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PREPARATION AND EVUALUATION OF AGMATINE CUBOSOMES (LIPID BASE NANO SYSTEM) NOSE TO BRAIN DELIVERY FOR EPILEPSY

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ABSTRACT

This study aimed to prepare and evaluate agmatine cubosomes nose-to-brain delivery for epilepsy. Agmatine alleviates epileptic seizures and Hippocampal neuronal damage. Glyceryl-monooleate and pluronic F127 (9:1 w/w) were melted at 60°C and mixed. To prepare the cubosome dispersion, the low-viscosity homogenous melt was either added drop-wise or injected into excess water. With continuous stirring, the concentration of lipids in the sample was approximately 8–10% (w/w). The particle size and zeta potential agmatine cubosomes of F7 were found to be -270.4 nm and 12.0 mV respectively. The maximum entrapment efficiency of the agmatine-loaded cubosomes was found to be 88.92±0.33 (% w/w) for batch F7 indicating that most of agmatine was encapsulated in cubosomes. Over 12 hours, more than 95% of the agmatine released from cubosomes was released, and the drug release is 0.00 0.00, 3.80 0.54, 6.79 0.86, and 15.44 0. 39, 23.56±0.51, 26.88±0.67, 29.65±1.39, 35.78±3.66, 43.89±1.32, 52.84±1.12, 61.79±1.24, 69.38±1.32, 78.94±1.14. After nasal administration of agmatine cubosomes, onset of convulsion and duration of convulsion were found to be 611±3.3 sec and 16±5.3 sec, respectively. This result demonstrated an increased onset of convulsion and decreased convulsion duration in comparison with intraperitoneal administration of diazepam (standard).

Keywords Cubosomes, Nose to brain, PTZ induced siezures, NDDS, Epilepsy

1. INTRODUCTION

A current trend in nanomedicine and drug delivery is to improve treatment specificity to improve efficacy and avoid side effects [1]. Nanoparticles have played a significant role in this trend because they can accumulate in target tissues passively via the enhanced permeation and retention effect or actively via surface-conjugated targeting ligands [2]. Cubosomes are self-assembled lipid-based liquid crystalline nanoparticles [LCNP] with an internal cubic phase structure that have gained interest as a drug delivery system because they can be loaded with both lipophilic and hydrophilic drugs and have the potential for on-demand reversible release, which offers advantages over more commonly used liposomes [3]. Amphiphilic lipids such as phytantriol and glycerol monooleate [GMO] can self-assemble in excess water to form thermodynamically stable liquid crystalline phases such as the bicontinuous cubic phase [4]. Cubosomes can then be formed by the dispersion of the ‘bulk" liquid crystalline phase, usually with the aid of a polymer stabilizer such as Pluronic F127 or F108. The internal structure of the particles and approaches to modification for drug delivery or imaging capabilities by incorporation of other agents such as lipids, phospholipids or metallic nanoparticles have been well studied [5], as has the influence of the stabilizer [6]. The cubosomes often have the same microstructure as the bulk liquid crystalline phases but have a larger surface area and are much less viscous, enabling their potential deployment as injectable drug delivery or imaging systems [7]. The internal structure of the cubic phase particles makes them particularly interesting as MRI contrast agents, as the bound water behaves very differently to bulk water, providing a boost in relaxivity [8].

Active targeting of drug carriers is a challenge, with the most common approach involving the use of antibodies or ligands for a specific cell surface receptor. In cancer therapy, an antibody or folic acid group targeting a receptor overexpressed by the diseased cells is often conjugated to the surface of the carrier particle [9]. Potential drawbacks of these approaches include being expensive, having poor stability, a lack of specificity if the target receptor is common or can mutate in the target cells, competition with other ligands, and poor pharmacokinetic consequences for the particle after injection [10].

