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Brain targeting efficiency of antimigrain drug loaded mucoadhesive intranasal nanoemulsion

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ABSTRACT

Zolmitriptan (ZT) is a well-tolerated drug in migraine treatment suffering from low bioavailability due to low amount of the drug that reaches the brain after oral and nasal delivery. Development of new nasal mucoadhesive nanoemulsion formulation for zolmitriptan may success in delivering the drug directly from the nose to the brain to achieve rapid onset of action and high drug concentration in the brain which is required for treatment of acute migraine. ZT mucoadhesive nanoemulsion were prepared and characterized for drug content, zeta potential, particle size, morphology, residence time and permeation through the nasal mucosa. The selected formula was tested in-vivo in mice for its pharmacokinetics in comparison with intravenous and nasal solution of zolmitriptan. Results showed that addition of chitosan as mucoadhesive agent in 0.3% concentration to the nanoemulsion enhanced its residence time and zetapotential with no significant effect on the globule size. All tested formulations showed higher permeability coefficients than the zolmitriptan solution through the nasal mucosa. In-vivo studies showed that the mucoadhesive nanoemulsion formulation of zolmitriptan has higher AUC_{0-8} and shorter T_{max} in the brain than the intravenous or the nasal solution. This was related to the small globule size and higher permeability of the formulation.

1. Introduction

Migraine headache is the most common neurological vascular headache disease which causes a throbbing and pulsating pain around the head. It usually includes abnormal sensitivity of arteries in the brain resulting in triggers which often lead to rapid changes in the artery diameter. As a result, other arteries in the brain and scalp dilate resulting in terrible pain in the head (Niranjan et al., 2015).

Zolmitriptan (4S-4-({3-[2-(dimethylammino)ethyl]-1H-indol-5-yl}methyl-1,3-oxazolidin −2-one) is second generation triptan which is an effective, well-tolerated treatment of acute migraine associated with menses, migraine with aura (Palmer and Spencer, 1997). It has a selective action on serotonin (5HT1B/1D) receptors and is very effective in reducing migraine symptoms. It is also effective in treatment of acute cluster headache (Cittadini et al., 2006; Mahakalkar and Upadhye, 2013)

Zolmitriptan (ZT) works by stimulating serotonin receptors in the brain which is a natural product in the brain that causes brain blood vessels to narrow. Zolmitriptan mimics this action of serotonin by stimulating its receptors, thus it acts peripherally inhibiting blood vessels dilatation and inflammation of cranial vessels.

Zolmitriptan is available commercially as a conventional tablet (ZOMIG®), an oral disintegrating tablet (ZOMIG-ZMT®) and a nasal spray (ZOMIG® nasal spray), in doses of 2.5 and 5 mg (Bankim et al., 2013; Patil et al., 2015). The oral administration of zolmitriptan showed many drawbacks, such as slow onset of action, low bioavailability (40%), nausea and incomplete pain relief with recurrence of headaches, short half-life (1–2 h) and first-pass metabolism (Ahonen et al., 2004; Goadsby and Yates, 2006; Mittal et al., 2014).

In addition, considerable ratio of migraine patients suffers from gastric stasis, severe nausea and vomiting during the migraine attack. The matters which causes erratic absorption of the drug from the gastrointestinal tract with delayed gastric emitting which makes the oral treatment is ineffective (Egla and Abd Al hammid, 2017; Vyas et al., 2005). Intranasal zolmitriptan has proved earlier onset of action than the oral formulation especially in acute cluster

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headache (Charlesworth et al., 2003). However clinical studies showed that zolmitriptan half-life and bioavailability after nasal administration do not significantly differ from those obtained after oral intake of the drug (Bigal et al., 2003; Goadsby and Yates, 2006; Mittal et al., 2014).

Recently, nose to brain delivery has become a novel noninvasive technique for transporting therapeutic agents directly to the brain depending on the unique connection provided by the olfactory and/or trigeminal nerve system present between the olfactory epithelium and the brain, bypassing the BBB (Pires et al., 2009) in addition to other intranasal rout benefits such as avoiding extensive hepatic and intestinal metabolism and better patient compliance due to ease of delivery and non-invasiveness (Mistry et al., 2009; Pandey et al., 2016; Stevens et al., 2011). However, the nasal transport of the drug from the nose to the brain is affected by many factors such as rapid clearance of the drug from the nose by the nasal mucosal membrane cilia (Kolsure and Rajkapoor, 2012; Raza et al., 2007), drug concentration, dosage form, and others (Hongbing et al., 2008). Modification of the dosage form in which the drug is delivered into the nasal cavity along with using absorption enhancer within the formula are two of the most important approaches used to enhance direct transfer of the drug from the nose to the brain. (Charlton et al., 2007; Dalpiaz et al., 2008; Hongbing et al., 2008)

Nanoemulsions are thermodynamically stable, isotropic, clear dispersions containing oil and water phases, stabilized by an interfacial film of surfactant and/or cosurfactant molecules (Rodrigues et al., 2015). Briefly, they are emulsions with droplet size on the order of 100 nm. The major differences between classical emulsions, nanoemulsions and microemulsions are in droplet size range and stability characteristics. Nanoemulsions are attractive for aforementioned applications because they are relatively the least sensitive to physical and chemical changes (Gupta et al., 2016; Pandey et al., 2016).

Nanoemulsion (NE) formulations enhance nose-to-brain drug delivery since they are able to protect the encapsulated drug from biological and/or chemical degradation, and increase its extracellular transport (Mahajan et al., 2014). They also provide higher surface area and can be formulated in different formulations such as liquids, sprays, foams, creams, ointments and gels (Sarker, 2005).

The objective of this work was to formulate nano-sized mucoadhesive drug delivery system of zolmitriptan in order to enhance its transport from nose to brain and have rapid onset of action to increase its efficiency in acute migraine management.

2. Methods

2.1. Materials

Capryol PGMC, Capmul MCM EP, Maisine 35-1, and Captex 200-P were obtained as a gift from GATTEFOSSE, France. Kolliphor® RH 40 (Polyoxyl 40 hydrogenated castor oil) and Kolliphor® El (Polyoxyl 35 hydrogenated castor oil) were purchased from Sigma (Sigma-Aldrich). Chitosan (MW:100,000–300,000 Da, Acros Organics, New jersy, USA). Tween 80, Brij 35, Ploxamer 188, Oleic acid, IPM, and Olive oil and other analytical reagents were purchased from El-Gomheria Co., Cairo, Egypt.

