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Preparation and evaluation of transnasal microemulsion of carbamazepine

Sheetal Porecha Acharya [a,*], *K. Pundarikakshudu* [a], *Aashish Panchal* [a], *Anita Lalwani* [b]

[a] *L. J. Institute of Pharmacy and Research Centre, Ahmedabad, Gujarat 382210, India*
[b] *Kadi Sarva Vishwavidyalaya, Sector 15/23, Gandhinagar, Gujarat, India*

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ABSTRACT

The objective of this study was to develop novel transanasl microemulsion containing carbamazepine for treatment of epilepsy. Oleic acid was selected as oil while Tween 80 and propylene glycol were selected as surfactant and cosurfactant respectively based on solubility results. Optimized ratio of Tween 80: propylene glycol was selected after developing pseudoternary phase diagrams for different ratio and microemulsions were prepared. The prepared microemulsions were evaluated for globule size, viscosity, pH, conductivity and % transmittance. *Ex-vivo* diffusion study for optimized microemulsion was performed through sheep nasal mucosa wherein diffusion flux and permeability coefficients were determined. Further pharmacodynamic performance was evaluated in rats by electrically induced seizures. It was found that optimized microemulsion was stable and transparent with average globule size of 190 nm and diffusion flux of 75.77 μg cm^{-2} min^{-1} and showed no toxicity during histopathological evaluation on sheep nasal mucosa. Pharmacodynamic evaluation also indicated lesser intensity of seizures in rats treated with optimized formulation in comparison to rats treated with oral carbamazepine microemulsion and nasal carbamazepine solution which suggested carbamazepine transnasal delivery system as an effective alternate therapy for treatment of epilepsy.

1. Introduction

Status epilepsy is a serious neurological emergency characterized by severe bouts of seizures. It requires rapid termination of seizure activity because if the episode of epilepsy remains untreated, it may lead to a permanent damage to the brain [1]. About 50 million people worldwide suffer from epilepsy and nearly two out of every three new cases are reported in developing countries. Epilepsy is more likely to occur in young children or people over the age of 65 years however it may occur at any age.

Treatments of epilepsy include treatment with antiepileptic drugs or surgery. Carbamazepine (CBZ) is a major antiepileptic drug for the treatment of different form of seizures [2]. Currently CBZ is available only in the form of oral dosage forms including tablets, capsules, suspensions etc. The major

* *Corresponding author*. Tel.: +91 9427844662; fax: +91 79 26890512.
E-mail address: sheetaltarak@gmail.com (S.P. Acharya).

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limitation with CBZ oral formulation is its slower and erratic absorption and thus a novel formulation of carbamazepine to overcome the stated limitation is mandatory [3].

In recent years, systemic drug delivery through nasal route has received a lot of attention, because of its advantages including rapid absorption, avoidance of hepatic first-pass metabolism and the ability for preferential drug delivery to brain via the olfactory region [4,5]. In comparison with oral administration, intranasal drug delivery may provide an improvement in bioavailability and rapid onset of action [6]. Thus, it is expected that intranasal delivery of CBZ may also achieve rapid onset of action and improved bioavailability by avoiding the first-pass effect in the liver and intestine. CBZ transnasal formulations are not available in market. Some researchers have worked on CBZ nasal formulations but thorough characterization with pharmacodynamic studies has not been reported by the researchers as per the authors' knowledge [7].

CBZ aqueous solubility is very poor. Hence solubility enhancement is necessary for intranasal delivery of CBZ as nasal delivery cannot permit administration of large volumes of liquids. Microemulsion (ME) seems to be convincing approach for administration of CBZ. ME is defined as a thermodynamically stable and transient dispersion consisting of oil, surfactant, cosurfactant and aqueous phases [8]. The advantages of ME as a drug delivery system are the enhancement of drug solubilization and absorption across mucosal membranes due to its lipophilic nature and smaller globule size [9].

The objective of this investigation was to prepare and optimize transnasal CBZ microemulsion by using various physicochemical parameters including globule size, % transmittance, pH, viscosity, etc. Optimized ME was further evaluated for *ex-vivo* diffusion study through sheep nasal mucosa. Pharmacodynamic activity of CBZ ME was evaluated in rats after inducing seizures electrically.

