Original Article
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Nasotransmucosal delivery of curcumin-loaded mucoadhesive microemulsion for treating inflammation-related CNS disorders

Enflamasyonla ilişkili CNS bozukluklarının tedavisi için kurkumin yüklü mukoadezif mikroemülsiyonun nazotransmukozal yoldan uygulanması

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Abstract
Objective
This investigation was aimed at designing an effective mucoadhesive microemulsion system to accomplish higher brain uptake of curcumin through intranasal route.
Methods
Mucoadhesive microemulsion of curcumin (MMEC) was developed using screened oil, surfactant and co-surfactant by Box-Behnken design and was evaluated for mucoadhesion, stability and naso-ciliotoxicity study. A comparative brain uptake of curcumin after nasal administration of MMEC and PCG and intravenous administration of PCS was studied by performing bio-distribution study in Swiss albino rats.
Results
The result showed that all formulation variables i.e., amount of Capmul MCM (X1), Smix (Accenon CC: Transcutol P) (X2) and % of aq. Polycarbophil (X3) had a significant effect ($p < 0.05$) on the responses. Developed MMEC was stable and non-ciliotoxic with 66.74 nm ± 3.46 and 98.58 % ± 1.21 as average globule size and drug content, respectively. Polydispersibility Index (0.133 ± 0.17) data and TEM study depicted the narrow size distribution of MMEC. Furthermore, following a comparative investigation of the brain uptake of curcumin among

MMEC, plain drug gel (PGC) and intravenous administration at 2.86 mg/kg, more brain uptake of curcumin was demonstrated for MMEC over intravenous application. Moreover, curcumin uptake in olfactory bulb after nasal administration of MMEC (31.11 ± 1.6) was than 9.44 times higher than intravenous injection of curcumin solution (3.25 ± 1.01). AUC represents the ratio of 2.86 mg/kg in brain tissue to plasma acquired afterward(s) the intranasal injection of MMEC (and it) was essentially greater than after the intravenous administration of curcumin solution.

Conclusion

Findings of the investigation revealed that optimal MMEC and intranasal route may be considered to be promising and an alternative approach for brain targeting of curcumin.

Keywords: Intranasal delivery; Microemulsion; Brain-targeting; MMEC; Mucoadhesion; TEM

ÖZET

Amaç

Bu araştırmada, intranazal yolla daha yüksek kurkumin alımını sağlamak için etkili bir mukoadhezif mikroemülsiyon sisteminin tasarlanması amaçlanmıştır.

Yöntem

Kurkuminin mukoadezif mikroemülsiyonu (MMEC), Box-Behnken tasarımı ile yağ, yüzey aktif madde ve yardımcı yüzey aktif madde kullanılarak geliştirilmiş ve mukoadezyon, stabilite ve nazo-siliotoksisite çalışması ile değerlendirilmiştir. MMEC ve PCG'nin nazal uygulamasından ve intravenöz PCS uygulamasından sonra kurkuminin beyin alımı, İsviçre albino sıçanlarında karşılaştırmalı bir biyolojik dağılım çalışması yapılarak incelenmiştir.

Bulgular

Tüm formülasyon değişkenlerinin (Capmul MCM (X1), Smix (Accenon CC: Transcutol P) (X2) ve Polikarbofil (X3) miktarı) yanıtlar üzerinde önemli bir etkiye sahip olduğu ($p < 0.05$) gösterilmiştir. Geliştirilen MMEC'nin ortalama globül boyutu ve ilaç içeriği sırasıyla 66.74 nm ± 3.46 ve %98.58 ± 1.21 olup, stabil olmadığı ve siliotoksik olmadığı belirlenmiştir. Polidispersibilite indeksi (0.133 ± 0.17) verileri ve TEM çalışması, MMEC'nin dar boyut dağılımını göstermiştir. Ayrıca, MMEC, düz ilaç jeli (PGC) ve 2.86 mg/kg'da intravenöz uygulama arasında beyinde kurkumin alımının karşılaştırmalı bir araştırmasını takiben, MMEC için intravenöz uygulamaya göre daha fazla kurkumin alımı gösterilmiştir.

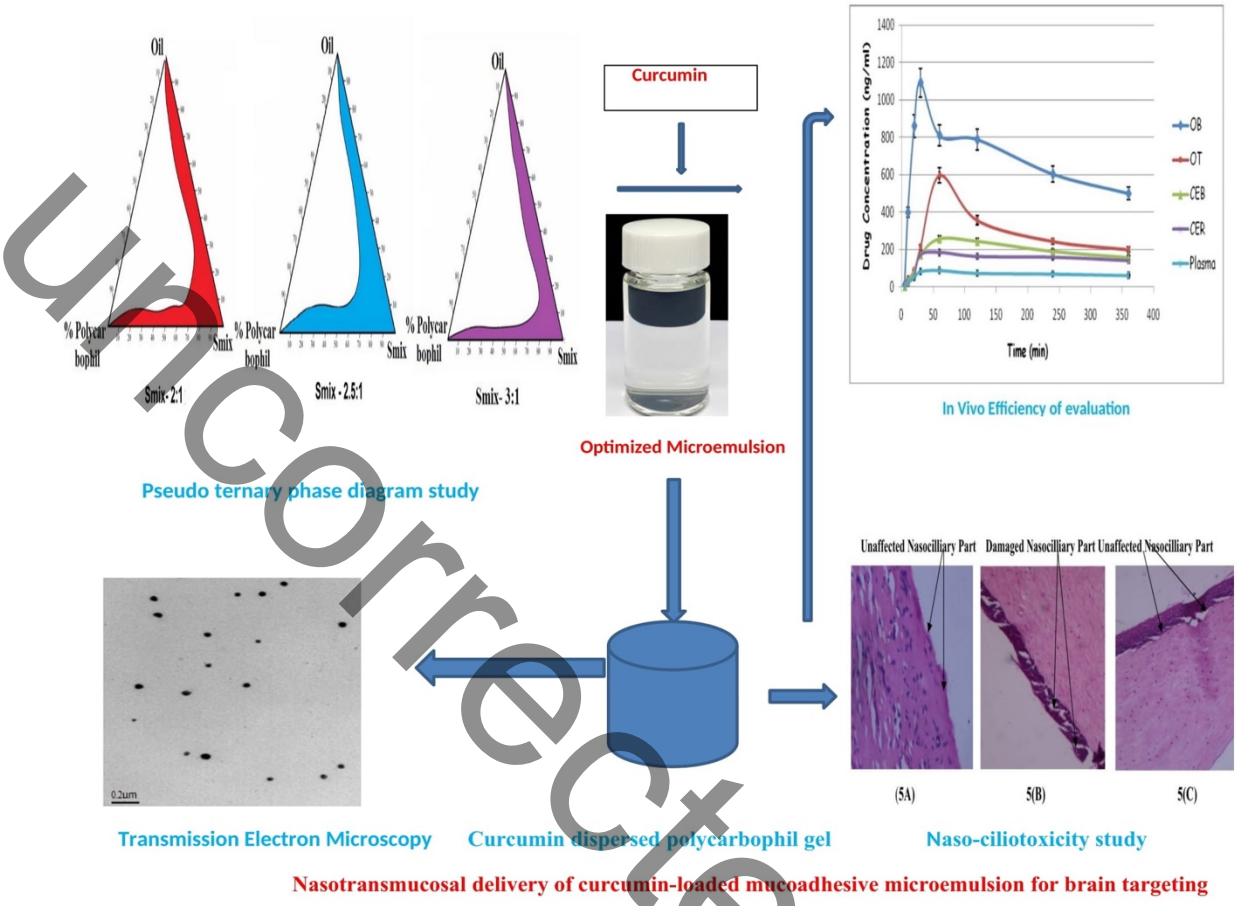
Ayrıca, MMEC'nin (31.11 ± 1.6) nazal uygulamasından sonra olfaktör ampulde kurkumin alımı, intravenöz kurkumin solüsyonu enjeksiyonu uygulamasından (3.25 ± 1.01) 9.44 kat daha yüksek bulunmuştur. Beyin dokusu/plazma oranını temsil eden AUC oranı, intranazal MMEC enjeksiyonundan sonra (2.86 mg/kg) kurkumin solüsyonunun intravenöz uygulamasına kıyasla daha yüksek bulunmuştur.

Sonuç

Çalışmanın bulguları, optimal MMEC ve intranazal yolun, kurkuminin beyin hedeflemesi için umut verici ve alternatif bir yaklaşım olarak kabul edilebileceğini ortaya koymuştur.