2.2. Spectrophotometric determination of zolmitriptan (ZT) in methanol

Stock solution of ZT in methanol was prepared by dissolving ZT in methanol into 100 mL volumetric flask to give the final concentration of 1 mg/mL. This solution was scanned against methanol at the UV range 200–400 nm using UV–vis Schimadzu spectrophotometer (Model UV-1601, Japan). Serial dilutions were done and the calibration curve was constructed in the range from 50 to 800 μg/mL.

For confirmative study, oils, surfactant and cosurfactants interferences with ZT estimation were studied using methanol as reference by measuring separately the absorbance of the oil, surfactant, and co-surfactants into which the maximum amount of ZT was dissolved at ZT ⩘max against methanol.

2.3. Solubility studies

1 Excess amounts of ZT was added into screw capped vials to 2 g of each of the following oils: (Capryol PGMC, Maisine 35-1, Capmul MCM EP, Oleic acid, IPM, and Olive oil), surfactants: (Kolliphor® RH 40, Kolliphor® El, Tween 80, Brij 35) and co-surfactants: (Transcutol-P, PEG 400, Captex 200-P, and Poloxamer 188).

The contents of each vial were mixed using vortex (BV1000 BenchMixer™) then shacked for 48 h using shaker (PSU-20i Orbital Multi-Platform Shaker) at room temperature. After equilibrium, solutions were centrifuged using laboratory centrifuge (Remi Laboratory Centrifuge *R*32A, Remi Equipment, Bombay, India) at 5000 rpm for 15 min and filtered by Whatman filter (0.45). From each supernatant, 0.5 mL was taken and diluted with 5 mL methanol then measured spetrophotometrically for ZT concentration. Each experiment was done in triplicate. The oil, surfactant and co-surfactant into which ZT has maximum solubility were selected for construction of the pseudo-ternary phase diagrams.

2.4. Pseudo-ternary phase diagrams construction

Based on solubility studies, Capryol PGMC was selected as the oil phase, Kolliphore® RH40 as the surfactant and Transcutol®-P as the cosurfactant along with distilled water as the aqueous phase for construction of the pseudo-ternary phase diagram.

Three ratios of the surfactant: cosurfactant (Smix) were used 1:1, 2:1, and 3:1 along with the oil in different ratios; 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 for construction of three phase diagrams in order to investigate the one has the maximum region of NE formation (Shah et al., 2014). Addition of water was done drop wise using the water titration method with mixing by a vortex until formation of clear transparent emulsion stopped with physical change appearance (usually occurs as turbidity,

Table 1
Composition and physicochemical characterization of prepared NE systems.

Oil (%w/w)	Smix (2:1) (%w/w)	Water (%w/w)	Globule size (nm)	PDI%	% T	Turbidity after centrifugation
10	30	60	180.53 ± 2.34	0.14 ± 0.02	89.65 ± 2.13	Phase separation
10	34	56	125.76 ± 3.72	0.23 ± 0.01	92.34 ± 1.85	turbid
10	38	52	76.62 ± 2.84	0.17 ± 0.04	97.68 ± 2.34	clear
10	42	48	67.57 ± 1.86	0.25 ± 0.01	99.46 ± 1.57	clear
10	46	44	61.27 ± 2.73	0.22 ± 0.03	99.67 ± 1.38	clear
10	50	40	54.63 ± 3.24	0.026 ± 0.03	100.08 ± 1.63	turbid

Values are expressed as mean ± SD, n = 3.

precipitation, phase separation or gel formation). Samples were left 24 h after each addition for equilibrium and then tested visually for clearance or turbidity. Phase diagram was constructed using CHEMIX- Ternary diagrams software.

2.5. Optimization of NE formulations

Based on the pseudo-ternary phase diagrams, the one which has the highest region of emulsion formation was selected. The oil was used as constant ratio while ratios of Smix and water were changed, Table 1.

The prepared formulations were examined for% transmission, average globule size, polydispersity index (PDI) and signs of phase separation or turbidity after centrifugation for 5000 rpm for 30 min.

2.6. ZT solubility determination in the NE optimized formulation

Excess amount of ZT was added to 5 mL of the optimized NE system, stirred on shaker at room temperature for 48 h. The system was centrifuged at 5000 rpm, filtered and the supernatant was examined for ZT concentration to determine the maximum amount of ZT that can be loaded into the NE system.

2.7. Preparation of mucoadhesive ZT loaded NE (MNE)

The optimized NE system was loaded with ZT. Chitosan was selected as mucoadhesive agent in two different concentrations 0.3 and 0.5% w/w.

Zolmitriptan MNEs were prepared using ultra probe sonicator (Sonifier 250 Bransan, USA) (5 min, 40 °C probe temperature, 90% amplitude) (Boche and Pokharkar, 2017). The specified amount of the drug was first dissolved into the oil and mixed thoroughly with Smix by vortex. Specified amount of chitosan was dissolved in minimum amount of water which was added dropwise to the oil and Smix upon sonication until give clear transparent NE.

2.8. Characterization of the prepared MNE formulations

2.8.1. Average globule size, polydispersity index (PDI) and zeta potential determination

Prepared emulsion systems were characterized for their average globule size (z-average) by laser diffraction technique (Zetasizer Nano ZS-90, Malvern instruments, Worcestershire, UK) (4 mW He–Ne laser, 642 nm), Scattering light was detected at 170° by the automated laser attenuation filters with a refractive index of 1.54. Size distribution was given and polydispersity index was calculated. The charge on the prepared systems was determined by Zetasizer (Zetasizer Nano ZS-90, Malvern instruments, Worcestershire, UK) through one hundred dilutions with distilled water.

2.8.2. Percentage (%) transmission

The percentage transmission (%) of the prepared formulations was measured using a UV–vis double beam spectrophotometer at 650 nm (Shahu et al., 2013; Sharma et al., 2015; Tandel et al., 2015) using distilled water as a blank.

2.8.3. Viscosity determination

Viscosity of the nanoemulsions was measured without dilution using cone and plate viscometer (Brookfield DV-III, USA) with spindle # 40 at 25 °C ± 0.5 and 50 rpm (Moghimipour et al., 2013).