2. Materials and methods

2.1. Materials

Carbamazepine was donated by Lincoln Pharma, Ahmedabad, India. Capmul MCM (glyceryl monocaprylate) and Labrafac (propylene glycol dicaprylocaprate) were obtained as gift samples from Gattefosse. Oleic acid, Tween 80, Tween 20, Span 80, polyethylene glycol (PEG 400) and propylene glycol (PG) were purchased from Sigma Aldrich. All other chemicals were of analytical grade and purchased commercially. Double distilled water was used throughout the study.

2.2. Determination of solubility of drug

The solubility of CBZ in various components (oils, surfactants, and cosurfactants) was determined by adding an excess of (1 g) CBZ to each cap vial containing 5 ml of the selected vehicles. The mixture was heated at 40 °C in a water bath to facilitate the solubilization. Formed suspensions were then stirred for 48 h on magnetic stirrer. Then each suspension was centrifuged at 3000 rpm for 5 min, and supernatant was taken and diluted with methanol and CBZ was quantified by UV spectrophotometer (UV 1800, Shimadzu) at wavelength of 284 nm.

2.3. Preparation of pseudoternary phase diagram

Pseudoternary phase diagrams were constructed to obtain the appropriate ratio of surfactant: cosurfactant which can result in to large existence of ME area. They were constructed using water titration method. Surfactant (Tween 80) and cosurfactant (propylene glycol) were mixed (Smix) in different weight ratios (1:1, 2:1 and 1:2). Oil (oleic acid) and Smix (Tween 80 and propylene glycol) were mixed thoroughly in different weight ratios from 1:9 to 9:1 in different glass vials and diluted with distilled water in a drop wise manner till it changed from transparent to opaque. By joining the change points, the boundaries of phases formed were obtained in the phase diagrams. All samples exhibiting a transparent and homogeneous state were assigned to an ME region, a monophasic area, in the phase diagram [10]. The pseudoternary phase diagrams were constructed by using CHEMIX software.

2.4. Physicochemical characterization of carbamazepine loaded MEs

Percentage transmittance of each sample was determined at 630 nm using distilled water as reference. One drop of MEs was placed on slide and refractive indices of MEs were measured by using Abbe refractometer (ELICO, India). Isotropic nature of MEs was verified by placing a drop of ME on slide with cover slip on it and observing it under polarized light using polarizing microscope (Carl Zeiss, Germany). Viscosity of the MEs was measured by a Brookfield viscometer at room temperature by using LV III spindle. Electrical conductivity of ME was measured using a conductivity meter (CM 180 ELICO, India) at ambient temperature and the pH of ME was measured by using pH meter (Systronic 335, India). Droplet size and Zeta potential distribution was measured using Malvern zetasizer (Nano ZS, Malvern Instruments, UK). Each sample was suitably diluted five times with filtered distilled water and placed in a disposable zeta cell [11]. Samples were centrifuged at 3000 rpm for 15 min to determine centrifugation stability. Experiments were performed in triplicate for each sample.

2.5. Ex-vivo diffusion studies

The use of natural membranes is very important for predicting the potential drug release characteristic. Freshly excised sheep nasal mucosa was obtained from slaughter house and dipped immediately in phosphate buffer (pH 6.4). Cartilages were removed properly and the mucosal membrane was isolated and washed with phosphate buffer (pH 6.4). *Ex-vivo* drug diffusion study was performed using a Franz-type diffusion cell with a diameter of 10 mm and mucosa thickness of 0.20 mm. The tissue was stabilized in phosphate buffer (pH 6.4). The receptor compartment was filled with 10 ml diffusion media (phosphate buffer pH 6.4 + 30% PEG 400) to maintain perfect sink condition while 2 ml ME (30 mg/ml) was placed in donor compartment. Continuous slow stirring was

maintained in receptor compartment. Similarly *ex-vivo* diffusion of pure drug was conducted by placing 2 ml of drug solution in PEG 400 (30 mg/ml). Samples from the receptor compartment were withdrawn at periodic time intervals, filtered through 0.45 μm nylon filter paper and analyzed using a UV–Visible spectrophotometer at 284 nm. Each removed sample was replaced by an equal volume of diffusion medium. The cumulative amount of CBZ permeated through the skin was determined by the following equation [12]:

$$Q_n = \frac{C_n \times V_o + \sum_{i=1}^{n-1} C_i \times V_i}{S}$$

Where C_n is the CBZ concentration of receptor medium after each sampling time, C_i is the CBZ concentration for i sample, V_o and V are the volumes of the receiver solution and sample, respectively, and S is the effective diffusion area. The steady state flux (J_{ss}) was calculated from the slope of the steady state portion of the line in the plot of drug amount permeated V_s time (min) [13]. Permeability coefficient (K_p) was calculated by dividing the flux with concentration of the drug in ME.

2.6. Nasal ciliotoxicity studies

The method described by Sheetal et al. was used for this study; pieces of freshly excised sheep nasal mucosa with a thickness of 0.2 mm were exposed to CBZ ME for 2 h followed by thorough rinsing with PBS pH 6.4. In two other different sets of experiments isopropyl alcohol (a strong mucociliary toxin) and PBS pH 6.4 were used instead of CBZ ME for arriving at a comparative analysis of the extent of damage caused by the preparation. These pieces of mucosa were fixed in paraffin blocks and fine sections were taken that were stained by eosin and hematoxylin. The prepared slides were examined with an optical microscope (Olympus, Model BX10, Japan) and photomicrographs (magnification 400×) were taken. Similar procedure was used in our previous study [14].

2.7. Pharmacodynamic studies

Maximal Electroshock:Sprague Dawley rats weighing between 200 and 250 g and exhibiting clear hind limb extension phase during electrically induced convulsions were included in the present study. The experimental protocol was approved by the Institutional Animal Ethics.

Committee (No. LJIP/IAEC/09/2011–2012). Rats were divided into 5 groups ($n = 6$). The first and second groups were treated intranasally with CBZ solution (60% PEG 400) and CBZ ME respectively containing CBZ equivalent to 8.18 mg/kg body weight (using a micropipette attached with low-density polyethylene (LDPE) tubing, having 0.1 mm internal diameter at the delivery site). In the third group CBZ solution containing CBZ equivalent to 8.18 mg/kg body weight was administered intraperitoneally (IP) while fourth group was treated with oral CBZ ME containing CBZ equivalent to 8.18 mg/kg body weight. The fifth group, not subjected to any treatment, served as control. Electroconvulsions were produced by applying current (150 mA, 0.2 s) through ear clip electrodes using electroconvulsiometer (INCO, Ambala, India) after 60 min of administration of formulations and different phases of seizures were measured. Briefly after application of current an immediate severe tonic phase (E phase) was observed which was characterized by maximal extension of the anterior and posterior legs. At the end of tonic phase clonic phase starts which was characterized by paddling movement of the hind limb and shaking of body. During stupor phase which was observed after tonic and clonic phase rat remained silent without any movement. Recovery time was recorded as total time from starting of tonic phase till animal regains its normal movement [15].

3. Results and discussion

3.1. Solubility of drug

The saturated solubility of CBZ in various oils was reported in Table 1. It is important that the nasal formulation have the least volume. It can be done if the components of the ME that are chosen have highest solubility for the drug. This was analyzed using one variable at a time (OVAT) type of optimization. The solubility of the drug was determined in each component of ME sequentially (oil, surfactant and then cosurfactant). Highest solubility was observed in oleic acid. Thus oleic acid was selected as oil for preparation of ME. Among the surfactants studied, Tween 80 showed the highest solubility for carbamazepine and previous studies have reported improved nasal absorption when Tween 80 was used as one of the ingredient [16]. Thus, Tween 80, surfactant with HLB 14, was selected as a surfactant and depending on the solubility results PG was selected as cosurfactant, which also acts as permeation enhancer [16].

3.2. The pseudoternary phase diagrams

The components that showed maximum solubility were further optimized using pseudoternary phase diagram as shown in Fig. 1. The zone of ME was obtained. Six formulations were then taken from each corner at random and the best formulation was characterized thoroughly. It was found that increasing concentration of Tween 80 incorporation of water can be increased but solubility of drug decreases while by increasing concentration of PG drug solubility increase but incorporation of water decreases and highest ME area was obtained with ratio of 1:1 and thus selected for further studies. According to the ME area in the phase diagram, the carbamazepine loaded ME formulations were prepared as per the composition shown in Table 2. ME systems were obtained by mixing oil, surfactant and cosurfactant together and adding

Table 1 – Solubility of CBZ in various excipients.