Anahtar Kelimeler: Intranazal taşıma; Mikroemülsiyon; Beyin hedefleme; MMEC; Mukoadezyon; TEM

Graphical Abstract:



Introduction:

Brain targeting through the nasal route becomes a fruitful research platform, and in recent times, it is attracting a lot of care around the globe. Drug delivery through the nasal route offers numerous benefits like quick bioavailability of the drug, circumvention of liver first-pass metabolic rate & better-delivering drug to the brain through the Olfactory region(OR) [1.] The olfactory part of the nasal route has a large surface area, i.e., 10 cm^2, and acts as a relatively more vital region of drug-carrying to Central Nervous System (CNS) and cerebrospinal fluid (CSF). The connective tissue of the olfactory area is known as lamina propria that comprises blood vessels and axons [2.] Several researches have been reported in literature of the brain targeting drugs that are insoluble in water via nasal route [3.] However, the intranasal administration of drug is connected with numerous intrinsic problems. The fundamental problem is its adequate nasal volume in humans, which is ≤400µl (200µl per nose) which undeniably a challenge for the formulators to develop a suitable formulation for poorly water-soluble drugs [4.] Secondly, the rapid proliferation of nasal mucosal layer (every 10 to 15 minutes) and ciliary movement facilitate the drug molecules clear from the nasal cavity [2.] Naso-ciliary clearance as a natural defense mechanism instead decreases the adhesion of the preparation to the nasal mucosa over a longer period of time.[5]

Curcumin is a bioactive constituent present in the rhizomes of Curcuma longa. Curcumin shows the pharmacologic effects of curcumin like anti-inflammatory, anticancer, antibacterial, antirheumatic, and antimalarial, antioxidant, cardioprotective, nephroprotective, neuroprotective,

and hepatoprotective effects [6.] But Curcumin is a phyto-constituents which are absorbed when given through the oral route leading to very poor oral bioavailability (less than 4%), which in fact is due to its poor aqueous solubility and erratic dissolution.[2,3.] Further, its incomplete oral absorption and high first-pass metabolism then difficult to enter the brain due to P-glycoprotein overexpression limit its clinical usefulness.[7-9] Therefore, we have tried here to improve brain targeting of curcumin by developing a new formulation and through the nasal route of administration.

Microemulsion (ME) is an anisotropically precise and thermodynamic stable liquid formulation with a globule size < 200 nm. It is composed of the oil and water phases, stabilized by a concrete mixture of surfactant and co-surfactant (S_{mix}).[10-14] Several research in the literature revealed the application of microemulsion for intranasal, topical, and parenteral, transdermal and oral drug delivery systems.[7,15.] In addition, o/w microemulsion is a better choice for drug incorporation with low water solubility due to its solution-like feature, which provides uniformity of dose. But, the microemulsion used for intranasal administration becomes the future area for the central nervous system targetting.[16-17] The addition of a suitable mucosal adhesive (Polycarbophil) such as a polyelectrolyte polymer helps to overcome the difficulties associated with the nasal route of drug delivery by retaining the microemulsion formulation on the nasal mucosa for a more extended period. The tissue appropriation and blood-cerebrum obstruction entrance information. The kinetics of tissue distribution and blood-brain barrier penetration data revealed that curcumin and nano-formulation were efficient enough for brain targeting.[3, 17-18] Moreover, used a quality by design approach to develop a suitable microemulsion formulation for intranasal delivery, which ultimately maximizing the brain targeting.[2-4, 16-20]

Therefore, the purpose of this research was to develop an optimal mucoadhesive microemulsion system (MMEC) and perform a comparative brain distribution study of curcumin following intranasal and intravenous administration in the rat. It is thought that MMEC can effectively distribute curcumin in the brain due to the unique connection between the brain and the nose, and also the controlled release capability of the developed formulation.[2-4, 18-20.] Thus, it can provide an innovative method for the treatment of inflammatory diseases of the CNS.

It is thought that MMEC might be effective enough to distribute curcumin within and into the brain due to the unique nose-brain connection and the controlled release capability of the formulation[2-4,] It can provide an innovative approach for treating inflammation-related CNS disorders.

Materials and Methods

Curcumin and Emodin were acquired from Arjuna Natural Pvt. Ltd. (Kerala, India). Capex, Capmul MCM, Cremophor EL, Accenon CC and Transcutol P were obtained from ABITEC Corporation (Columbus, USA). Polycarbophil was received from Lubrizol India Pvt. Ltd, (Navi Mumbai, India). Labral M 1944CS and Labrafac CC were acquired from Gattefosse, Navi Mumbai, Isopropyl Myristate, PEG 400, Tween 80, PEG 600, glycerol, isopropyl alcohol, Tween 60, Oleic Acid, isobutyl alcohol, were procured from Sigma Aldrich (Bangalore, India). Propranolol was obtained from Torrent Pvt Ltd. (Ahmedabad, India) Hexane, Diethyl Ether, NaH_2PO_4, Na_2HPO_4, p-phenyl phenol, and Acetonitrile (HPLC grade) acquired from Merck Life Science Pvt (Bengaluru, India). All the reagents were used of analytical grade. Double distilled pure water was used for present research.

Experimental Method:

Animals

All animal experiments were performed with NIH (National Institute of Health instructions for the Care and Use of Laboratory Animals publishing # 85-23, revised 1996). The Institutional Animal Ethical Committee approved the animal experiment (CPCSEA No. 984/14/11/CPCSEA), New Delhi, India. Albino rats (weighing 230–270g) were obtained from Zydus Cadila Healthcare Ltd, Moraiya, and Ahmedabad India. The rats were fasted for approximately 12-18 hours with free access to water and quality food through research. The rats were maintained at constant room temperature (25±2^{0}C) and air humidity (50±10%) with a light/dark cycle of 12 h.

Preformulation Study

The selection of oil as internal phase and core phase for intranasal o/w mucoadhesive microemulsion was mainly made on drug solubility in nasomucosal compatible oils.[3] The solubility of drug in various screened oils such as Labrafil M 1944CS, Isopropyl Myristate, Capmul MCM, Labrafac CC, and Oleic acid for the intranasal drug delivery was determined through saturation solubility technique. Surfactants such as tween-60, Tween-80, Captex-355, Accenon CC, and Cremophor RH 40, having HLB values ranging in between 12 to 16, were screened for curcumin solubility. Screening of co-surfactants was based on their ability to form stable and transparent microemulsions at a minimum concentration, and few reported co-surfactants like PEG 400, PEG 600, Propylene glycol, Glycerol, Isobutyl Alcohol, Isopropyl alcohol, and Transcutol P were screened.[7,21] Excess of curcumin was added to each cap vial containing 5 mL of each of the selected vehicles. After sealing, mixtures were shaken with a shaker at 37^{0}C for 24 h. After reaching equilibrium, centrifuged each vial at 8000 rpm for 15 min, and excess insoluble curcumin was separated by filtering the supernatant by Whatman filter (0.45µm). Solubilized drug concentration from the supernatant was quantified by UV-VIS spectrophotometer (Shimadzu UV-1800)[2, 5]

Preparation of MMEC

Amount of oil and surfactant-cosurfactant ratio (S_{mix}) were selected from pseudo ternary phase diagrams data. Mucoadhesive microemulsion was then prepared by water titration method using the screened formulation compositions and was optimized by Box Behnken design of Design-Expert® Software (Stat-Ease, Inc., Minneapolis, Minnesota, USA, V 7.1.0)
[7, 11] Three independent factors such as % amount of Capmul MCM (X1), Smix (Accenon CC: Transcutol P, X2), and % measure of Polycarbophil (X3, as far as % w/v in water) with their three levels. It is taken from preliminary experiments. [8] A total of 15 formulation compositions of MMEC were obtained. The polynomial model and equation were experimentally interpreted basing on significant terms ($p < 0.05$) and non-significant lack of fit data as provided by Design-Expert® software to define the influence of independent variables on the responses.[22, 23.] The experimental design was quadratic, and details of their three levels as taken are demonstrated in Table 1. MMECs were prepared experimentally using the compositions of all model MMECs as summarized in Table 1. Smix, i.e., a mixture of Accenon CC and Transcutol P (3:1), was mixed well with drug dissolved Capmul MCM solution. The above mixture was then titrated with different aqueous polycarbophil concentrations with gentle and continuous stirring at room temperature using a magnetic stirrer.[10, 21, 23] Finally, It was decided on standard droplet size, flux, retention time, and drug release (%) to be measured experimentally for all fifteen batches. Flux was estimated from the ex-vivo permeation study. Plain curcumin dispersed polycarbophil gel (Polycarbophil Curcumin Gel(PCG) 3.0 mg/mL) was prepared by dispersing 30 mg of curcumin to the already prepared 0.5% aqueous-based plain polycarbophil based gel with continuous stirring.

Optimization of MMEC

Responses like average globule size, flux, mucoadhesive potential, and drug release (%) were selected for numerical and graphical optimization. It was decided to choose the maximum flux and drug release (%) while a minimum of average globule size with suitable mucoadhesive potential to obtain an optimized formulation. From the overlay plot, the best composition as MMEC was visually selected. Finally, for verification, checkpoint batches were prepared experimentally and predicted values of all four responses were compared with the observed value. The best-suited composition was considered as an optimized batch and was used for further study.

Evaluation of dependent variables

Droplet size, zeta potential, and polydispersity index (PDI)

The droplet size, zeta potential, and PDI of optimized MMEC were determined by Zetasizer (Nano Z.S.; Malvern Instruments Inc, Malvern, U.K.). Therefore, in order to identify the type of microemulsion formed, and a dilutability test was carried out by dispersing 1 gm formulation in 100 mL of distilled water and was evaluated for phase separation.[22]

The Mucoadhesive Potential

The mucoadhesive potential, indicated by the residence time of developed nasal formulations, was evaluated as per reported method[23-25.] Briefly, 100 mg MMEC was kept at on the focal point of the different agar plates at room temperature at (1% w /w, prepared in phosphate buffer solution, pH 6.4). After 10 min, the agar plates were linked with the USP disintegration test equipment and moved up and down at a speed of 30 ±2 times in phosphate buffer solution at 37ºC±1ºC.The time taken by the formulations to isolate from the agar plates was noted outwardly as the residence time of the formulations. [22]

Flux

Flux was quantified as the amount of permeated curcumin from the unit area of the nasal mucosa. For this study, a Franz diffusion cell with an effective diffusion area of 7.06 cm^2 and volume 30 mL was used.[26-29.] The sheep nasal mucosa with the same thickness was collected from slater house in formalin, prepared, and mounted on the receptor compartment with 30 mL of phosphate buffer (pH-6.4) as diffusion medium. The donor compartment was amassed to it and was stacked with 1 mL of MMECs, PCG, and PCS (≈ 3 mg of curcumin). Diffusion was done at 37 ± 1 °C and 50 rpm. At predetermined intervals of 10 mins, an aliquot of 0.5 mL diffusion medium was drawn from the receptor medium and was dissected by UV-VIS Spectrophotometer at 422 nm. The results are obtained in three times and the mean value is considered.