2. MATERIALS AND METHODS

2.1. MATERIALS

Agmatine was purchased from Alkem, Mumbai. Pluronic F127 was purchased from Loba Chemie, Mumbai. Sodium hydroxide and methanol from Hi-Media Laboratories, Mumbai. Swiss albino mice, weighing between 25-30 g, were ethically approved by the Research Ethics

Committee of KBHSS IOP (No: 1566/PO/Re/S/11/CPCSEA) which is held on 10/04/2023. The animals were kept in a standard laboratory setting at 25 1 °C with free access to food and water. The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethical Committee. All other ingredients were of analytical grade.

2.2. METHODS

Bulk cubic phases were prepared from glyceryl monooleate and pluronic F127 melt with or without the drug. Glyceryl-monooleate and pluronic F127 (9:1 w/w) were melted at 60°C and mixed. The drug was loaded by first preparing a solution in ethanol. This drug solution was then added to a previously prepared homogeneous mixture of glyceryl-monooleate and Pluronic® F127. To this mixture, 5.0 mL of ethanol was added as a hydrotropic solvent, which helps to bind the hydrophilic and lipophilic phases, to form a bi-continuous lipid bilayer. To prepare the cubosome dispersion, the low-viscosity homogenous melt was either added drop-wise or injected into excess water. With continuous stirring, the concentration of lipids in the sample was approximately 8–10% (w/w). The samples were then homogenized for 2 min in a homogenizer at 1500–2000 rpm [11,12,13].

Table 1. Composition of agmatine lipid base nano system

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8
Agmatine	100mg	100mg	100mg	100mg	100mg	100mg	100mg	100mg
Pluronic F127	2gm	2gm	2gm	3gm	4gm	5gm	5gm	5gm
Ethanol	3ml	4ml	5ml	4ml	4ml	4ml	4ml	4ml
Glycerylmonooleate	7ml	7ml	7ml	7ml	7ml	7ml	8ml	9ml
Distilled water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

2.3. CHARACTERIZATION OF CUBOSOMES

2.3.1. Particle size and polydispersity Index (PDI)

The particle size of cubosomes was determined by using Zetasizer version 6.20 (Malvern Instruments, Malvern, UK) All the prepared batches of cubosomes were viewed under microscope to study their size and polydispersity index (PDI). Size of cubosomes from each

batch was measured at different location on slide by taking a small drop of lipid nano system on it and average size and PDI of nano suspension were determined [14].

2.3.2. Zeta potential

This method is used to determine charge on empty and drug loaded cubosomes using Zetasizer version 6.20 (Malvern Instruments, Malvern, UK). Analysis time was kept for 60 seconds and average zeta potential and charge on the cubosomes was determined. The obtained value indicates that the surface of lipid nano system is dominated by the anions and proved that prepared cubosomes have sufficient charge to hold aggregation of vesicles [14].

2.3.3. The entrapment efficiency

The entrapment efficiency i.e., the Agmatine content encapsulated in cubosomes. 1 mL of cubosomes containing agmatine were added into the reservoir of Centricon® (Model: YM-100, Amicon, Millipore, Bedford, MA, USA). After centrifuging the cubosomes dispersion at 15,000 rpm for 40 min, the filtrate containing free Agmatine was removed. The filtered dispersion was then diluted with methanol and analyzed for Agmatine content using UV. The UV analysis was used to compute the total concentration of Agmatine (Ct), and the concentration of Agmatine contained in the filtrate after centrifugation (Cf). [15]

The entrapment efficiency was calculated using the following equation:

$$\text{Entrapment efficiency}(\%) = \left[\frac{C_t - C_f}{C_t}\right] \times 100$$

2.3.4. Field Emission Scanning Electron Microscopy (FE-SEM)

Field Emission Scanning Electron Microscopy (FE-SEM) FE-SEM was used to examine the shape and morphology of a cubosomes formulation (JSM-7610F, JEOL., Tokyo, Japan). The samples were made by coarsely dusting an adequate amount onto glass slides and then dropping a little drop of the lipid nano system (cubosomes)on top. These slides were then mounted on an aluminum stub and coated with a fine Platinum layer in an argon atmosphere using a cold sputter coater to a thickness of 400 A. After that, photomicrographs were taken at a voltage of 5.0 kV [15].

