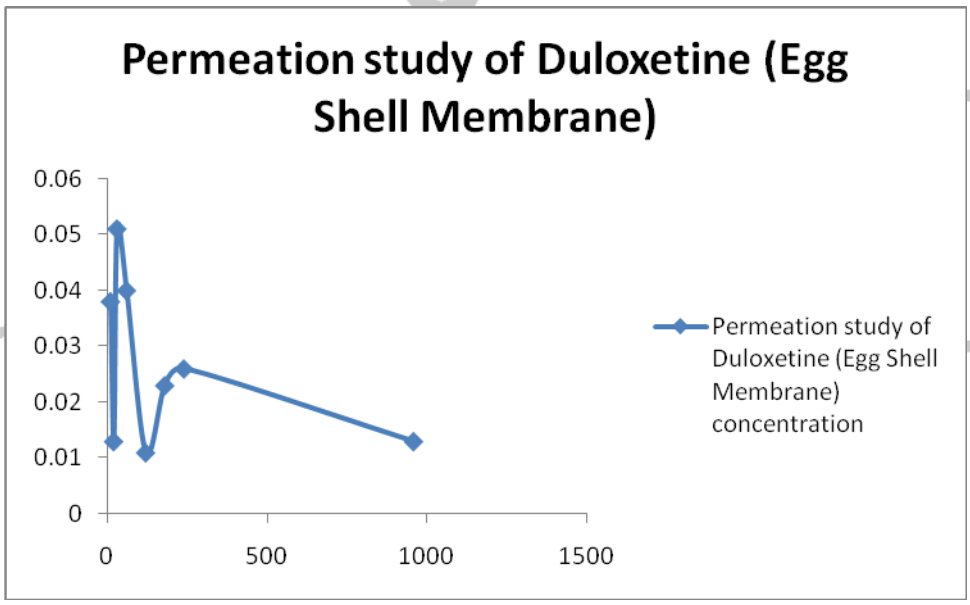
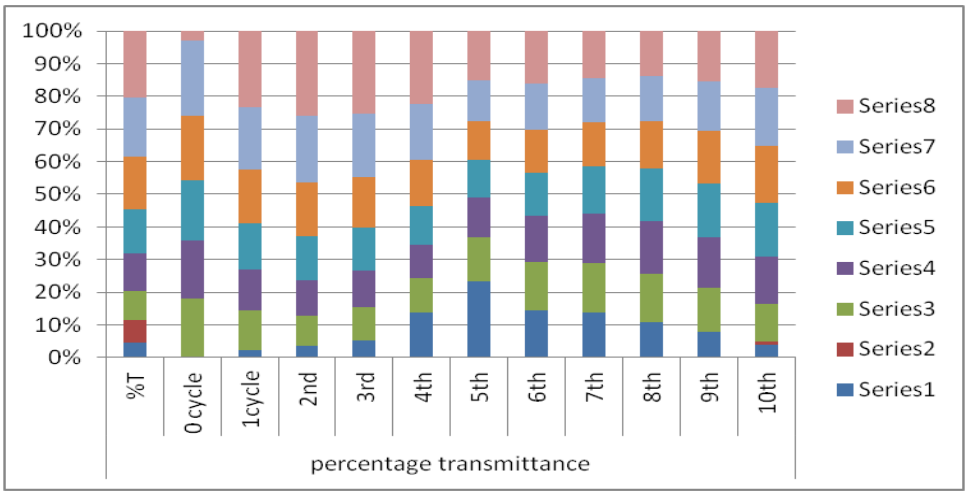


Fig II Nanosizing:



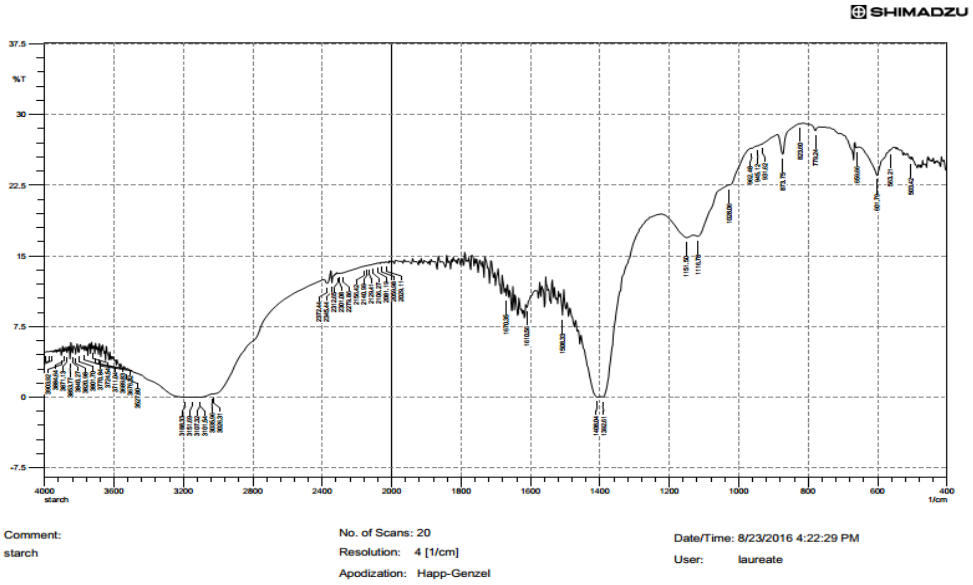


Fig3: IR spectra of Tagetes Patula

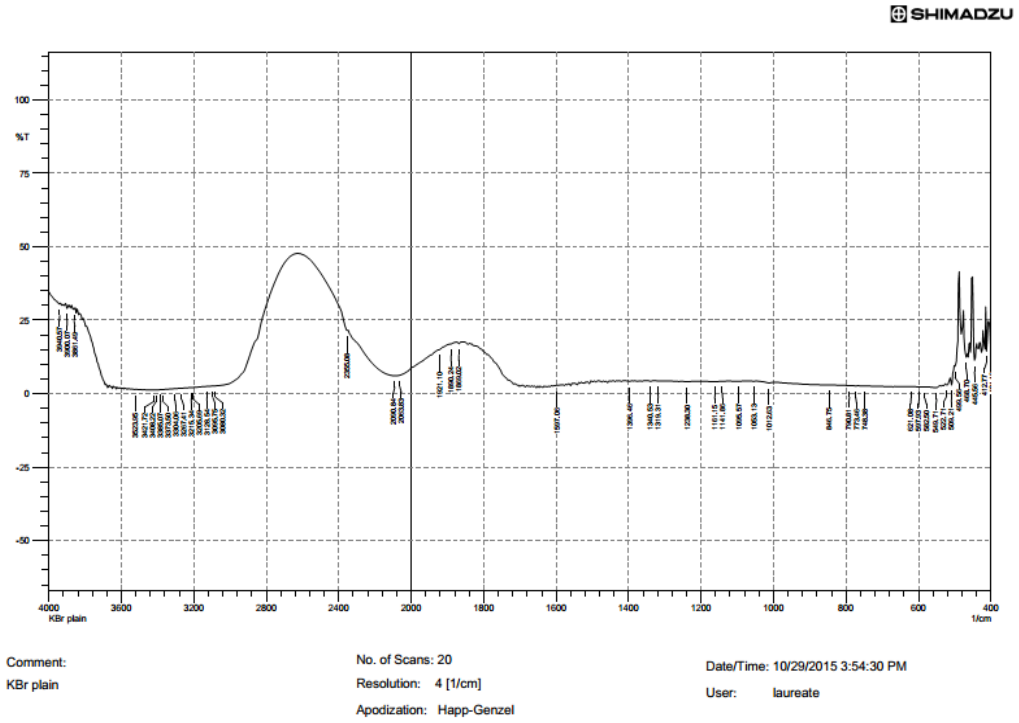


Fig3a: IR spectra of the formulation