2.8.4. pH measurement

pH meter (CG 820 Schott-Gerate, W.Germany) was used to measure the pH of the prepared MNE formulations at 25 °C ± 0.5

2.8.5. Drug content

Fixed amount (0.1 mL) of each formulation was diluted to 10 mL methanol to give clear solution. The solution was suitably diluted and measured for ZT content using spectrophotometer against methanol as blank.

2.8.6. Morphological characterization

Morphological examination of the optimized ZT nanoemulsion was done by transmission electron microscopy (TEM JSM-JEOL, Tokyo, Japan). MNE formulation was dispersed in water, and one drop was placed on a 200-mesh carbon-coated copper grid which was dried at room temperature and was stained with 2% phosphotungstic acid for 15 min and washed three times with ultrapure water and dried. After that, samples were examined under the transmission electron microscope and photos were taken.

2.8.7. In-vitro mucoadhesion study

Mucoadhesion of the prepared mucoadhesive nanoemulsions was measured by previously mentioned method (Pisal et al., 2004; Mandal et al., 2016) with slight modification as here we used sheep nasal mucosa instead of using agar. Sheep nasal mucosa was obtained from local slaughterhouse immediately after slaughtering. The mucosa was cut into longitudinal sections (1 × 2 cm) and 100 mg of each formulation were centered on the mucosa and left for 2 min to assure attachment. Mucosal sections were attached to USP disintegration test apparatus (USA) and moved up and down in PB pH 6.4 at 37 ± 1 °C. The time taken by the formulations to separate completely from the mucosa was recorded as residence time (RT).

2.8.8. Ex-vivo permeation studies

Ex-vivo permeation studies of the prepared zolmitriptan MNE and zolmitriptan solution were done on Franz diffusion apparatus (Franz diffusion cell, Hanson Research Corporation (HRC), USA) using cheep nasal mucosa as permeation membrane (Pidaparthi and Suares, 2016).

Cheep nasal mucosa was obtained from local slaughterhouse. The nasal mucosa was carefully removed and soaked in phosphate buffer pH 6.8 (Basu and Maity, 2012) and kept in deep freezer at −20 °C until being used. Nasal mucosa was mounted on Franze cell with permeation area of 1.77 cm^2 keeping the mucosal side facing towards the donor compartment. The tissue was stabilized with the phosphate buffer (pH 6.8) in both donor and receptor compartments and allowed to stir for 15 min by magnetic stirrer (Prajapati et al., 2015). The solution was removed from both compartments and the donor compartment was filled with amounts of the MNE and aqueous solution equivalent to 10 mg ZT. The receptor compartment was filled with 7.5 mL of phosphate buffer which was adjusted to 37 ± 0.5 °C by means of circulating water bath. The medium was magnetically stirred at 50 rpm. Aliquots of 1 mL were withdrawn and filtered at pre-determined time intervals up to 6 h, suitably diluted with methanol and measured for ZT content by means of UV spectrophotometer. Experiments were done in triplicates and amount permeated was plotted against time. Permeability coefficient (p) was calculated by the following equation:

$$P = \frac{dQ/dt}{C_o X A} \quad (1)$$

Where, dQ/dt is the flux or permeability rate (mg/h), C○ is the initial concentration in the donor compartment, and A is the effective surface area of nasal mucosa.

2.9. Stability studies

Physical stability of the prepared ZT MNEs was examined by storing them for 6 months at room temperature, samples were withdrawn at 1, 3, and 6 month and analyzed for% transmission and drug content after their visual examination for any signs of drug precipitation, phase separation or turbidity (Zhao et al., 2013).

3. In-vivo biodistribution studies

Based on pharmaceutical evaluation, formulation MNE1 was selected for in-vivo evaluation in rats in comparison with IV solution and nasal solution of zolmitriptan.

Animal handling was in accordance to the guidelines of the Research Ethical Committee of the National Organization for Drug Control and Research (NODCAR, Cairo, Egypt) and in accordance to the ethical procedures and policies approved by Ethical Research Committee of Faculty of Pharmacy, Cairo University, Cairo, Egypt and complied with the Guide for the Care and Use of Laboratory Animals (ILAR, 1996). ILAR (Institute of Laboratory Animal Resources) (1996) Guide for the Care and Use of Laboratory Animals. NIH Publication No. 85-23 (revised 1996). National Academy Press, Washington, D.C. Available from www.nap.edu/openbook.php?record_id = 5140

Adult male (54) Sprague-Dawley rats weighing 200–210 g were divided into 3 groups. Animals were allowed free access to standard diet and tap water ad libitum and were housed at room temperature and natural light/dark conditions for one week before experiment. At the day of experiment, the animals were mildly anaesthetized under isoflurane and were administered to the tested treatments as follow:

Group 1: intranasal (i.n) aqueous solution of ZT equivalent to 3 mg/kg body weight (10 μL in each nostril).

Group 2: intravenous (i.v) aqueous ZT solution equivalent to 3 mg/kg body weight was injected through the tail vein of the rats.

Group 3: MNE1 ZT equivalent to 3 mg/kg body weight (10 μL in each nostril).

Intranasal administration (i.n) was done using a micropipette (10 μL, fixed) (Robfield-Gmbtt Kobenicker, Strabe 320 Deutsch Land) attached with the polyethylene tube (0.1 mm) and was performed on rats laid on their backs in a slanted position (Abed-Alaal et al., 2016). The procedure was performed gently, allowing the animals to inhale all the preparation.

The experiments were done for 8 h, at each predetermined time intervals, 0.25, 0.5, 1, 2, 4, and 8 h, three rats were sacrificed, blood samples taken by cardiac puncture (Tubesha et al., 2013) and collected into-heparinized test tube then centrifuged at 1000 rpm for 20 min to separate plasma. On the other side, brains were dissected, washed three times with normal saline, made free from any adhering tissues or fluids and weighed. Each brain tissue was homogenized with normal saline using tissue homogenizer (Thomas® Scintifica, USA) at 20,000 rpm for 5 min to a final concentration of 10% w/v. Plasma samples and brain homogenates were stored at −20 °C until use.