***In-vitro* drug diffusion study**

In-vitro drug diffusion study of MMEC was carried out in modified dissolution apparatus containing 400 mL of dissolution media, i.e., phosphate buffer saline (PBS), pH 6.4.[32, 33,35.] The temperature was maintained at 37 ± 1 °C and set rpm at 50. Dialysis membrane of cut-off weight 10,000 D was soaked in PBS (pH 6.4) overnight before the experiment. Different formulations (Mucoadhesive Micro emulsion (MMEC), Polycarbophil Curcumin Gel (PCG), and Plain Curcumin Solution (PCS)) equivalent to 30 mg of curcumin were put in separate diffusion bags tied to both ends. Aliquots (5 mL) were withdrawn at an interval of 30 minutes for the first one hour and then one h interval for the rest of the study period, i.e., 10 h, and supplanted with a similar measure of new phosphate buffer solution. After proper dilution with dilution media, samples were evaluated at 422 nm by UV-VIS spectrophotometer.[30-34] The results were obtained in three times and the mean value was considered.

pH

The pH of the optimal MMEC was measured using a digital pH meter (Welltonix digital pH meter PM100). Accurately weigh 1 g of MMEC and dispersed in 10 mL of purified water. The calibrated pH meter electrode was inserted into the sample 10 min before reading at room temperature. The pH value was measured in triplicate and the mean value was calculated.[32]

The content analysis of drug

For drug content, 0.5 g equivalent weight of curcumin in a 100 mL volumetric flask and dissolved in 50 mL of ethanolic phosphate buffer (70 mL ethanol and 30-mL PBS- pH 6.4). The volumetric flask was kept for 2 h and shaken well in a shaker to mix it properly. It was diluted appropriately and analyzed on a UV-VIS Spectrophotometer (Shimadzu UV 1800) at 422 nm.

Spreadability

For MMEC gel spreadability study, 0.5 g of MMEC was placed on the glass plate within a premarked circle of 10mm diameter. A second plate was put over this first petridish, and 50g weight was permitted to rest on the upper petridish.[2, 26] Spreading of the gel with respect to the increase in the diameter was noted.

Viscosity

The viscosity of the developed MMEC was measured in triplicate at $25^0C \pm 1^0C$ by Brookfield viscometer (Model HVT).[2,32] The prepared MMEC 50gm was placed in a container and permitted to equilibrate for 5 minutes prior estimating dial measurements using TC shaft spindle # 64 at 20 rpm.

Transmission Electron microscopy

The morphology of emulsion droplet for the optimal MMEC was observed using TEM (JEM 1010, JEOL Ltd, Tokyo, Japan) with an acceleration voltage of 80 Kv. The optimal MMEC was diluted with water (1:1000). One drop of the sample wad directly plain matrix, which has a cross-section size of 300, discolored to two percent weight by volume tungstic acid for 2 minutes after drying the MMEC. It was extra dried at R.T. and then inspected using transmission electron microscopy. The interaction was determined at image was intensified 1, 50000 times at 8000 times.[2, 32]

Nasal Ciliotoxicity

The nasal ciliary toxicity studied was carried out using extracted sheep nasal mucosa to evaluate the ingredients' safety to the mucosal layer utilized in the formulation. In a brief, the nasal mucosa of the sheep with the exception of the diaphragm was collected from the slaughterhouse in phosphate buffered saline (pH 6.4). At that point, three different parts of the nasal mucosa (NP1, NP2, NP3) having an equal thickness was mounted on a Franz dispersion cell and then were exposed with 2 mL of MMEC (test sample, 2860 µg/mL Curcumin), phosphate buffer saline (pH 6.4) (Negative control) and isopropyl alcohol, a serious nasal mucociliary toxicity agent (Positive control) [23, 26] for 2 h respectively. All three nasal samples were washed with purified water after 2 h. and the cross-section of the mucociliary was examined with optimal microscope (Nikon Fx35A Japan) then the sample was stained with hematoxylin and eosin.[23, 26, 30]

Animal Experiment

The rats were maintained at temperature (25 ± 2 °C) and humidity (45 ± 5%) and were supplied with standard laboratory diet and water ad libitum on a 12 h light/dark cycle. Rats were assigned to five groups with six animals in each group.

Group I - Saline treated (Normal control).

Group II - Intranasal administration of optimized MMEC at 2860µg/kg of body weight at two hour intervals were administered through intraperitoneal route for two weeks.

Group III Animals were treated animal PCG 2860µg/kg in of body weight at two hour intervals were administered through intraperitoneal route for two weeks.
Group IV- Animals were first treated with MMEC 2860µg/kg in the same above method followed by intranasal applied plain drug solution (PDS) at 2860µg/kg of curcumin/kg of body weight for two weeks.
Group V- Animals were treated with 2860µg/kg of curcumin/kg of body weight for two weeks
In brief, the animals were anesthetized using intraperitoneal pentobarbital injection (40 mg/kg of body weight) and were maintained their body temperature by keeping them on a heating pad set at 37 °C. 40 µL of MMEC and PCG containing curcumin equivalent to 2.86 mg/kg each were administered through the intranasal route with the assistance of a micropipette (200 µL) connected to low density polyethylene tubing with 0.1 mm inner measurement at the delivery site. For intravenous administration, 0.5mL of PCS was delivered (portion comparable to 2.86 mg/kg) through the femoral vein. At 5, 15, 30, 60, 120, 180, 240, and 360 min after the portion, blood was gathered in the anesthetized condition by cardiac puncture in heparinized Eppendorf tube (Eppendorf India Pvt Ltd Bangalore). Blood samples were then centrifuged at 6000 rpm and -4 °C for 15 min to obtain plasma. At each time point, the animals have sacrificed by euthanasia. At that point, the skull was cut open, and the olfactory bulb (OB), olfactory plot (OT), cerebrum (CRB), and cerebellum (CEB) were carefully excised. All four excised brain tissue were rinsed with saline followed by blotted up with channel paper to take out blood spoil and plainly visible veins however much as could reasonably be expected. After gauging, the cerebrum tissues were homogenized independently with 5 mL of saline in a tissue homogenizer (BD-144 Tissue Homogenizer, India). All tissue samples, such as aliquots of plasma and cerebrum tissue homogenates, were put away in a freezer (- 20 °C) until HPLC analysis. Measurements of curcumin were made using 3 rats at each time point.

Pharmacokinetic data analysis

A non-compartmental pharmacokinetic analysis method was used to investigate the pharmacokinetic behavior of curcumin. Microsoft Excel was used to calculate the pharmacokinetic parameters from the experiments. The total area under the plasma concentration time curve was determined by the trapezoidal rule using plasma curcumin concentration *vs.* time data from time zero to the last sampling time, *i.e.* 6 h plus the extrapolated area (from the last experimental time to infinity). The relative bioavailability of the representative MMEC to the control was calculated as follows:

Relative bioavailability % = $[(AUC_{MMEC} \times Dose_{control}) / (Dose_{MMEC} \times AUC_{control})]$.

Where,

AUC_{MMEC} means the area under the plot of plasma concentration of a drug versus time after MMEC gives insight into the extent of exposure to a MMEC and its clearance rate from the body.

The AUC Control represents the total curcumin solution exposure across time.

The apparent elimination half-life ($t_{1/2}$) was calculated from the estimated elimination rate constant (kel) by linear regression of the log of the plasma concentrations as in $0.693/_{Kel}$. The elimination rate constant (kel) can be calculated directly from those parameters using the equation kel equals clearance divided by volume of distribution. The maximum plasma concentration (c_{max}) and time to maximum concentration (t_{max}) after oral administration were determined directly from the concentration versus time curve.

Analytical Method

Curcumin in plasma as well as brain tissue was quantified by HPLC method.[27] Curcumin is extracted from plasma by protein precipitation method [28, 38.] To 0.2mL of processed plasma samples, 10 µL Emodin (suitably diluting in methanol) was added as an internal standard and was sonicated in a bath sonicator for foe 120 seconds. The HPLC system consisted of LC-10AD VP HPLC Pump (Shimadzu, Japan) equipped with a U.V. detector with lab-solution software. The column used was the Agilent C18 column (Inertsil, 250 mm x 4.6 mm, particle size 5µ, USA). Chromatographic analysis was carried out at 1mL/min flow rate of mobile phase i.e., Acetonitrile - 5% Acetic acid (75:25, v/v). The mobile phase was prepared by mixing acetonitrile and 5% acetic acid and was further separated by a 0.22 µ membrane filter, followed by degassing by sonication before use. Elution of the drug was identified at 425 nanometers. 80-400 µl of brain tissue homogenates, 30 µg / ml of Emodin were added and stirred for 2 min. At this point of centrifugation at 10,000 rpm for 10 min, the drug was removed from the supernatant by adding 0.7 ml of n-hexane and diethyl ether (1: 1). Next, the separated organic phase was evaporated at 40 °C until dryness. The residue reconstituted in 100 µL mobile phase, and afterward, 20µL was injected onto a HPLC framework. The residue was reconstituted in 100 µL mobile phase, and then 20 µL was implemented into an HPLC framework.[2.] The blood samples were analyzed using the same mobile phase and chromatographic conditions.
The retention time was 5.8 min for curcumin and 3.2 minutes for the interior standard (i.e. Emodin). The linear range of curcumin is 40-600 ng / mL, and the linear range plasma and brain tissue is 20-400 ng/g. Extraction recoveries of curcumin from plasma and tissue homogenates were more than 86.7 % and 82.6 %, respectively.[33]

Statistical analysis

All data were displayed as mean ± S.D., and the distinctions between the groups were tested utilizing the Student's t-test at the significance level of $P < 0.05$. More than two groups were compared at using ANOVA, and differences greater at $P < 0.05$ were considered significant.