Material	Solubility (mg/ml)
Sunflower oil	9.55 ± 0.25
Soybean oil	16.22 ± 0.97
Labrafac	5.46 ± 0.20
Capmul MCM	10.11 ± 0.47
Oleic acid	32.08 ± 2.54
Tween 80	29.05 ± 1.13
Propylene glycol	54.18 ± 1.76
Alcohol	51.55 ± 1.79

All values are expressed as mean of three readings.

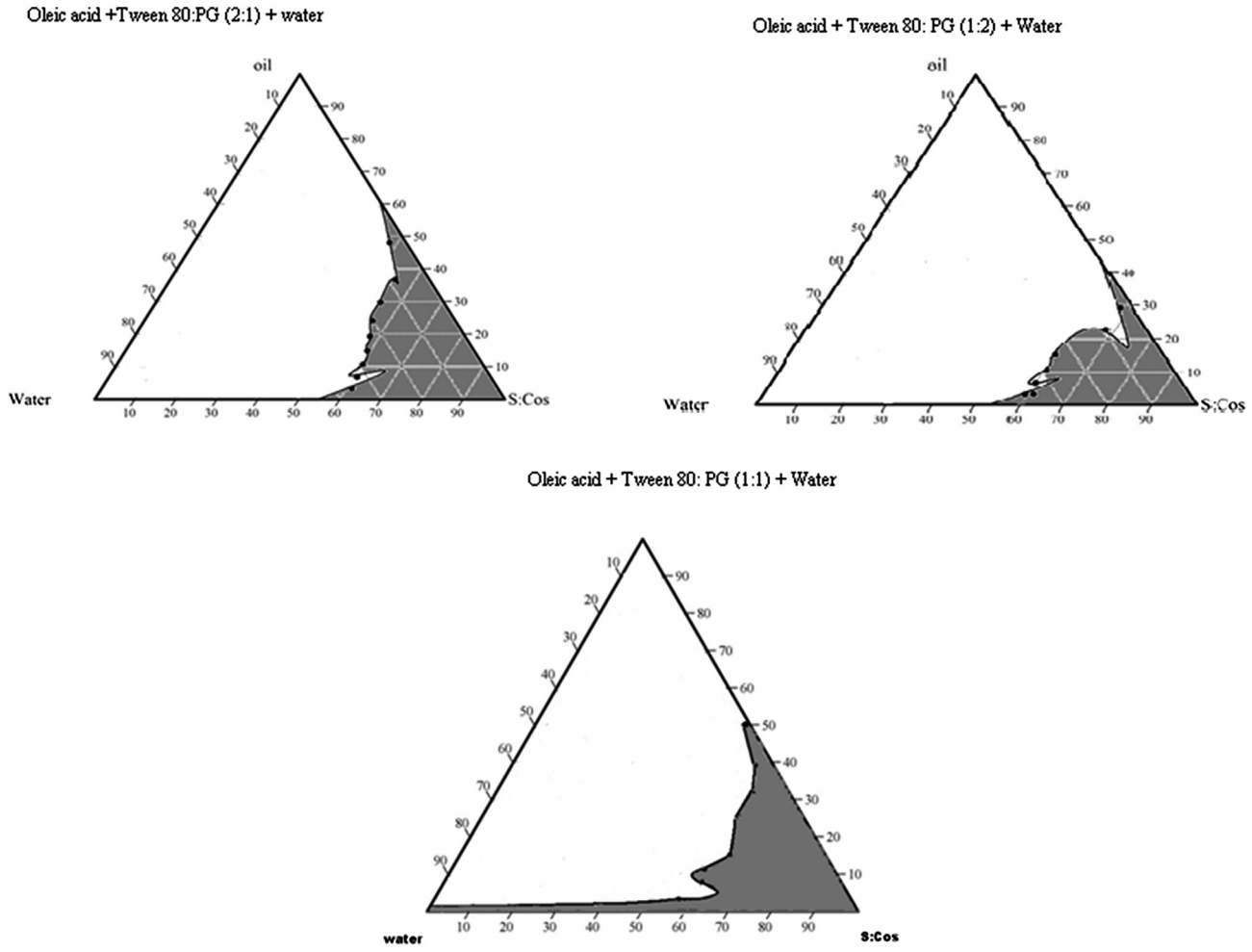

Fig. 1 – Pseudoternary phase diagram using oleic acid as oil, Tween 80 as surfactant, propylene glycol as cosurfactant and water (Tween 80: propylene glycol = 2:1, 1: 1 and 1:2).

appropriate quantity of CBZ and adding precisely distilled water drop by drop to these oily phases with magnetic stirring at ambient temperature. The final concentration of CBZ in ME systems was 30 mg/ml.

3.3. *Physicochemical characterization of carbamazepine loaded MEs*

The microemulsions for nasal administration are expected to show higher permeation rate with minimum globule size. Prepared microemulsions are expected to have good physical stability with respect to phase separation and/or flocculation.

Table 2 – Composition of selected ME.

	S1	S2	S3	S4	S5	S6
Carbamazepine (g)	3	3	3	3	3	3
Oleic acid (%)	10	10	10	15	15	15
Tween 80 (%)	32.5	35	37.5	32.5	35	37.5
PG (%)	32.5	35	37.5	32.5	35	37.5
Water (%)	25	20	15	20	15	10

This can be achieved when zeta potential values are negative The pH values have to be close to nasal secretions (4.5–6.5) and viscosity has to be moderate. pH and viscosity are important factors affecting mucociliary action which if affected may cause another set of complications. Additionally the pH deviations may cause irritation to the patient [7]. Higher viscosity is preferred as it increases residence time but permeation rate also decreases with increase in viscosity and hence formulation should have moderate viscosity. Reported values indicates viscosity in between 100 and 200 cps is suitable for nasal administration [16]. It also has been observed the formulation containing water in external phase shows less irritancy on nasal mucosa. Based on the above rationale critical quality attributes and their desirable ranges were defined as follows. i) External medium – water ii) globule size – minimum