3.1. ZT HPLC chromatographic determination (Awari et al., 2013)

Zolmitriptan detection was done using High performance liquid chromatography (Agilant 1100) equipped with an HPLC solvent delivery pump (model M-6000a), a sample injector (Model U6 K) and a UV detector with an 8-μl flow cell and 254 nm filter (Model 440). The column used was C18 reverse-phase HPLC column (Hibar, 250 × 4.6 mm, 5 μ). The mobile phase consisted of methanol: 0.01 M di-potassium hydrogen orthophosphate pH 3.2 (23:77, v/v). Detection was done at ⩘max 231 nm at flow rate of 1 mL/min. ZT standard stock solution was prepared by dissolving 10 mg of ZT in 10 mL methanol. ZT stock solution was diluted with the mobile phase to suitable concentrations to obtain series of standard solutions.

Blank plasma (200 μL) was added to 300 μL ZT solution in a centrifuge tube. To this mixture, 200 μL of 10% perchloric acid was added and centrifuged at 4000 rpm for 15 min from the supernatant, 20 μL was injected into the HPLC system and the peak area was determined where the retention time was found to be 6.2 min. Calibration curve of ZT in plasma was constructed by plotting peak area against ZT concentration. In the same manner such as plasma, calibration curve of ZT in brain homogenates was constructed.

3.2. Pharmacokinetic and bio-distribution study

Concentrations of ZT in plasma and brain samples were determined. Different pharmacokinetics parameters after i.v and i.n administrations were calculated by WinNonlin software program (Ver. 1.5, scientific consulting Inc., Cary, NC) by non-compartment analysis (Authier et al., 2016).

3 Brain targeting with ZT after intranasal administration was estimated by calculating the drug targeting efficacy (DTE%) and nose to brain direct transport percentage (DTP%) (Kumar et al., 2008; Md et al., 2013). The higher the DTE is, the further degree of ZT targeting to brain can be expected.

Drug targeting efficiency (DTE%)

$$DTE\% = \left(\frac{\left(\frac{AUCbrain}{AUCblood}\right) i.n}{\left(\frac{AUCbrain}{AUCblood}\right) i.v.} \right) x100 \quad (2)$$

Nose-to-brain direct transport (DTP%)

$$DTP\% = \left(\frac{Bi.n. - Bx}{Bi.n.} \right) x100 \quad (3)$$

Where Bx = (B i.v/P i.v) x P i.n, Bx is the brain AUC fraction contributed by systemic circulation through the BBB following intranasal administration.

B i.v is the AUC0–240 (brain) following intravenous administration.

P i.v is the AUC0–240 (blood) following intravenous administration.

B i.n is the AUC0–240 (brain) following intranasal administration.

P i.n is the AUC0–240 (blood) following intranasal administration

3.3. Histopathological study

Studying the effect of the seleted ZT nanoemulsion formulation, MNE1, on the integrity of the nasal mucosa in mice was done according to the method described by Abdel-Bar et al. (2013).

Adult male (15) Sprague-Dawley rats weighing 200–220 g were divided into three groups and subjected to intranasal administration of 10 μL/nostril after mild anesthesia as follows:

Group 1: phosphate buffer pH 6.4 (negative control). Group 2: Isopropyl alcohol (positive control). Group 3: nanoemulsion formulation (MNE1).

Dosing was done once daily for two weeks after which the rats were sacrificed by cervical dislocation and the nose was dissected and suspended in 10% v/v formalin solution and embedded in paraffin. Approximately 4–5-μm thick sections of nasal mucosa were taken. The sections were treated using standard histopathologial techniques, stained with hematoxylin and eosin dye and observed under × 400 magnification using a light microscope (Motic® BA210, USA).

3.3.1. Statistical analysis

Data of all the experiments were expressed as the mean value ± SD. Statistical data were analyzed by one-way analysis of variance (ANOVA) and $P < 0.05$ was considered to be significant with 95% confidence intervals.

4. Results

4.1. Spectrophotometric determination of ZT in methanol

After investigation of the interference effect of oils, surfactants and co-surfactant with spectrophotometeric determination of ZT against methanol, calibration curve of ZT at ⩘max = 223 nm was constructed with correlation co-efficient ($r^2 = 0.9998$) and linear correlation in the range of 50–800 μg/mL of ZT.

4.2. Solubility study

Solubility of ZT in different oils, surfactants and cosurfactants is shown in Fig. 1. ZT has highest solubility in Capryol PGMC (20.54 ± 2.2 mg/mL) and Capmul MCM EP (17.69 ±2.1 mg/mL) with no significant difference between each other (P-value = 0.1832) among the tested oils, highest solubility in Kolliphor® RH 40 (38.64± 1.4 mg/mL) and Tween 80 (37.20 ± 2 mg/ mL) with no significant difference between each other (P-value = 0.055) among the tested surfactants, and the highest solubility in Transcutol®-P (31.56 ± 2.1 mg/mL) among the screened cosurfactants.

4.3. Pseudoternary phase diagram construction

One phase diagram was constructed for each surfactant: cosurfactant ratio, Fig. 2. Increasing the ratio of Smix from 1:1 to 2:1 resulted in increasing the nanoemulsion region while upon further increase to 3:1, the nanoemulsion region decreased. Smix ratio of 2:1 was chosen for further preparations.

4.4. Optimization of NE formulations and ZT-solubility in the optimized NE

According to characterization tests of the prepared nanoemulsions, Table 1, it was observed that increase the Smix ratio resulted in decrease in the average globule size of the NE with constant ratio of the oil until it gives the smallest size (54.63 nm ± 3.24) with the highest ratio of Smix (50%). But this formulation was unstable upon centrifugation and become turbid. So, the formulation contain 10% oil, 48% Smix and 42% water was selected for preparation of the drug-loaded NE. ZT maximum solubility in this formulation was found to be 30.57 ± 0.68 mg/mL. High solubility gives high drug loading.

4.5. Preparation and characterization of ZT-MNE

ZT-MNE with two ratios of chitosan, 0.3 and 0.5%, were prepared along with ZT-NE without addition of chitosan, for comparative study, using ultra sonication method which is one of the high-energy methods for nanoemulsions preparation. These methods consume significant energy to make small droplets (Fryd and Mason, 2012).