Stability Study

The stability of MMEC was carried out as per the ICH guideline (Q1A, R2) for 6 months [30, 36] the optimal microemulsion was stored at cold temperature (4-8 °C, 45% R.H. ±5), (25 ± 2 °C, and 60 % R.H. ± 5) and at accelerated temperature (40 ± 2 °C, 75 % R.H. ± 5). After each 3 months for half year, MMEC was analyzed for droplet size, Particle size distribution, Mucoadhesion, and % drug content. Since curcumin is reported to light-sensitive, the formulation was kept in an amber-colored container.[37]

Results and discussion:

Preformulation study

Capmul MCM is a compatible hydrophobic vehicle to the nasal mucosa, which exhibited maximum curcumin dissolving volume (42.17 mg/mL ± 3.12) compared to other screened oils to develop a microemulsion system. Therefore, it was designated as the oil phase. However, Accenon CC, which has HLB value of 15.6, was chosen as surfactant and has relatively low drug solubility compared to other surfactants. The selection of Capmul MCM based on more reservoirs and a more negligible effect partitioning effect of curcumin. Because curcumin remained in the core phase as a dissolved state, curcumin has less soluble in Accenon CC, which leads to sustained drug release.[7, 30] The longer retention time of curcumin may be due to the loading of curcumin in mucoadhesive microemulsion, as a reserve source for sustained release of curcumin in brain tissue. The results from the release studies confirm this controlled release. The accumulation of curcumin in the brain may be due to the micro sizes of the particles and the presence of surfactant on mucoadhesive microemulsion surface which may cause the brain

uptake reduction of lipid carriers by reticuloendothelial system. The predicted result can be compared to data obtained from the literature.[20.] In his results, Bashara pointed out the controlled release of Buspirone and high concentration of the same Buspirone in the brain, which approving the transport of blunt nasal passages to the brain after an intranasal loading microemulsion of Buspirone.[30.]

Results of the pseudo-ternary phase study exhibited that Accenon CC and Transcutol P (Smix) revealed no significant changes in the existing microemulsion region from 2:1, 2.5:1, and 3:1. For the development of ME, Smix of 2.5:1 was chosen since another two proportions (2:1 & 3:1) having more surfactant, which may not help the supply the reservoir properties of the formulation. Then, at that point, we obtained an appropriate microemulsion, which did not aggravate on the nasal mucosa, Smix having relatively less surfactant was not selected. Pseudo-ternary phase diagram was shown in Fig. 1. This data can be compared to the findings of the results of the literature.[30- 32]

Optimization of MMEC

In order to formulate the best MMEC and to observe the effect of independent variables on the responses, such as normal droplet size, flow rate, and retention time as mucosal adhesion possible and drug release (%), three independent variables (X1, X2, and X3) are based on RSM to Design Expert® programming[14] and the consequences of various regression analysis are summed up in Table 2. In addition, as shown in Table 1, we also sees that the normal droplet size, transition, retention time, and curcumin drug discharge (%) are significantly affected by the independent factors described in polynomial equation 1 to 4.

The evaluation of all the responses showed the suitability of the quadratic model ($p < 0.05$). Final conditions for all responses, i.e. mean droplet size, movement, hold time, and drug discharge (%) in terms of their respective coded value were obtained by running ANOVA as follows.

$$\text{Droplet Size} = + 77.88 + 3.47 \times A - 2.63 \times B + 3.89 \times C - 0.64 \times (A \times B) + 1.12 \times (B \times C) + 2.54 \times (A \times C) + 1.52 \times A^2 - 1.67 \times B^2 + 2.37 \times C^2 \quad \text{----------(1)}$$

Equation 1 exhibited that the average droplet size of MMEC was generally influenced by Capmul MCM, Accenon CC: Transcutol P concentration ratio and Polycarbophil with their interactions. For formulating a suitable intranasal drug delivery system, droplet size plays a vital role as it impacts the *in-vivo* absorption of the drug from the formulations [4, 24.] The droplet size of the microemulsion formulation is a key factor because it affects the release rate of the drug and the *in vivo* profile of the drug. In this way, the experimental of optimizing a microemulsion with droplet size usually smaller at confirms that rapid penetration through mucous layers is an objective of the present research. The observed droplet size of the formulations ranged between 55.82nm to 90.13 nm (Table 1). The results were obtained that the increasing of the oil volume from 0.1 mL to 0.5 mL and the concentration of the mucosal adhesion polymer from 0.25- 0.75 % caused a significant increase in globule size of microemulsion formulation. It could be because it didn't diminish the interfacial tension among oil and external phase with maximum concentration with a similar Smix. Additional, at higher Smix concentration, the lipophilic property of oil was well masked, resulting about low interfacial tension, and therefore, droplet size reduces.[23] Mucoadhesive polymer also show to upsurge the droplet size, which may be due to the fact that it was capable of absorbing water and swell which in turn disturb the hydrophilic-lipophilic balance of the system.

$$\text{Flux} = + 88.46 + 2.47 \times B - 1.83 \times C + 0.58 \times (A \times B) - 1.67 \times (A \times C) + 1.55 \times C^2 \quad \text{----------} \quad (2)$$

Capmul MCM due to its reservoir action and polycarbophil due to the viscosity enhancing property showed negative effect on release rate. So, the drug concentration gradient across the

permeation barrier (nasal mucosa) is not high and hence the flux. The results obtained in this study indicated that oil concentration (X1) and mucoadhesive polymer concentration (X3) has significant effect on the flux through sheep nasal mucosa (p – 0.0001). However, Smix showed positive effect on flux due to the fact that both surfactant and cosurfactant capable of altering the permeation behavior of the membrane by changing the fluidization of lipid enabling the drug molecule to permeate through rapidly.

Retention time = + 47.37 + 3.55 × C + 2.79 × (A × C) + 0.88 × (B × C) – 0.76 × B^2 + 1.04 × C^2 ---------- (3)

Polycarbophil due to its mucoadhesive property along with viscosity enhancing property, showed noticeably positive effect on retention time of MMEC on the nasal mucosa while oil and Smix showed non- significant effect as shown in equation 3. Mucoadhesive nature of the polymer may be because of the presence of high density of hydrogen bonding groups which could combine with mucin more strongly as shown in equation 3 (p - 0.0021) [36]

Drug release (%) = + 93.87 – 1.89 × A + 2.01 × B – 3.11 × C + 0.88 × (A × B) – 1.95 × (A × C) – 1.89 × (B × C) + 0.51 × A^2– 0.67 × B^2 –1.34 × C^2 ---------- (4)

Drug release from MMEC was inversely affected by Capmul MCM and Polycarbophil concentration, while mixture of Accenon CC and Transcutol P was found to facilitate the release of curcumin from the developed formulation as revealed in equation 4. Drug release from the model MMEC batches after 8 h was ranging from 79 % to 96 % as shown in Table 1. Capmul MCM showed to retard the drug release from the formulation due to its partitioning and reservoir property, while polycarbophil because of its viscosity enhancing property, kinetics of drug molecule reduced leading to the slow release. However, Smix (Accenon CC and Transcutol P mixture) was found to increase curcumin release because it increased the water solubility of curcumin by reducing the interfacial tension. This increase in drug release may further be due to nano globule size with narrow size distribution.

The rationale of optimization through factorial design was to obtain the ranges of all independent factors, establish their influence on responses, and find a robust composition for intranasal delivery of curcumin. In this study, globule size, viscosity, and retention time (RT indicating the mucoadhesive property) were set to the maximum without affecting release, while flux and release were set to maximum. Confirming the desirability of the optimized nasal formulations, three MMECs, so obtained from the overlay plot were prepared experimentally, and all responses were evaluated as given in Table 3. It was observed that experimentally found data matched the predicted responses for all three MMECs, and hence, the optimization process was verified.[32, 33, 34]

MMEC characterization

MMEC with 0.5% w/v polycarbophil, 0.3 mL Capmul MCM, 3.70 mL Smix (2.5:1 ratio) exhibited the smallest droplet size (55.82-90.12 nm) , the highest Flux and drug release (80-96.1%) are demonstrated in Table 3 and closer to the observed results than predicted. Therefore, was considered as optimal formulation. The average globule size was 66.74 ± 3.46 nm with PDI equivalents to 0.133 ± 0.017, as revealed in Fig. 2. Besides, the PDI value (<0.3) showed the monodisperse property of the formulation.[37]

Zeta potential of MMEC was -21.4 mV ± 4.11 as shown in Fig. 3. This data is depicting neither the stability of the formulation which might be due to the fact that moderate negative surface charges neither resulted into strong aggregation nor repulsion of the globules [7]. So individual nano globules increased the surface area which in turn helped in the nasal absorption of drug and

hence the stability of formulation and brain targeting. TEM data further supports the nano size with narrow size distribution as illustrated in Fig. 4.