2.3.5. In vitro release study

The Agmatine release from the cubosomes dispersion was evaluated by measuring the diffusion of the drug across a cellophane membrane using Franz diffusion cell. The cell consisted of two compartments i.e., the donor compartment and the receptor compartment. A previously activated semipermeable membrane was placed between these two compartments. The dispersion formulation was added on to the donor compartment above the membrane. The receptor compartment contained 18 mL phosphate buffer saline solution (PBS, pH 7.4),

maintained at 37±0.5°C, as a release medium. At predetermined time intervals, aliquots of the release medium were withdrawn and replaced with an equal volume of fresh release medium. The drug concentrations in the release medium at various time intervals were analyzed using UV [16].

2.4. EVALUATION OF ANTI-EPILEPTIC ACTIVITY OF PREPARED CUBOSOMES

Swiss albino mice weighing between 20-25 g, were obtained from Wockhardt Ltd, Aurangabad. Animals were housed under standard laboratory conditions of temperature 25 ± 1°C with free access to food (Amrut rat and mice feed, Sangli, India) and water. The experiments were performed during the light cycle (12 h). The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethical Committee.

2.5. EXPERIMENTAL PROTOCOL

The animals were divided in following experimental groups, each group comprising of six animals...

Group I: Animals served as vehicle control and received saline

Group II: Animals served as toxic and received PTZ

Group III: Animals served as Standard and received Diazepam + PTZ.

Group IV: Animals served as Treated and received Agamatin + PTZ

Group V: Animals served as Treated and received Cubosomes +PTZ [17].

3. RESULTS AND DISCUSSION

3.1 FTIR ANALYSIS OF AGMATINE:

The FTIR spectrum of agmatine (Figure 1) showed the characteristic peaks of absorption at 1279 cm-1 (C-H stretching), 1388 cm-1(S=O stretching), 1662 cm- 1 (C=N stretching) 3361cm-1 (N-H stretching) and 1476 cm-1 (C-H bending) as illustrated in Table 2.

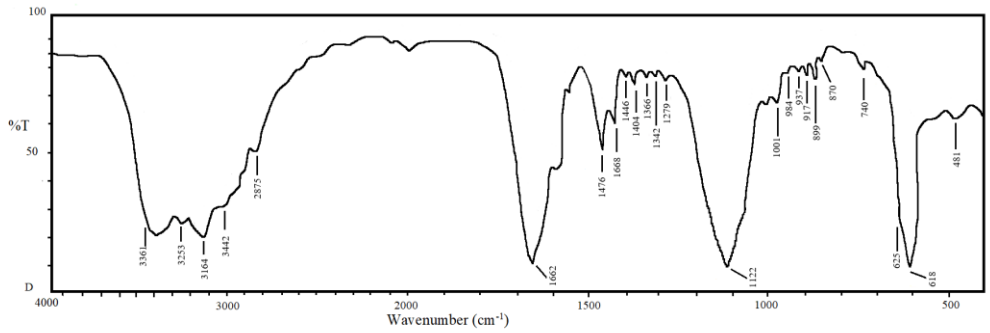

Figure 1. FTIR spectra of pure agmatine

Table 2. Frequency of functional group of pure agmatine

Sr. No.	Type of vibration	Peak (cm-1)
1	C-H stretching	1279
2	S=O stretching	1388
3	C-H bending	1476
4	C=N stretching	1662
5	N-H stretching	3361

3.2 UV SCANNING OF AGMATINE

The UV scanning of agmatine showed maximum absorbance at 334 nm (λmax) as shown in Figure 2.