PDI indicates the uniformity of droplet size distribution in the nanoemulsion system. The higher the value of polydispersity, the lower the uniformity of globule size of nanoemulsion (Meor Mohd Affandi et al., 2011). The prepared NE and MNE formulations have low PDI, Table 2, indicating narrow size distribution and monodisperse property of the formulations (Rao and Mcclements, 2011). The formulations have acceptable drug content values and pH values compatible with the nasal pH (4.5–6.5) (Jagdale et al., 2016) which is required to prevent the nasal damage or irritation of the nasal mucosa.

NE formulation without chitosan has small negative zeta potential (ZP) which may be related to the drug particles as the used surfactant and cosurfactant are non-ionic while those with chitosan have positive zeta potential values related to presence of the positively charged polymer chitosan.

Addition of 0.3% chitosan to the prepared nanoemulsion, formulation MNE1, has increased its viscosity, RT, and ZP while the globule size and the% transmission were un-significantly changed. Increase viscosity and zeta potential can enhance the NE formulation mucoadhesion ability and stability respectively. Further increase of chitosan to 0.5%, formulation MNE2, has significantly increased the ZP, RT, globule size, and viscosity while it decreased the% transmission. Increase the globule size and decrease the% transmission aren't in accordance with the target of preparation of highly clear nanoemulsion with smaller globule size.

4.6. Morphological characterization

Morphological characterization of the prepared MNE1 is shown in Fig. 3. It appears that the oil droplets have almost spherical shape with low size variations. Also, the globule size extracted from the Transmission Electron Microscope is highly comparable to the z-average globule size of formula MNE1, Fig. 3.

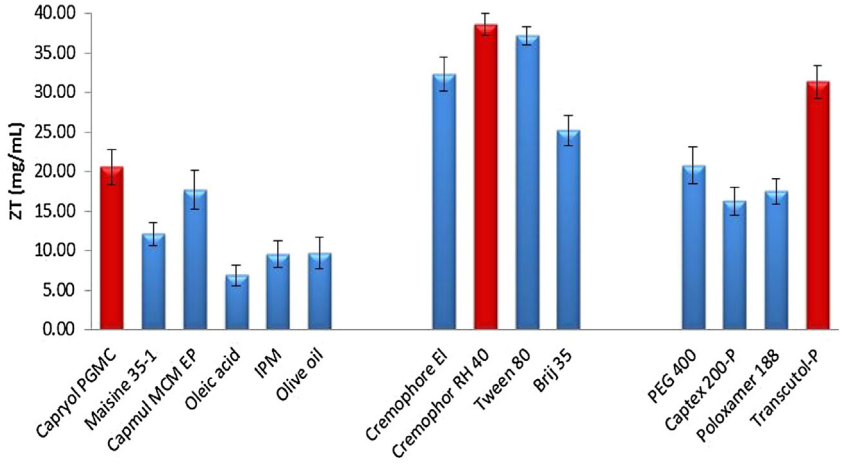

Fig. 1. Solubility of ZT in different oils, surfactants and cosurfactants.

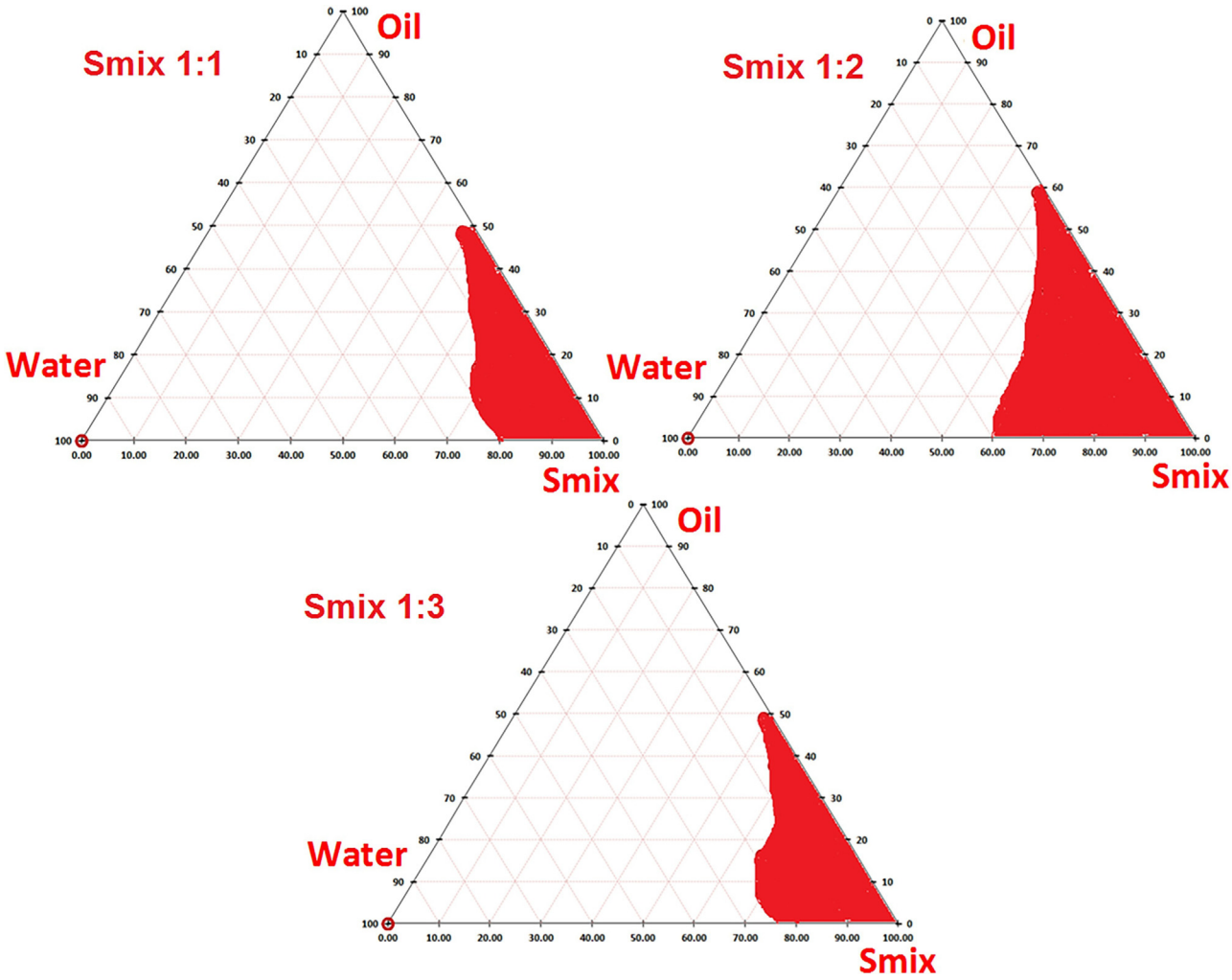

Fig. 2. Pseudoternary phase diagrams for different surfactant and co-surfactant ratios.