Curcumin content was discovered to be 97.82 % ± 0.44, 98.11 % ± 0.51 and 99.32 % ± 0.22 for MMEC, PCG and PCS individually. The pH (6.7 ±0.18), thickness, and mucoadhesive strength data showed the appropriateness for the nasal application of MMEC. The stability studies showed no significant changes in droplet size; size distribution, retention time, and phase separation over six months were observed from the stability study. Hence developed mucoadhesive nasal formulation of curcumin was considered physically stable for 6 months.

Optical microscopic pictures showed no mucociliary damage by MMEC as shown in Fig. 5 (C) which revealed non-ciliotoxicity profile of developed MMEC while complete cilia destruction was observed in the isopropyl treated mucosa. Hence, developed MMEC was considered suitable for nasal application. This may be due to the fact that all formulation compositions were of GRAS and having no interaction.

The prepared gel produces excellent spreadability and mucoadhesive strength. The drug content of the MMEC was found to be 98.67 % ± 0. 44 and pH was found to be 6.58 ± 0. 29. Viscosity of the gel was found to be 18.7 Ps ± 2.11 at 10 rpm. Spreadability, pH, viscosity and mucoadhesive strength data were showing the suitability for intranasal application of MMEC.

From the stability study, as shown in Table 4, no significant changes of globule size, size distribution, retention time and phase separation over a period of six months was observed. Hence developed mucoadhesive nasal formulation of curcumin was considered physical stable for 6 months.

Pharmacokinetic Study

Curcumin concentration in plasma and brain tissue concentration after nasal administration of MMEC and PCG and intravenous administration of PCS at 2.86 mg/kg body weight of rat was estimated. The pharmacokinetic parameters obtained by non-compartmental pharmacokinetic analysis ((n=3; mean±SD) were given in Table 5.

Following the IV administration of PCS, plasma curcumin fixations arrived at the maximum level before 15 min and then decreased rapidly over time, as shown in Fig. 6. After intranasal administration of MMEC, maximum concentration of curcumin was achieved even before 30 min in OB while 60-120 min in other brain parts like OT, CRB and CEB. Curcumin content was differed considerably in different brain regions. The highest concentration was observed in the OB (the peak drug level was 1109.1 ng/g ± 56.4), followed by OT, then the cerebellum and finally in cerebrum as shown in Fig. 6. These findings support the existence of a nose–brain direct pathway following the intranasal administration. As shown in Table 5, Curcumin concentration particularly in OB was noticeably higher than other tested parts of brain following nasal administration of MMEC.

AUC0→360 for MMEC was 2.81 times (56449 ng. min/g ± 3113) more that obtained after IV injection of PCS (20088 ng. min/g ± 1241). However, PCG showed comparatively less curcumin uptake than that of MMEC which was a clear indication of only mucoadhesive property was not enough for nasal delivery to brain.

Curcumin uptake in other brain parts (OT, CRB, and CEB) after nasal dosing of MMEC was lower than OB ((n=3; mean±SD) as shown in Table 5 and Fig. 6, which may be due to its anatomical position from the nasal turbinates[33,35]. After nasal dosing of MMEC, Curcumin transport from the nasal turbinates into the olfactory region may occur through three pathways, i.e., transcellular between the sustentacular cells by endocytosis, paracellularly through tight junctions between the sustentacular cells, and intracellular axonal transport via olfactory nerve

pathway following endocytosis or pinocytosis into the olfactory bulb. MMEC increased the aqueous solubility of lipophilic curcumin, enhancing the drug permeation through the nasal cells by combining all three above described methods and reaching the therapeutic area[36, 37].
Curcumin uptakes into other brain regions like OT, CBR, and CEL after nasal dosing of MMEC were lower than i.v. dosing of PCS as shown in Table 5 and Fig. 6. These may be attributed to the comparative lower plasma levels from MMEC, which might reduce curcumin distribution into the brain. In contrast to CBR and CEL, the mass of the OB is small. The distribution of higher curcumin from OB into other brain regions could be counteracted by its small masses, thus leading to the significant increase in Curcumin content in different brain tissues (olfactory pathway). Due to the controlled release of curcumin from MMEC and mucoadhesive nature, prolonged curcumin absorption was observed, indicating the nose–direct brain transport. These obtained data were also similar to the data given in the literature[36-38] showing the unique nasal mucosa-brain connection. In order to evaluate the brain targeting through nasal delivery of MMEC, the brain-to-plasma Curcumin AUC ratios at 10, 30, and 360 min following intravenous and intranasal routes were calculated. Results exhibited that the ratio of AUC in brain tissues to that in plasma after intranasal application of MMEC was significantly higher ($P < 0.05$) than those after intravenous injection of PCS and PCG. For instance, at 10 min after nasal dosing, the AUC ratio was 5.83 times higher than after intravenous dosing (19.85 ± 1.5 v/s 3.4 ± 1.1) in OB, as shown in Fig. 7[A].
Similarly, 9.17 times (60.41 ± 2.8 v/s 6.58 ± 1.3) and 9.56 times (31.11 ± 1.6 v/s 3.25 ± 1.01) increment of curcumin concentration in OB following intranasal and intravenous delivery at the time points 30 min and 360 min (n=3; mean±SD) respectively as shown in Fig. 7[B] and Fig. 7[C]. Following intranasal administration, 87.22 % of curcumin was transported to the brain via the olfactory pathway at six hours.[37, 38] therefore; it can conclude that the nasal route of administration and mucoadhesive formulation may help curcumin enhance its brain uptake.

Conclusion

Results confirmed that polycarbophil based mucoadhesive microemulsion system including Capmul MCM (3% v/v), Accenon CC (26% v/v) and Transcutol P (9% v/v) was optimal for transnasal delivery of curcumin. The developed microemulsion system was non ciliotoxic, physical stable at ambient conditions for 6 months and was suitable for controlled curcumin delivery to brain. Results of brain distribution study confirmed that a fraction of Curcumin could be transported directly into the brain after nasal delivery which may decrease the dose and frequency of dosing and hence maximize its therapeutic index. However, clinical benefits to risk ratio of this mucoadhesive formulation so developed in this investigation will decide its effectiveness in the clinical practice.

Abbreviations:

MMEC- Mucoadhesive Microemulsion of Curcumin
TEM- Transmission Electron Microscopy
PGC - Plain Drug Gel
CSF- Cerebrospinal Fluid
PEG-Polyethylene Glycol
PBS- Phosphate Buffer Saline
OB- Olfactory Bulb
OT- Olfactory Tract
ANOVA- Analysis of Variance

$AUC_{0-\alpha}$- Area under Curve
C_{max}- Maximum Peak Plasma Concentration
PDI- Polydispersity Index
PCG- Polycarbophil Gel
PCS-Plain Curcumin Solution
M.E- Microemulsion
OR- Olfactory region
Smix- Accenon CC and Transcutol P
O/W- oil in water
MME- Mucoadhesive Microemulsion
Accenon CC -ACC
Cremophor RH 40-CRH40
X1- Capmul MCM
X2- Accenon CC and Transcutol P
X3-% polycarbophil
RSM-Response Surface Method
PEG -Polyethylene glycol

References

1. Behl CR, Pimplaskar HK, Sileno AP, Demeireles J, Romeo VD. Effect of physicochemical properties and other systemic nasal drug delivery factors. Adv. the drug delivers rev.1998; 29: 89-116.
2. Mandal, S.D., Mandal, S., Krishna Chutani, K., & Subudhia, B.B. The mucoadhesive microemulsion of ibuprofen: design and evaluation for brain targeting efficiency through intranasal route. Braz. J. of Pharma Sci. 2015: 51(3):721-731.
3. Mandal, S.D., Mandal, S. & Patel, J .Brain targeting efficiency of curcumin loaded mucoadhesive microemulsion through intranasal route. J. of Pharm. Investig. 2016; 46:179–188.
4. Pathak R, Dash RP, Misra M, Nivsarkar M. Role of mucoadhesive polymers in enhancing the delivery of nimodipine microemulsion to the brain via the intranasal route. Acta Pharmaceutica Sinica. B. 2014; 4: 151-160.