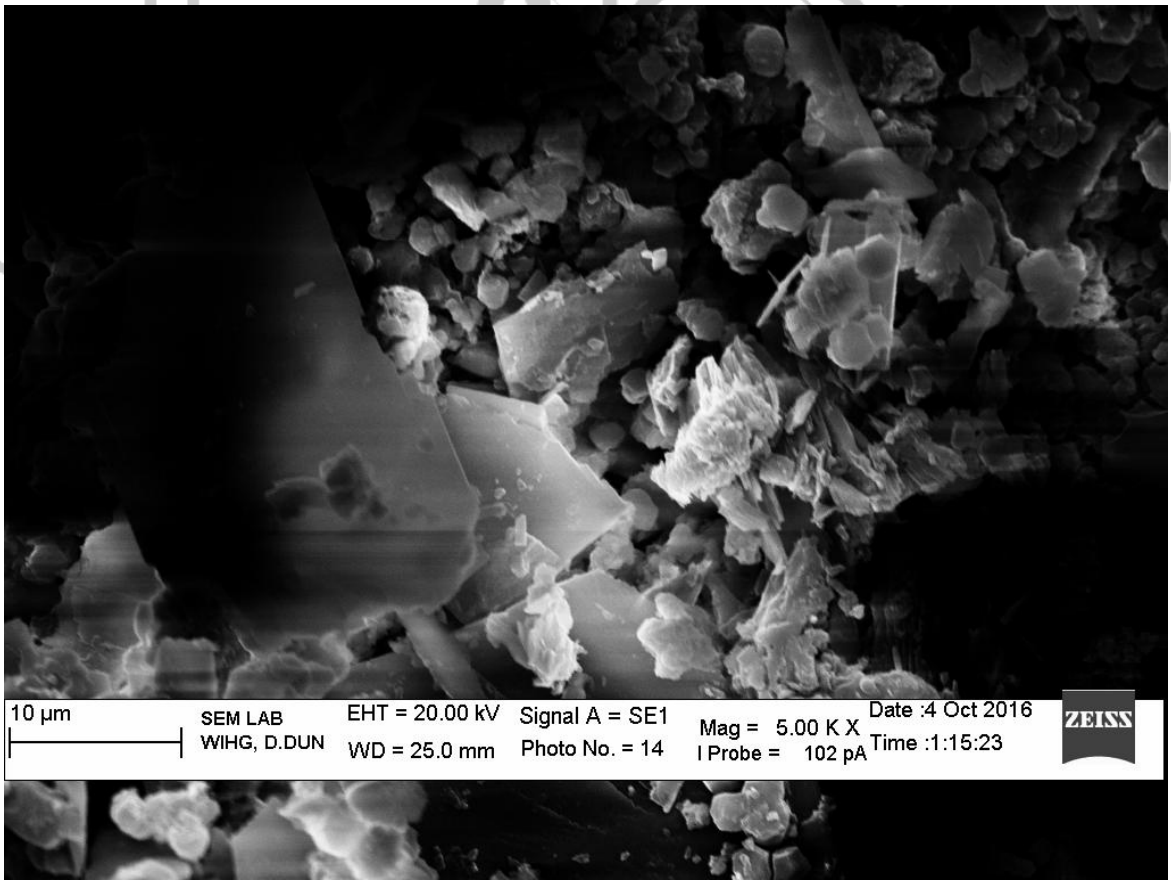


Fig4: SEM image of Tagetes patula

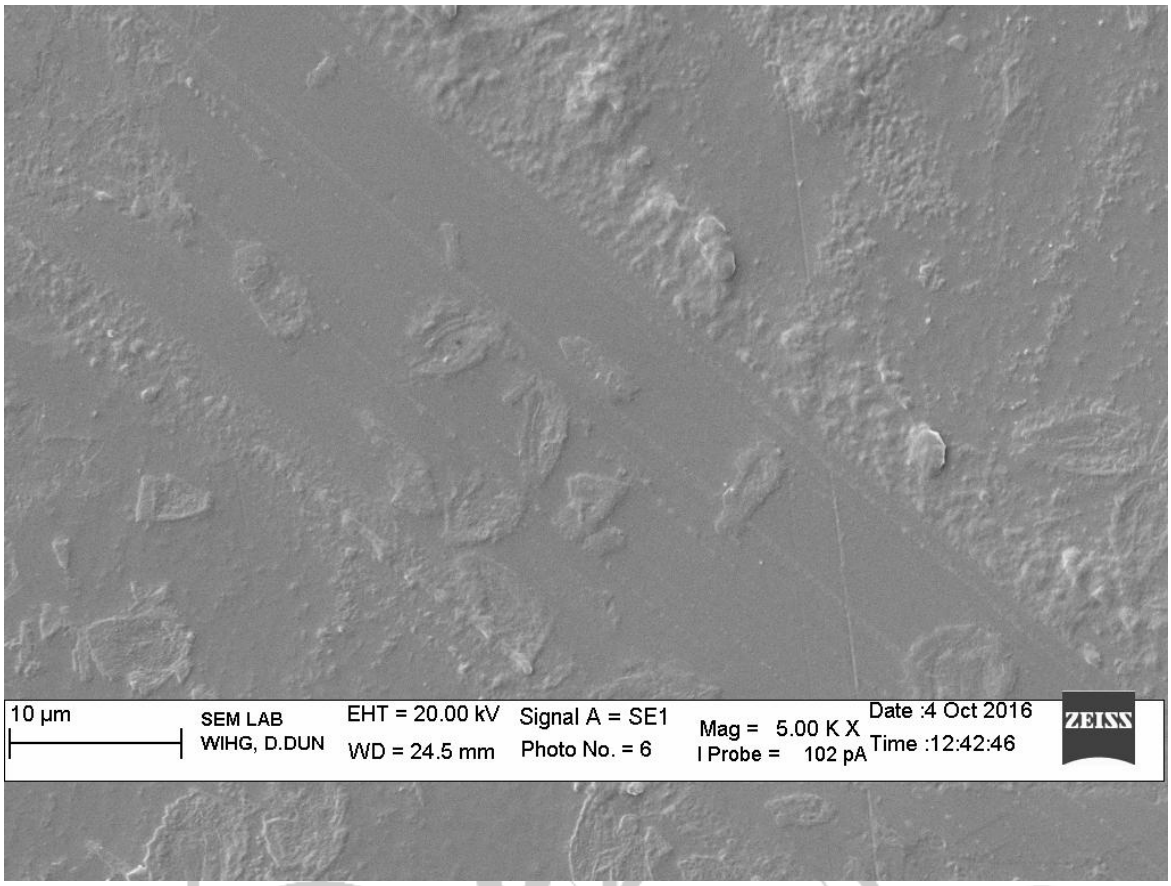