Table 2
Composition and physicochemical characterization of ZT loaded MNE systems.

Composition and characterization	NE	MNE1	MNE2
ZT (mg/mL)	30	30	30
Chitosan (mg/mL)	–	0.3	0.5
Zeta potential(ZP) (mV)	−0.086 ± 0.014	5.21 ± 0.85	8.42 ± 0.73
Globule size (nm)(z-average)	42.93 ± 1.53	43.45 ± 1.89	60.53 ± 2.34
PDI%	0.17 ± 0.01	0.18 ± 0.02	0.21 ± 0.02
% T	99.58 ± 0.82	99.65 ± 1.24	96.46 ± 0.86
Viscosity (mPa s^{-1})	50.47 ± 1.61	100.86 ± 2.31	119.37 ± 2.63
Drug content (%)	100.53 ± 0.38	99.83 ± 0.67	99.57 ± 0.72
pH	6.5 ± 0.24	6.3 ± 0.32	6.7 ± 0.27
Residence time (RT) seconds	7 ± 2	26 ± 4	42 ± 5
Permeability coefficient (cm/h)[a]	0.344 ± 0.013	0.3815 ± 0.011	0.3708 ± 0.014

Values are expressed as mean ± SD, n = 3.
[a] Permeability coefficient of ZT solution = 0.0517 ± 0.0032.

4.7. Ex-vivo permeation study

Permeation profiles of prepared NE and MNE along with ZT solution are represented in Fig. 4 and their permeability coefficient values are collected in Table 2. It was observed that the drug solution showed the lowest permeability coefficient (0.052 ± 0.002 cm/h) and lowest cumulative amount permeated after 6 h (16.83% ± 2.54) compared to the NE formulations ($p < 0.05$) which confirmed that formulation of ZT in nano-emulsion form has enhanced its permeability.

Formulations MNE1 and MNE2 with 0.3% and 0.5% MNE2 chitosan showed significant higher ZT permeability coefficient than formula without chitosan NE, with no significant difference between each other. Formula MNE1 was selected for studies as it has optimized physical properties and permeation through the nasal mucosa.

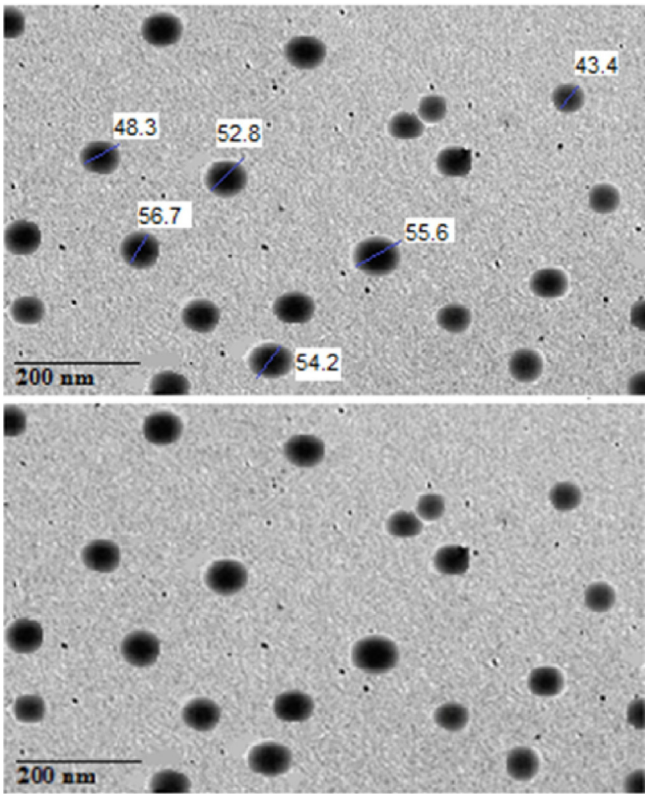

Fig. 3. Transmission Electron microscope photos of zolmitriptan MNE1 formulation.

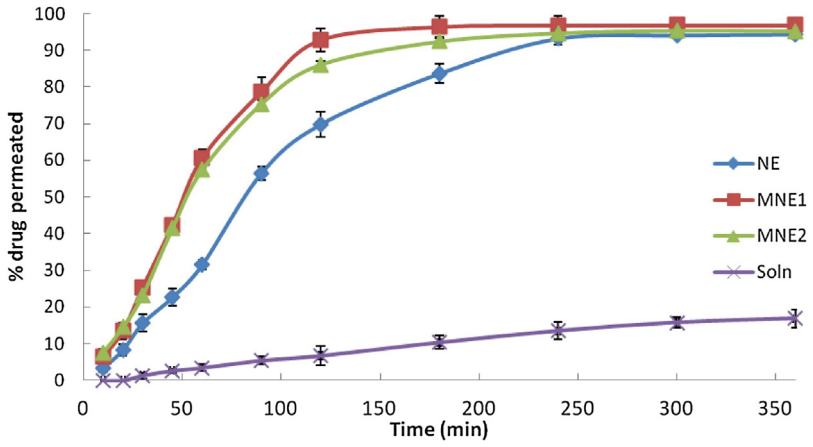

Fig. 4. Cumulative amount permeated of ZT from different formulations across nasal mucosa.

4.8. Stability study

Stability of nanoemulsion can be checked through% transmission measurements as transmission acts as a function of droplet size and droplet size distribution (Choudhury et al., 2014). No significant difference (P-value < 0.05) was found either in drug content nor% transmission of the stored MNE1 formulation, Table 3, indicating good stability at these storage conditions.

Table 3
Stability results of MNE1 formulation after storage for 1, 3, and 6 months at room temperature.