5. Aggarwal B, Harikumar K. Potential therapeutic effects of curcumin, the anti-inflammatory agent, are against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune, and neoplastic diseases. J. Biochem. Cell Biol. 2009; 41: 40-59.
6. Lawrencea MJ, Reesb GD. Microemulsion-based media as novel drug delivery systems. Adv drug delivers rev. 2000; 45: 89–121.
7. Tenjarla SN. Microemulsions: An overview and pharmaceutical applications. Critical Reviews in Therapeutic Drug Carrier Systems. 1999; 16: 461-521.
8. Mandal S, Mandal SD. Design and development of carbamazepine mucoadhesive Microemulsion for intranasal delivery: An ex-vivo study. International Journal of Pharmaceutical Sciences Review and Research.2010; 3: 56–60.
9. Mandal SD, Mandal S, Patel J. Development of curcumin loaded microemulsion drug delivery System for improving its dissolution profile. International Journal of Pharmaceutical Formulation and Analysis.2013; 4(2): 101-107.
10. Soni H, Patel SS, Mishra K, Nayak G, Singhai AK. Qualitative and quantitative profile of curcumin from ethanolic extract of Curcuma longa. International Research Journal of Pharmacy. 2011; 2(4): 180-184.
11. Bitter, C.; Katja, S.Z.; Surber, C. Nasal Drug Delivery in Humans. Curr. Probl. Dermatol. 2011; 40:20-35.
12. Stevens, J.; Ploeger, B.; Graaf, P.H.; Danhof, M.; Elizabeth, C.M. Systemic and direct nose-to-brain transport pharmacokinetic model for remoxipride after intravenous and intranasal administration. Drug metabolism and disposition 2011; 39:2275-2282.
13. Mohammad, S.; Khan, R.A.; Mustafa, G. Bromocriptine loaded chitosan nanoparticles intended for the blunt nose to brain delivery: pharmacodynamics, pharmacokinetic and scintigraphy study in mice model. Eur. J.Pharm. Sci. 2013; 48:393-405.
14. Anant, P.; Anshuman, A.A.; Bhimrao, K.J.; Mahadik, K.R. Characterization of curcumin–PVP solid dispersion obtained by spray drying. Int. J. Pharm. 2004;271: 281-286.
15. Dhupia, S.V.; Hanson, L.R.; Frey, W.H. Intranasal delivery to the central nervous system: mechanisms and experimental considerations. J Pharm Sci 2010; 99: 1654–1673.
16. Jeffrey P.; Summerfield, S. Assessment of the blood-brain barrier in CNS drug discovery. Neurobiol. Dis. 2010;37: 33–37.
17. Tsai, Y.M; Chiena, C.F.; Lina, C.L.; Tsai, T.H. Curcumin and its nano-formulation: The kinetics of tissue distribution and blood-brain barrier penetration. Int. J. Pharm. 2011; 416:331-338.
18. Shah, B.; Khunt, D.; Bhatt, H.; Misra, M.; Padh, H. Application of quality by design approach for intranasal delivery of rivastigmine loaded solid lipid nanoparticles: Effect on formulation and characterization parameters. Eur. J. Pharm Sci. 2015; 78:54-66.
19. Swamy, N.G.N.; Abbasb, Z. Mucoadhesive in situ gels as nasal drug delivery systems: an overview. Asian Journal of Pharmaceutical Sciences. 2012;7:168-180.
20. Wang, X.; Chi, N.; Tang, X. Preparation of estradiol chitosan nanoparticles for improving nasal absorption and brain targeting. Eur. J .Pharm. Biopharm. 2008;70:735-740.
21. Mandal, S.D., Mandal, S. & Patel, J. Intranasal mucoadhesive microemulsion for the neuroprotective effect of curcumin in MPTP induced Parkinson model. Braz. J. Pharm. Sci. 2017;53(2):e15223/
22. Aqil M, Kamran M, Ahad A, Imam SS. Development of clove oil-based nanoemulsion of olmesartan for transdermal delivery: Box–Behnken design optimization and pharmacokinetic evaluation. Journal of Molecular Liquids. 2019;214: 238-248.

23. Thakkar HP, Patel AA, Chauhan NP. Formulation and optimization of mucoadhesive microemulsion containing mirtazapine for intranasal delivery. Chronicles of Young Scientists. 2014; 4(1): 25-32.
24. Joshi HM, Bhumkar DR, Joshi K, Pokharkar V, Sastry M. Gold nanoparticles as carriers for efficient transmucosal insulin delivery. Langmuir. 2006; 22: 300-305.
25. Kan P, Chen ZB, Kung RY, Lee CJ, Chu IM. Study on the formulation of o/w emulsion as carriers for lipophilic drugs. Colloids Surf B: Biointerfaces. 1999;15: 117-125.
26. Acharya S, Pundarikakshudu K, Panchal A, Lalwani A. Preparation and evaluation of transnasal microemulsion of carbamazepine. Asian J Pharm Sci. 2013;8: 64–70.
27. Kumar M, Misra A, Babbar AK. Intranasal nanoemulsion based brain targeting drug delivery system of risperidone. Int J Pharm. 2008;24: 285-291.
28. Li J, Jiang Y, Wen J.A rapid and simple HPLC method for determining curcumin in rat plasma: Assay development, validation, and application to a pharmacokinetic study of curcumin liposome. Biomed Chromatogr.2009;23(11): 1201-1207.
29. Wang D, Hu J, Lu L. Enhanced inhibitory effect of curcumin via reactive oxygen species generation in human nasopharyngeal carcinoma cells following purple-light irradiation. Oncol Lett. 2013; 6(1):81-85.
30. Bshara HN, Ahmed RO, Holayel SM, El-Shamy AA. Improvement of the bioavailability of buspirone HCl using intranasal delivery systems. J Pharm Sci. 2012;45: 86-102.
31. Karasulu HY, Sanal ZE, Ertan G. Permeation studies of Indomethacin from different emulsions for nasal delivery and their possible anti-inflammatory effects. AAPS PharmSciTech. 2008; 9(2): 342-348.
32. Kaur P, Kim K .Pharmacokinetics and brain uptake of diazepam after IV and intranasal administration in rats and rabbits. Int J Pharm. 2008; 364: 27-35.
33. Rao J, Mcclements DJ. Formation of flavor oil microemulsions, nanoemulsions, and emulsions influence composition and preparation method. J Agric Food Chem.2011; 59(9): 5026-5035.
34. Dey S, Mahanti B, Mazumder B, Malgope A, Dasgupta S. Nasal drug delivery: An approach of drug delivery through nasal route. Der Pharmacia Sinica. 2011;2(3): 94-106.
35. Vyas TK, Babbar AK, Sharma RK. Singh S, Misra AN. Preliminary brain-targeting studies on intranasal mucoadhesive microemulsions of sumatriptan. AAPS Pharm SciTech. 2016;7: E1-E6.
36. Chen X, Zhi F, Zhang X. Enhanced brain targeting of curcumin by intranasal administration of a thermosensitive poloxamer hydrogel. J Pharm Pharmacol. 2013; 65(6):807-816.
37. Tian XH, Lin XN, Wei F. Enhanced brain targeting of temozolomide in polysorbate-80 coated poly butyl cyanoacrylate nanoparticles. Int J Nanomed. 2011;6: 445-452.
38. Mandal S, Mandal SD, Chuttani K, Dharamsi A, Subudhi B. Transnasomucosal mucoadhesive microemulsion of zaltoprofen: A comparative brain distribution study. J. Drug Deliv. Sci. 2017;39:237e246.

List of Tables:

Table 1:

Batch	X_1	X_2	X_3	Globule Size (nm)	Flux ($\mu g/cm^2$. h)	RT (min)	Drug release (%)
F1	0.10	1.55	0.50	59.62	13.8	17.6	96.1
F2	0.50	1.55	0.50	76.36	18.3	21.5	83.1
F3	0.10	1.65	0.50	55.82	16.3	23.4	80.0
F4	0.50	1.65	0.50	62.59	14.2	22.1	86.2
F5	0.10	1.60	0.25	56.44	15.4	16.2	90.4
F6	0.50	1.60	0.25	68.74	17.5	21.8	76.4
F7	0.10	1.60	0.75	79.22	14.6	24.7	86.2
F8	0.50	1.60	0.75	90.13	16.1	27.8	79.3
F9	0.30	1.55	0.25	60.64	16.4	21.5	85.8
F10	0.30	1.65	0.25	56.54	24.2	23.7	90.4
F11	0.30	1.55	0.75	71.89	12.4	23.2	87.3
F12	0.30	1.65	0.75	63.33	22.0	23.1	85.7
F13	0.30	1.60	0.50	64.12	25.9	22.6	88.3

F14	0.30	1.60	0.50	62.89	25.8	22.5	88.6
F15	0.30	1.60	0.50	63.75	25.6	22.4	88.1
Variables					Low	Medium	High
X_1= Capmul MCM (Oil)					0.1mL	0.3mL	0.5mL
X_2= Accenon CC: Transcutol P (S_{mix})					1.55	1.60	1.65
X_3= % aq. Polycarbophil					0.25%	0.5%	0.75%

Amount of curcumin = 30 mg; Total volume of MMEC = 10 mL.
RT- Retention time (time required for formulations to separate from the agar plates)

Table 2:

Regression Coefficient	Coefficient Estimate			
	Globule Size (nm)	Flux (µg/cm^2. h)	RT (min)	Drug Release (%)
A- Capmul MCM (X1)	3.47	-0.47	0.39	-1.89
B- S_{mix} (X2)	-2.63	2.47	-0.21	2.01
C- % aq. Polycarbophil (X3)	3.89	-1.83	3.55	-3.11
AB (X1 X2)	– 0.64	0.58	0.35	0.88
AC (X1 X2)	2.54	– 1.67	2.79	– 1.95
BC (X1 X2)	1.12	- 0.43	0.88	– 1.89
A^2	1.52	– 0.45	0.37	0.51
B^2	– 1.67	0.43	-0.76	- 0.67
C^2	2.37	1.55	1.04	-1.34
Model (*p* Value)	0.0002	0.0003	0.0021	0.0002
Coefficient of variation	0.991	0.999	0.962	0.999
R^2	0.973	0.998	0.976	0.997
Adjusted R^2	0.6710	0.2891	0.8771	0.0580
Lack of Fit (*p* Value)	3.47	-0.47	0.39	-1.89

Table 3:

No	Components	Globule Size (nm)	Flux [µg/cm2.h]	RT (min)	Drug Release (%)

	O	S_{mix}	P	Pre.	Obs.	Pre.	Obs.	Pre.	Obs.	Pre.	Obs.
1	0.30	3.80	0.50	48.6	47.4	24.0	23.9	22.9	22.2	93.9	95.6
2	0.31	3.80	0.52	48.4	47.5	24.7	23.8	22.8	22.0	89.8	93.0
3	0.30	3.70	0.50	46.7	46.3	25.4	25.8	22.9	22.8	94.1	94.8

O- Oil; Smix- Mixture of surfactant and co-surfactant; P- Polycarbophil
(Pre.- Predicted and Obs.- Observed)

Table 4

Temperature & Relative humidity	Evaluated parameters of MMEC							
	Globule size (nm)		Retention Time (min)		PDI		Phase separation	
	3^{rd} Month	6^{th} Month	3^{rd} Month	6^{th} Month	3^{rd} Month	6^{th} Month	3^{rd} Month	6^{th} Month
2 – 8 ˚C & 45 + 5 %	75.5± 4.2	83.3± 3.7	19.8± 2.2	22.3± 1.9	0.31± 0.02	0.36± 0.07	No	No
25 + 2 ˚C & 60 + 5 %	62.7± 4.8	69.6± 5.3	22.5± 1.27	22.8± 1.85	0.201± 0.02	0.217± 0.03	No	No
45 + 2 ˚C & 75 + 5 %	63.9± 4.9	61.8± 4.4	18.9± 1.87	20.1± 1.37	0.364± 0.21	0.343± 0.017	No	Yes

(Result = mean ± SD, n =3).