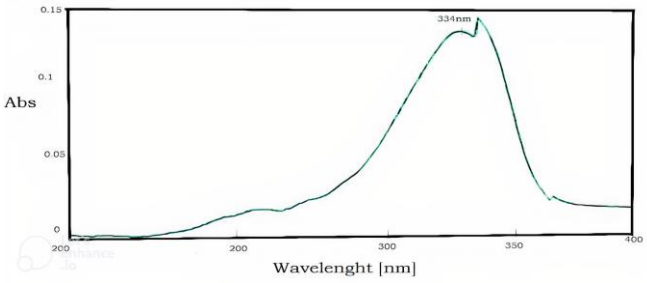

Figure 2. UV Spectrum of agmatine

3.3 STANDARD CALIBRATION CURVE OF AGMATINE

Table 3 and Figure 3 show absorbance for agmatine in pH 6.6 phosphate buffer. The absorbance was measured at λmax 334 nm in the concentration range 2–12 µg/ml. The calculation of in vitro drug diffusion, drug content and drug permeation study were based on the calibration curve. The curve obeys Beer-lambert's law within concentration range 2 -12 µg/ml in pH 6.6 phosphate buffer. The correlation coefficient (R2) value for agmatine calibration curve was 0.9978 in pH 6.6 phosphate buffer.

Table 3. Absorbance for calibration curve for agmatine

Sr. No.	Concentration (µg/ml)	Absorbance
1	2	0.011
2	4	0.026
3	6	0.043
4	8	0.057
5	10	0.072
6	12	0.089

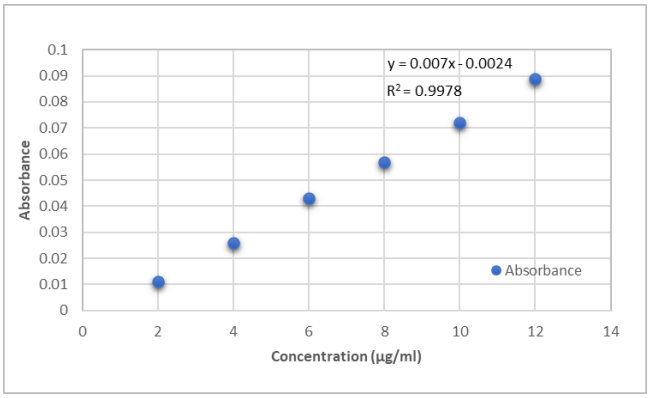

Figure 3. Calibration curve for agmatine

3.4 CHARACTERIZATION OF CUBOSOMES

3.4.1 Particle size

The particle size was determined by using Zetasizer version 6.20 (Malvern Instruments, Malvern, UK). In the current study, the size of agmatine loaded cubosomes was found to be within the nanometer range. As shown in the Figure 4, the particle size of agmatine

cubosomes of F7 was found to be 270.4 nm. This resultant particle is suitable for deposition in the nasal cavity. Smaller particles offer larger surface area.

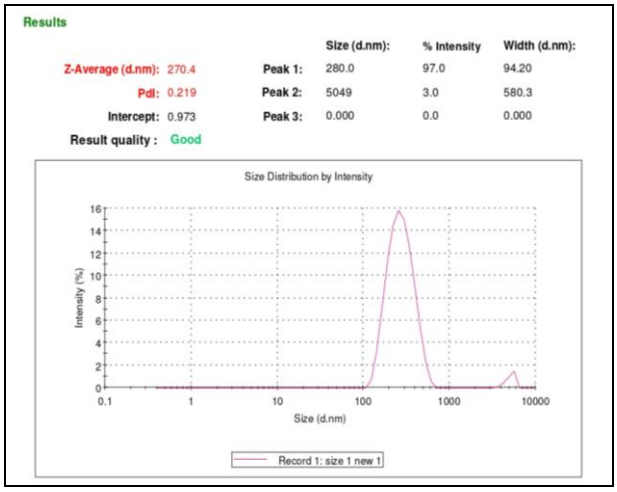

Figure 4. Particle size

3.4.2 Zeta Potential

Zeta potential analysis carried out to detect the surface charges acquired by cubosomes. High zeta potential values, either positive or negative, should be achieved in order to ensure stability and avoid aggregation of the particles. The electrostatic repulsive force between the nanoparticles depends on the charge which is present on the surface of the nanoparticles. When they are negatively charged, the process prevents the nanoparticles from agglomeration in the medium, leading to long term stability. While the surface charge more than 30 mV may cause cytotoxicity. As shown in figure 5, the zeta potential agmatine cubosomes of F7 were found to be -12.0 mV severally.