Physicochemical characterization	Fresh MNE1	1st Month	3rd Month	6th Month
% Drug content	99.83 ± 0.67	99.75 ± 0.42[a]	99.32 ± 0.53[a]	99.04 ± 0.48[a]
% Transmission	99.65 ± 1.24	99.63 ± 1.33[a]	99.20 ± 1.05[a]	98.84 ± 1.22[a]
Turbidity or phase separation	No	No	No	No

Data are expressed as mean ± SD, n = 3.
[a] Non-significant difference from the values of fresh formulation.

4.9. In-vivo biodistribution studies

ZT concentrations in plasma and brain at different time intervals after administration of different dosage forms to mice are shown in Fig. 5(A & B) while its pharmacokinetic parameters as well as DTE (%) and DTP are reported in Table 4.

It was observed that the mucoadhesive nanoemulsion formulation of ZT (MNE1) have significant higher C_{max} and $AUC_{(0-8)}$ with shorter T_{max} than both the i.v and i.n solution of ZT. As a result, MNE1 has higher DTE% and DTP% than the ZT nasal solution. Higher $AUC_{(0-8)}$ of ZT after i.v administration in plasma than brain indicates high distribution of the drug into the plasma while lower concentrations can pass to the brain by passive diffusion in longer time. High values of DTE% and DTP% for ZT MNE1 formulation prove the ability of the formula to transport ZT direct to the brain with higher concentrations and more rapid onset of action.

4.10. Histopathological study

The nasal mucosal tissues of the −ve control group showed normal nasal mucosa (Fig. 6A), while the mucosal tissues of the +ve group (isopropyl alcohol group) exhibited hyperplasia of epithelial lining with nuclear changes including pleomorphism, margination of chromatin, enlargement of nucleoli and mitotic figures appearance (Fig. 6B). In comparison to the −ve and +ve groups, the nasal mucosal tissues after application of the mucoadhesive nanoemulsion formulation MNE1, preservation of the ciliated respiratory epithelium was obvious (Fig. 6C).

5. Discussion

For nasal drug delivery, it is important to select all formulation excipients to be safe, non-irritating and non-sensitizing. For nanoemulsion, drug solubility in the oil phase is important to maintain drug in the solubilized form and thus the solubilizing capacity of an oily phase is the perspective consideration regarding oil selection (Mahmoud et al., 2013). Higher solubility is required to decrease the amount of oil used and thus don't require higher amounts of the surfactant. So, Capryol PGMC was selected to achieve maximum solubility of ZT in the oil phase.

In general, non-ionic surfactants are less toxic than ionic ones, so all surfactants and cosurfactants used in this study were of non-ionic grads. ZT was found to have high solubility in Kolliphor® RH 40, with hydrophile − lipophile balance (HLB) value lies between 14 and 16, as surfactant. It is previously known that the stability of the emulsion is affected by the HLB of the surfactant and the oil/surfactant ratio. An HLB range of 10–15 has been suggested for stable emulsions with finer droplet diameter (Matsaridou et al., 2012). It was also stated that surfactants with high (HLB) values were reported to produce fine nanoemulsions when they are introduced in aqueous environment (Umeyora et al., 2016).

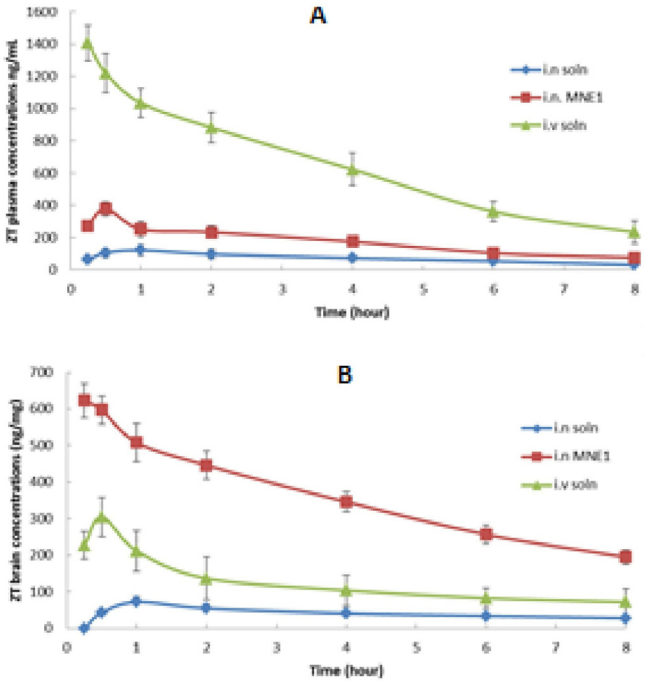

Fig. 5. ZT concentrations in mice after different formulations: (A) Plasma concentrations, (B) Brain concentrations.

Table 4
ZT pharmacokinetic parameters in plasma and brain.

Formula	C_{max} (ng/g)		T_{max} (min)		$AUC_{(0-8)}$ (ng/g.hr)		DTE%	DTP%
	Plasma	Brain	Plasma	Brain	Plasma	Brain		
MNE1	$779 \pm 45^{\#}$	$532 \pm 52^{*,\#}$	30	$15^{*,\#}$	$1384 \pm 132^{*,\#}$	$2831 \pm 163^{*,\#}$	$1064.77^{\#}$	$90.61^{\#}$
i.n ZT solution	543 ± 49	$194 \pm 37^{*}$	30	30	$581 \pm 65^{*}$	$331 \pm 57^{*}$	296.49	66.27
i.v. ZT solution	–	306 ± 43	–	30	5108 ± 204	983 ± 108	–	–

Data of C_{max} and $AUC_{(0-8)}$ are expressed as the mean ± SD, n = 6.
*Significant difference ($P < 0.05$) from the i.v. solution.
#Significant difference ($P < 0.05$) from the i.n. solution.