Table 5:

Parameters and Routes	Plasma	OB	OT	CBR	CEL
C_{max} (ng/mL.g) (Intranasal)	112.3±21.5	956.8±71.2	345.6±101.6	256.7±21.2	2037 ±19.8
C_{max} (ng/mL.g) (Intravenous)	954.5±56.4	3087.8±72.5	2807.7±87.3	2857.9±99.7	464.7±169.3
$AUC_{0\rightarrow360}$ (ng.min//mL.g) (Intranasal)	21311±871	62458±1867	8876.1±928	6542.8±298.7	3894.2±228.6
$AUC_{0\rightarrow360}$ (ng.min//mL.g) (Intravenous)	72561±4427	26857±971	13241.2±366	19288.1±558	14389.6±237
% [AUCi.n. / AUCi.v.]	29.36	232.56	67.03	33.92	27.06

OB- Olfactory bulb; OT- Olfactory tract; CBR-Cerebrum; CEL- Cerebellum.
(Result; Mean± SD, n =3)

List of Figures:

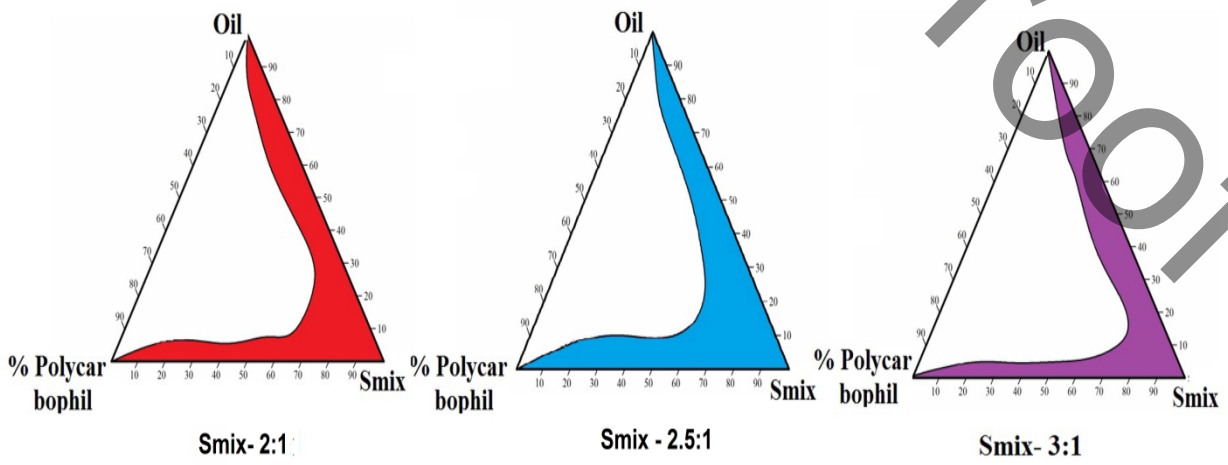


Figure 1. Pseudo-ternary Phase diagram of MMEC showing microemulsion existing region with 2.5:1 S_{mix}.

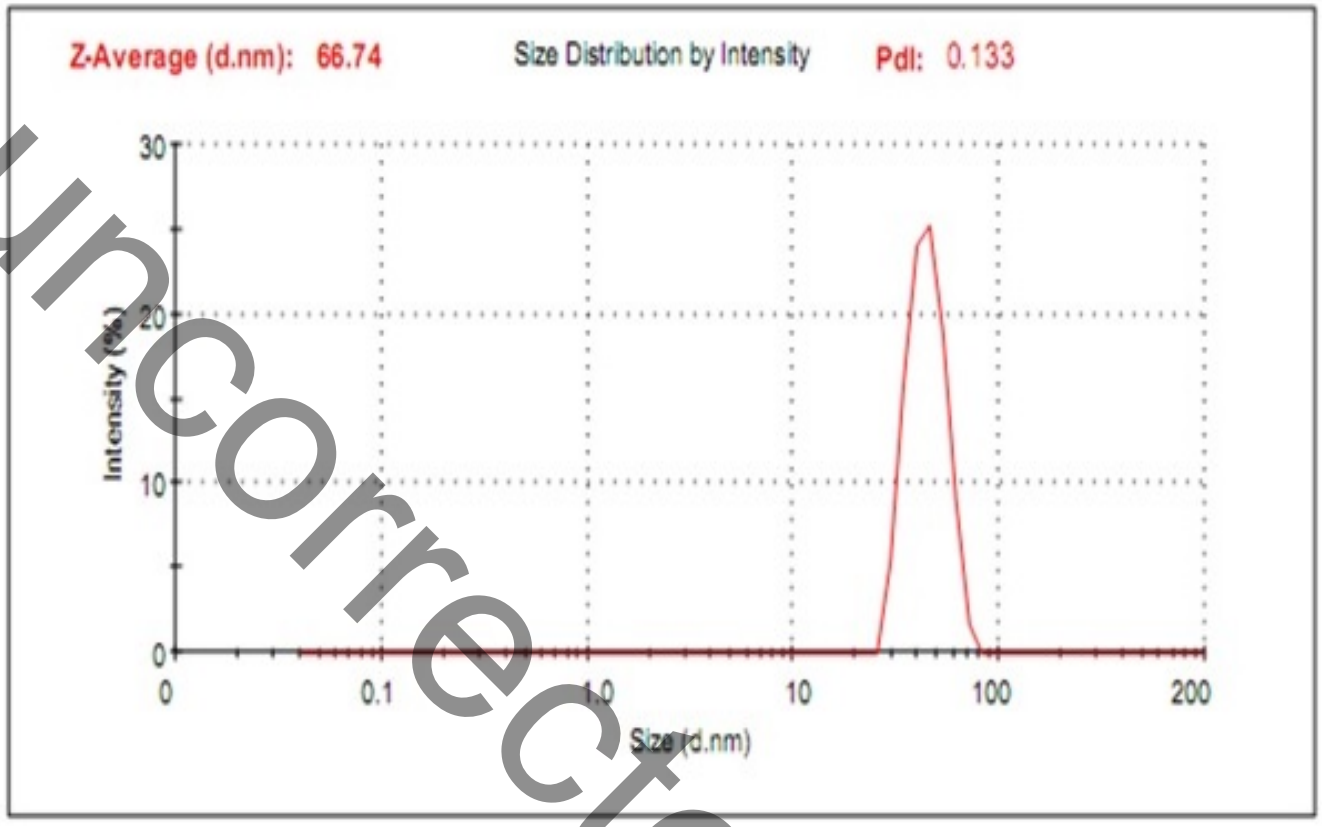


Figure 2. Result of globule size with size distribution indicating the nano size range of optimal MMEC.