iii) pH – 4.5–6.5 iv) viscosity – 100–200 cps. The pH, viscosity, conductivity, globule size and zeta potential of the prepared formulations are shown in Table 3. Result of globule size indicated that smallest globule size was obtained with formulation S3 with PDI 0.20, which is close to zero, indicating that the prepared ME had uniform globule size and thus it was selected for further studies as faster permeation is expected when the globule size is small. The pH of the ME S3

Table 3 – Physicochemical parameters of ME.

	S1	S2	S3	S4	S5	S6
% Assay	99.66 ± 0.65	99.47 ± 0.55	99.58 ± 0.42	98.89 ± 0.33	99.27 ± 0.25	98.76 ± 0.32
pH	5.31	5.29	5.45	5.69	5.27	5.67
Globule size (nm)	370 ± 21	300 ± 15	190 ± 8	411 ± 23	375 ± 12	325 ± 10
Conductivity (ms/cm)	0.25 ± 0.04	0.25 ± 0.03	0.24 ± 0.02	0.24 ± 0.04	0.23 ± 0.04	0.22 ± 0.05
Viscosity (cps)	190 ± 5	195 ± 3	193 ± 2	177 ± 5	189 ± 2	200 ± 5
% Transmittance	98.88	99.43	99.87	99.21	99.33	99.98
Zeta potential	−0.2093	−0.1895	−0.1105	−0.4929	−0.1856	−0.1756

All values are expressed as mean of three readings.

was also near to above stated limit which indicated less chances of irritancy on nasal mucosa. The refractive index was 1.44 and % transmittance was found to be greater than 99% which confirmed that prepared CBZ ME was transparent. Isotropic nature of ME was also proved as ME appeared completely dark under polarized microscope [17]. The conductivity of the results confirmed the formation of solution type of ME with water in continuous phase. Viscosity of the optimized formulation was 193 cps which is suitable for nasal administration. Zeta potential was negative which indicated the stability of formulation as there were less chances of globules aggregation. After centrifugation cycle it was found that ME S3 was stable and no separation was observed which indicated centrifugation stability. The optimized ME S3 remained clear and transparent even after 3 months of storage. ME S3 was optimized from the prepared formulations as per the attributes decided earlier in this section. *Ex-vivo* permeation study and pharmacodynamic study was performed by using optimized ME S3.