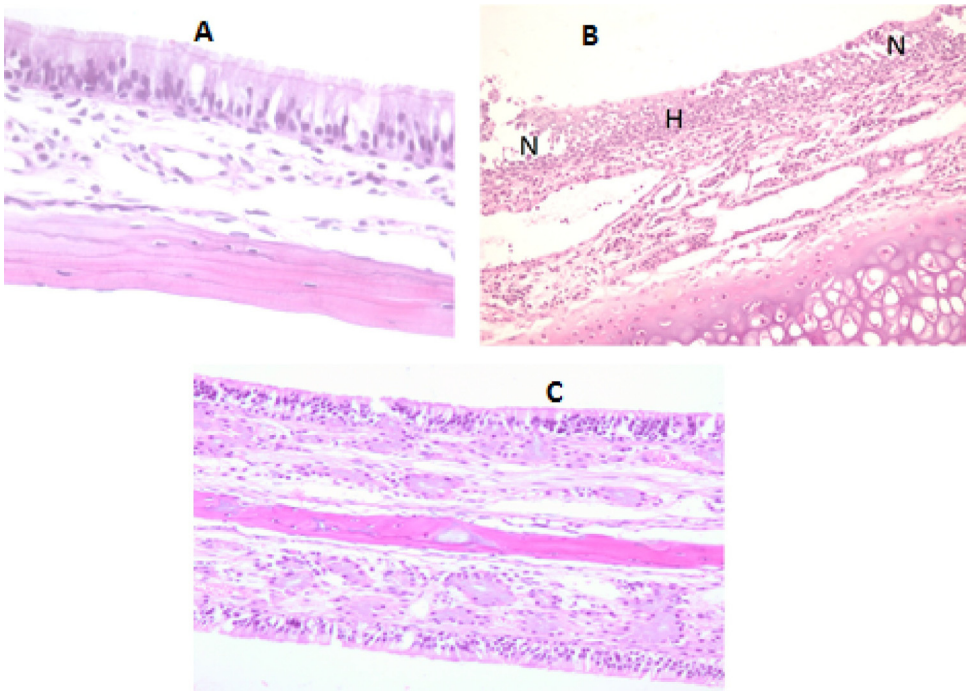

Fig. 6. Histopathological examination of the nasal mucosa: (A) Nasal mucosa of the −ve control showing normal nasal mucosa, (B) Nasal mucosa of the +ve group showing hyperplasia (H) and necrosis of nasal mucosa (N), (C) Nasal mucosa of the MNE1 group showing preserved ciliated respiratory epithelium.

ZT was found to have the highest solubility in Transcutol®-P, with HLB value of 4, as cosurfactant. To construct nanoemulsion with wide variety of compositions, addition of co-surfactant to the surfactant is required in order to minimize the mixing stress of the interface and gives the interfacial film sufficient flexibility by penetrating into the surfactant film creating void among surfactant molecules and leading to improved entrapment and solubilization of the drug molecules (Kawakami et al., 2002; Mahmoud et al., 2013).

As co-surfactant have amphiphilic nature, they can accumulate at the interfacial layer and decrease the interfacial tension, the surface energy per unit area, which gives them the ability to increase the fluidity of the interfacial film and penetrate the surfactant monolayer (He et al., 2010; Patel et al., 2013). This action of cosurfactants allows the oil phase to penetrate into the hydrophobic region of the surfactant monomers (Warisnoicharoen et al., 2000). Nanoemulsion formulation was selected with the oil, surfactant and co-surfactant in which ZT has the highest solubility in order to provide the maximum drug loading of the drug.

Increasing the surfactant concentration relative to the cosurfactant enhanced water penetration into oil droplets but further increase causes interfacial disruption and ejection of oil droplets into the aqueous phase and formation of lesser micells resulting in decreasing the nanoemulsion region (Shah et al., 2014). Also, the high oil-surfactant interaction may lead to increased adsorption of surfactants onto the oily droplets rejecting the water particles (Umeyora et al., 2016).

4 According to the biopharmaceutical classification system, ZT belongs to class III which has high solubility and low permeability (Prajapati et al., 2015). For these drugs, they are solvated fast but the permeation remains the rate limiting step of their absorption (Zhou and Qiu, 2009).

Higher efficient permeation of the NE formulations over the drug solution explained by small particle size, larger surface area of the NE and presence of surfactants which enhance drug permeation through the mucosa (Mahajan et al., 2014; Pangeni et al., 2014). All NE formulations contain Transcutol®-P which has been reported to be non-toxic biocompatabile permeation enhancer (Pandey et al., 2014; Paudel et al., 2010).

Higher and faster transport of the drug from nasal MNE1 formulation to the brain is related to its small globule size which allow drug to be transported transcellulary through olfactory neurons to the brain via the various endocytic pathways of sustentacular (supporting) or neuronal cells in the olfactory membrane (Mistry et al., 2009; Shah et al., 2013). There are two factors which characterize the mucoadhesive nanoemulsion from ZT aqueous nanosized nasal spray which are presence of surfactant and cosurfactant in the MNE1 formulation acting as solubilizing agents and penetration enhancers thus increase drug permeation through the nasal mucosa (Gonjari et al., 2009; Pardeshi and Belgamwar, 2013) and addition of chitosan which can enhance the contact between the formulation and the nasal mucosa resulting in longer nasal clearance time and prolonged residence time (Aspden et al., 1997; Aspden et al., 1997; Maggio, 2014). Also, it was reported that chitosan is a positively charged mucoadhesive agent that forms hydrogen bond with the negatively charged nasal mucosa (Illum, 2002; Wydro, 2007) and can produces a transient opening of tight junctions of confluent Caco-2 cells (Artursson et al., 1994) which enhanced ZT permeation through the nasal mucosa in accordance with the results previously obtained by Naik and Nair (2014). Furthermore, chitosan is a biodegradable polysaccharide that is known by its nontoxicity, biocompability and biodegradability. It is broken down in the human system to harmless products (amino sugars) which can be easily absorbed (Li et al., 2016).

Preserved normal nasal mucosa in mice after application of the formulation MNE1 for 14 days in continuous way indicated its safety and absence of cilio-toxic effect of the formulation on the nasal mucosa.

6. Conclusion

In the present work, zolmitriptan mucoadhesive nanoemulsion was successfully prepared and characterized to be suitable for use

by the intranasal delivery. The prepared nanoemulsion showed small average globule size, long residence time and enhanced drug diffusion through the nasal mucosa. In-vivo testing of the formula in mice resulted in enhanced direct drug delivery to the brain with higher drug concentration and faster onset of action than the intravenous or the nasal solution. The formula showed high DTP% and DTE% resulting in high bioavailability of the drug in the brain. In addition, it showed no abnormality in the nasal mucosa of mice after 14- day application. From the results, it seems that formulation of zolmitriptan as mucoadhesive nanoemulsion is a promising drug delivery system to enhance its bioavailability and treatment efficacy.

Conflict of interest

The authors report no conflicts of interest in this work.

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