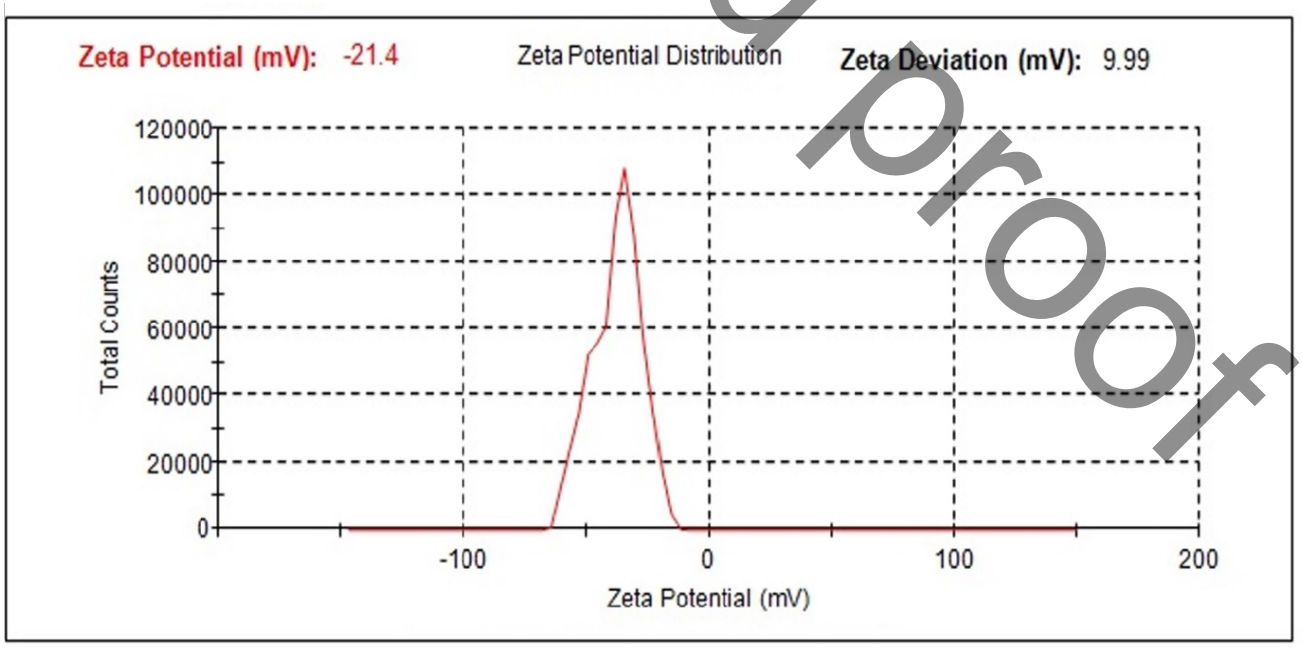


Figure 3. Zetapotential data representing the physical stability of MMEC.

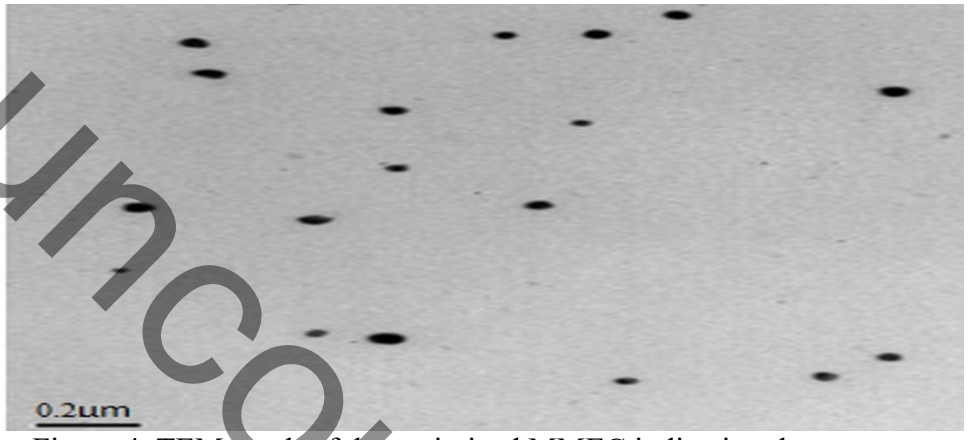


Figure 4. TEM result of the optimized MMEC indicating the narrow particle size with uniform distribution.

(5A) **5(B)** **5(C)**

Figure 5. Result of naso-ciliotoxicity study displaying the nontoxicity of developed MMEC. 5[A], 5[B] and 5[C] are representative of PBS (pH-6.4), Propranolol and developed MMEC treated naso mucosal part individually.

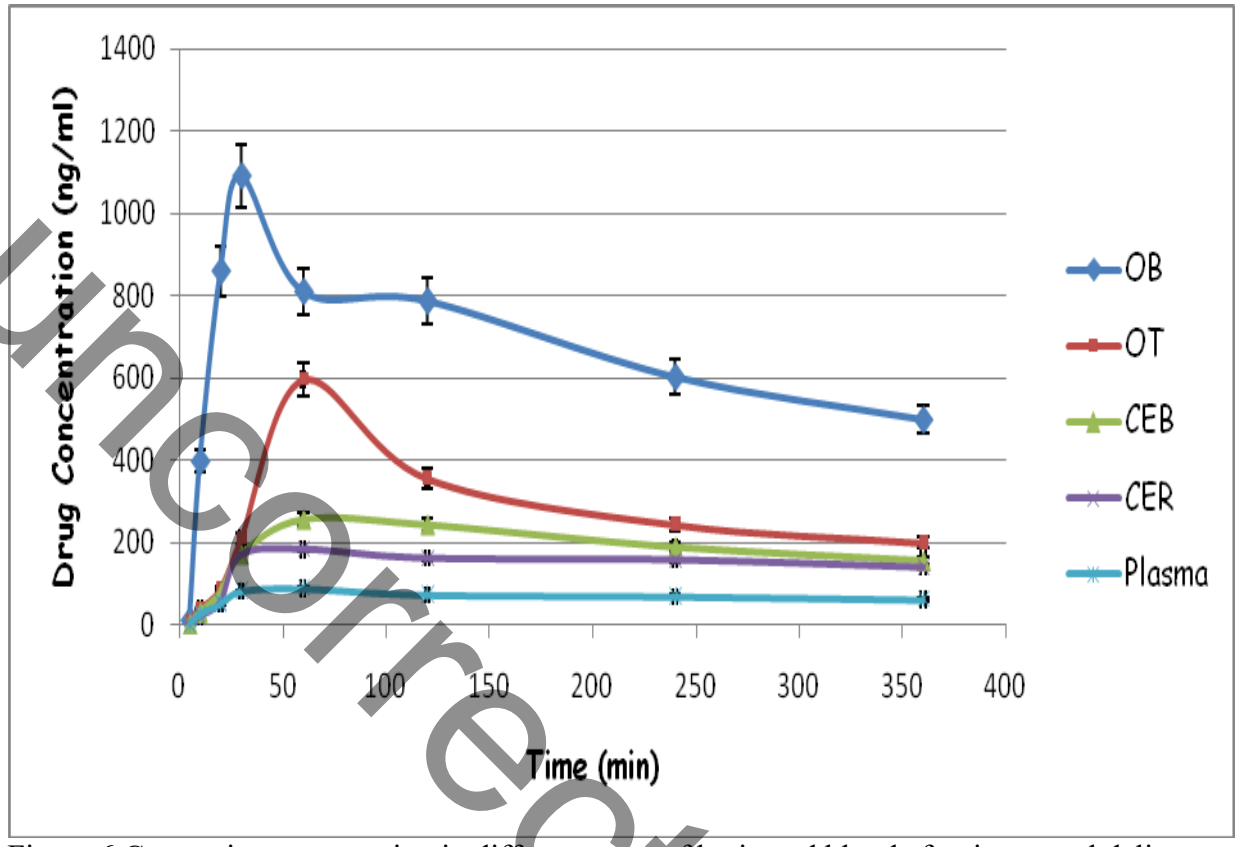


Figure 6.Curcumin concentration in different parts of brain and blood after intranasal delivery (n=3; mean±SD).

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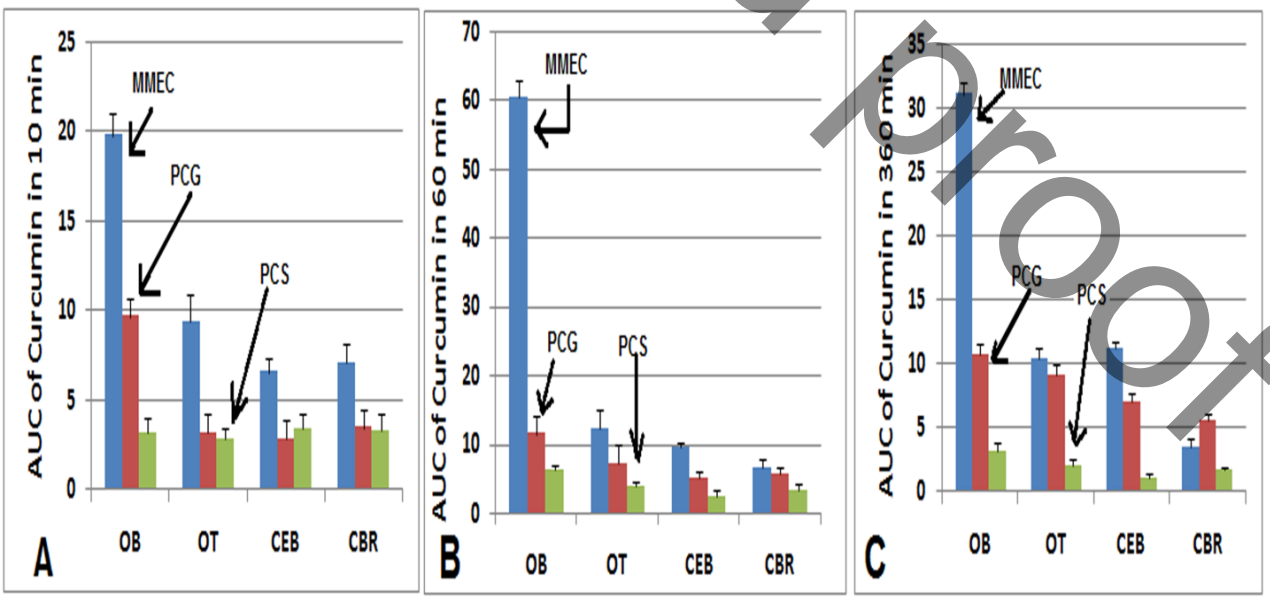

Figure7. Plasma curcumin concentration and in different parts of brain after intranasal administration of MMEC, PCG and intravenous dosing of PCS after 10 min (A), 30 min (B) and 360 min (C) respectively(n=3; mean±SD).