3.4. Ex-vivo *diffusion through sheep nasal mucosa*

Ex-vivo diffusion study was performed by using sheep nasal mucosa for optimized formulation S3 and CBZ solution prepared in 60% PEG 400 and results were shown in Fig. 2). It was found that cumulative amount of CBZ permeated through sheep nasal mucosa was32744. 5 μg with a flux of 75.77 μg cm^{-2} min^{-1} and permeability coefficient was found to be 0.00253 cm^{-2} $min^{-1}$from CBZ ME while in case of CBZ solution cumulative amount of CBZ permeated through sheep nasal mucosa was 24537.06 μg with a flux of 57.26 μg cm^{-2} min^{-1} and permeability coefficient was found to be 0.00191 cm^{-2} min^{-1}. The results clearly indicated faster diffusion of CBZ from CBZ ME in comparison to CBZ solution. The diffusion is faster due to presence of oleic acid, Tween 80 and PG which act as permeation enhancers. A biphasic release profile was obtained in which initial faster release was due to solubilized drug in continuous phase while slower rate was due to CBZ release from the oil droplets. CBZ from ME permeates rapidly (more than 50% of drug in 90 min) through nasal mucosa in comparison CBZ solution (less than 40% in 90 min).

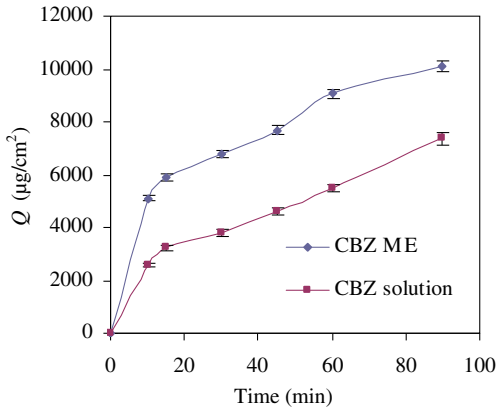

Fig. 2 – *Ex-vivo* CBZ diffusion through sheep nasal mucosa from CBZ ME and CBZ solution.

3.5. *Nasal ciliotoxicity studies*

Results of nasal ciliotoxicity studies were shown in Fig. 3. Nasal ciliotoxicity studies revealed that nasal mucosa treated with PBS pH 6.4 (negative control) showed intact epithelium layer without any necrosis while nasal mucosa treated with isopropyl alcohol (positive control mucociliary toxic agent) showed complete destruction of epithelium layer, necrosis and even the deeper tissue parts were also destroyed. CBZ ME prepared in our studies did not exhibit any toxicity as no change could be noticed in the gross morphology and histology of the nasal mucosa. Our results are on similar lines of the observations reported by other workers on toxicity of oleic acid and Tween 80 which are the major components of our preparation [18].

3.6. *Pharmacodynamic studies*

The antiepileptic activity was assessed by observing the extent of different stages of seizures including duration of seizure, extension phase (E), clonus phase (F) and stupor phase (S) and results were represented in Figs. 4 and 5. Significant differences between the control and treated groups were calculated using one way ANOVA followed by Tukey test for exact comparison in pharmacodynamic study. Significant reduction in E phase, clonus, stupor and duration of seizure was observed in the rats treated with CBZ ME by intranasal route and in comparison to group of rats treated CBZ solution administered IN and CBZ ME administered intranasally ($p < 0.05$, $n = 6$). The results clearly indicated lesser intensity