Fig5: SEM image of the best formulation.

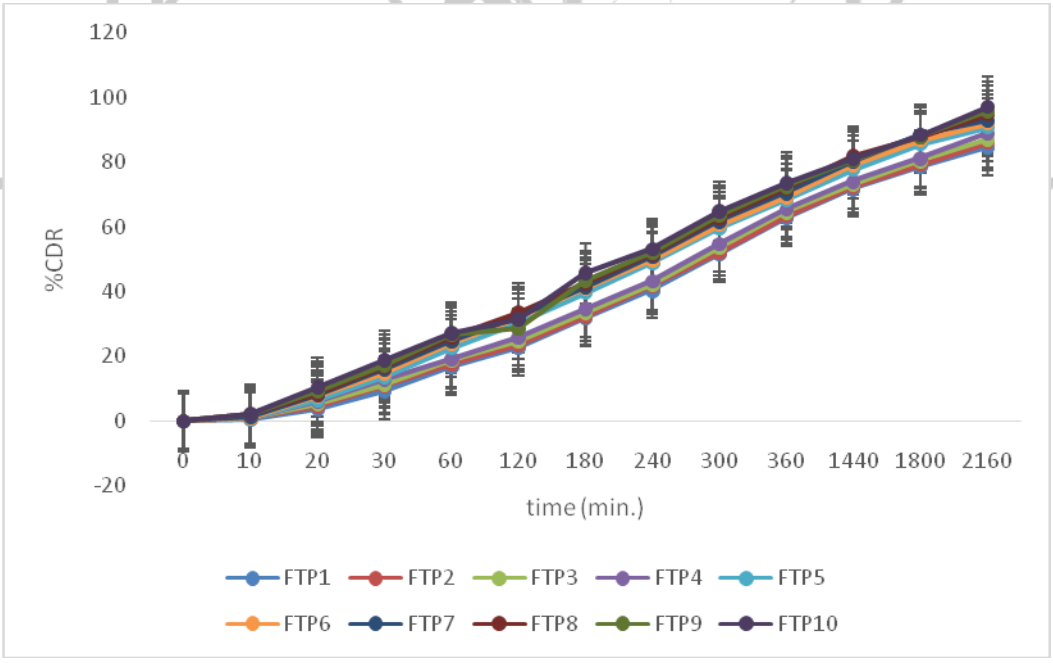


Fig no 6 . *In-vitro* release of Duloxetine loaded bionano gels of *Tagetes patula*