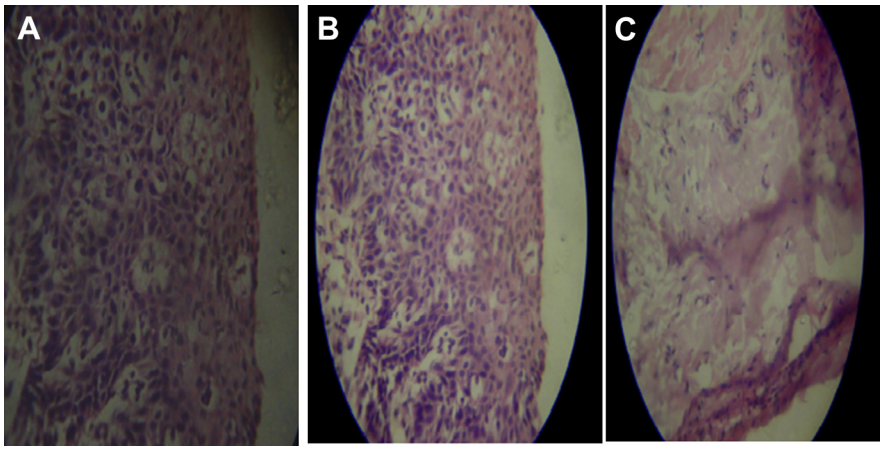

Fig. 3 – Nasal ciliotoxicity study for CBZ ME. (A) Nasal mucosa treated with PBS pH 6.4. (B) Nasal mucosa treated with CBZ ME. (C) Nasal mucosa treated with IPA.

of seizure and rapid recovery from seizures in the rats treated with intranasal CBZ ME.

4. Conclusion

The carbamazepine loaded transnasal ME demonstrated lesser intensity of seizures which may be due to larger extent of selective nose to brain delivery of drug in comparison to oral ME, oral solution and nasal solution of carbamazepine. This may help in decreasing dose and frequency of administration of drug and may possibly maximize therapeutic benefits and may also reduce cost of therapy. However detailed animal study followed by thorough clinical trials is required to establish clinical safety and efficacy of this formulation.

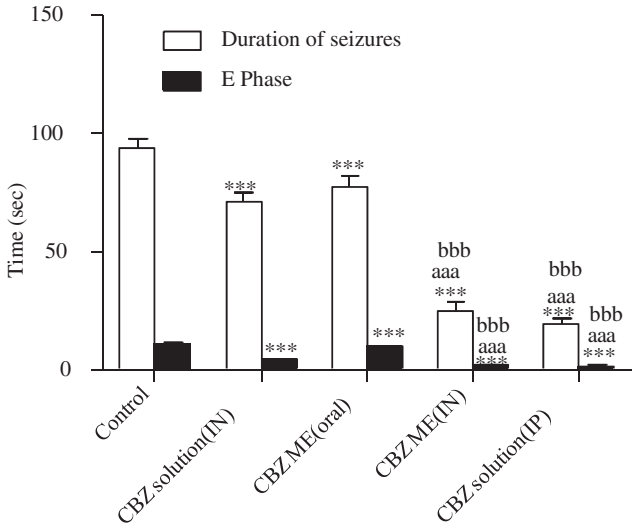

Fig. 4 – Duration of seizure and E phase for different treatments of carbamazepine where, * indicate significant difference in comparison to control, aaa indicate significant difference in comparison to CBZ solution (IN), bbb indicates significant difference in comparison to CBZ ME (oral).**

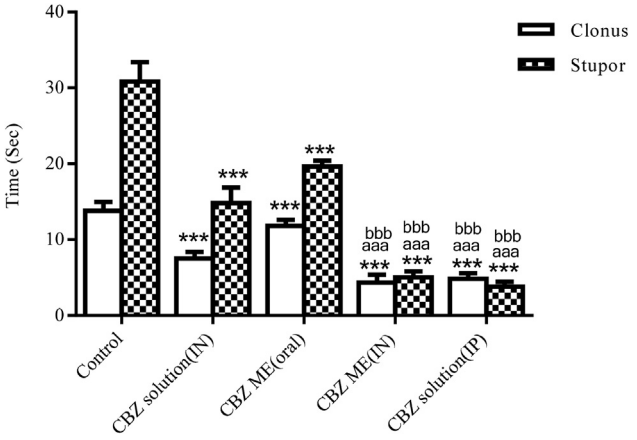

Fig. 5 – Duration of clonus and stupor phase for different treatments of carbamazepine where, * indicate significant difference in comparison to control, aaa indicate significant difference in comparison to CBZ solution (IN), bbb indicates significant difference in comparison to CBZ ME (oral).**

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