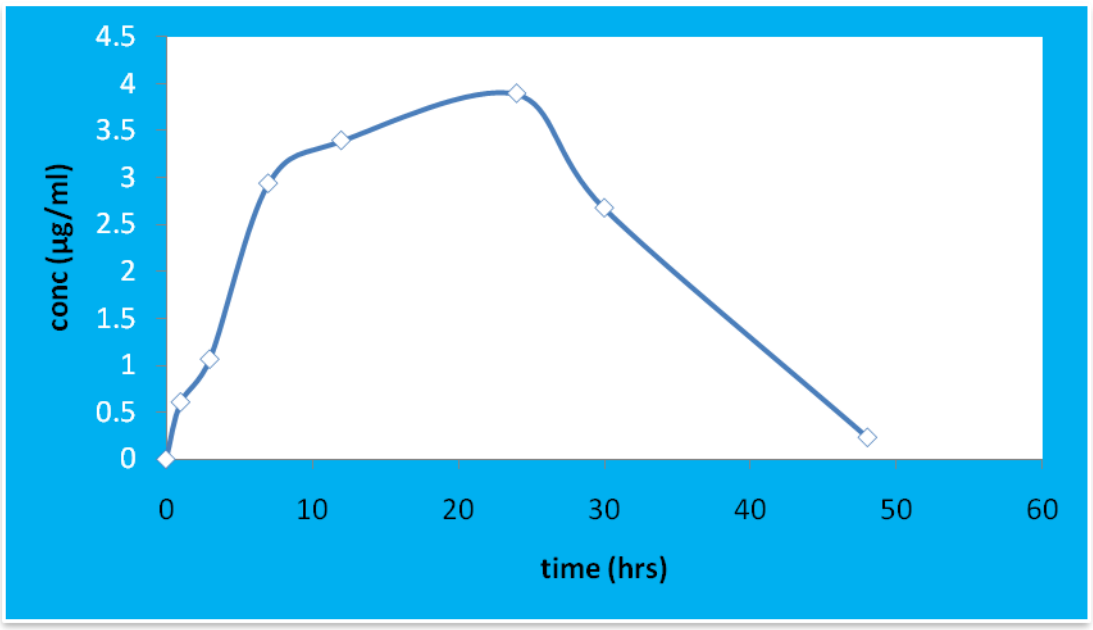


Fig no. 6a *In - vivo* release of bio nanogel FTP1 (1:1)

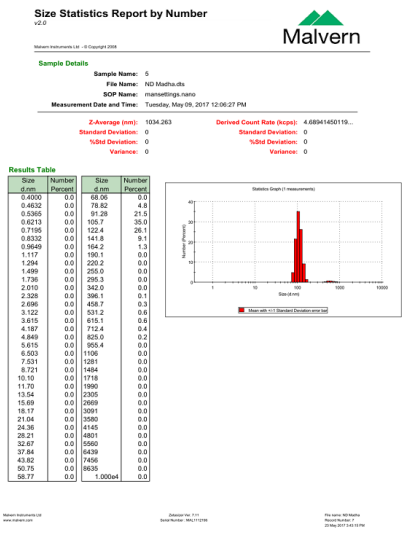


Fig VI Particle size analysis of Tagetes Patula

Conclusion

The current research work an innovative approach for delivery of nanosized Duloxetine via EAM (External acoustic meatus) platform is an innovative research work made to deliver Duloxetine is an acid labile drug, degrades at acidic pH of stomach. Duloxetine shows comparably very low concentrations in cerebrospinal fluid, due to the fact that the drug crosses the blood–cerebrospinal fluid barrier much worse than other antidepressants do, suggesting a low ability of Duloxetine to enter the brain. Our *In-vitro* release pattern reveals that over an extended period of significant amount of drug reaches 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 he precipitation of the disease from the chronic stage. The long term therapy and multiple dosing are the main reason for the discomfort of the patient. All above-mentioned problem can be overcome by the instilling of Duloxetine loaded nano-suspension into the ear which Is targeted directly to the brain via inter and intra neural pathway. Proposed mechanism based on the research out coming, for drug targeting to the brain from ear may achieve via a neural pathway which is located ear and brain through vestibular ganglion to verve VIII and from nerve VII to left cochlear neuron which is located in the brain. This route can be used for drug targeting to the brain.

Declaration:

A. Conflict of Interest: NA

B. Ethical Approval: NA

C. Clinical trial registration: NA

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