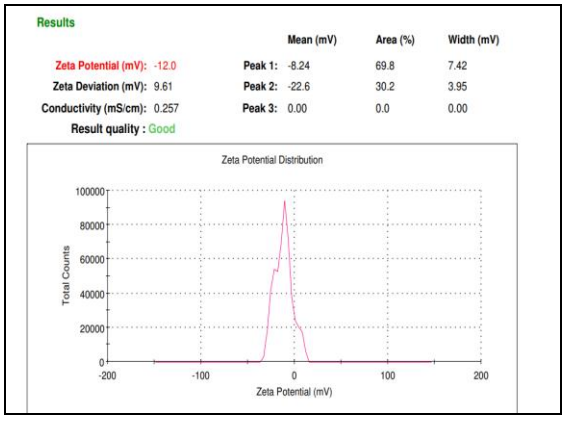

Figure 5. Zeta potential

3.4.3 Entrapment efficiency:

The drug entrapment efficiency is an important parameter for drug delivery systems. Figure 6 depicted the maximum entrapment efficiency of the agmatine -loaded cubosomes was found to be 88.92±0.33 (% w/w) for batch F7 indicating that most of agmatine was encapsulated in cubosomes as shown in table 4.

Table 4. Entrapment efficiency

Formulation	Entrapment Efficiency
F1	60.07±1.21
F2	64.28±1.73
F3	67.0±0.83
F4	72.92±0.92
F5	57.33±0.31
F6	81.79±1.28
F7	88.92±0.33
F8	59.09±0.83

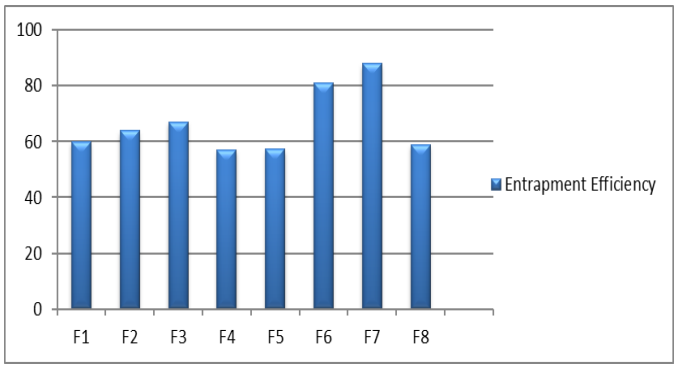

Figure 6. Entrapment efficiency

3.4.4 In-vitro drug release:

The results from the in-vitro drug-release study is shown in Figure 7. The release of agmatine from cubosomes over a period of 12 h, at the end of 12 h, over 95% of agmatine was released from the cubosomes (table 5) and drug release is 0.00± 0.00, 3.80±0.54, 6.79±0.86, 15.44±0.39, 23.56±0.51, 26.88±0.67, 29.65±1.39, 35.78±3.66, 43.89±1.32, 52.84±1.12, 61.79±1.24, 69.38±1.32, 78.94±1.14.

Table 5. In-vitro release

X	Mean ± SD
0	0.00± 0.00
1	3.80±0.54
2	6.79±0.86
3	15.44±0.39
4	23.56±0.51
5	26.88±0.67
6	29.65±1.39
7	35.78±3.66
8	43.89±1.32
9	52.84±1.12
10	61.79±1.24
11	69.38±1.32
12	78.94±1.14

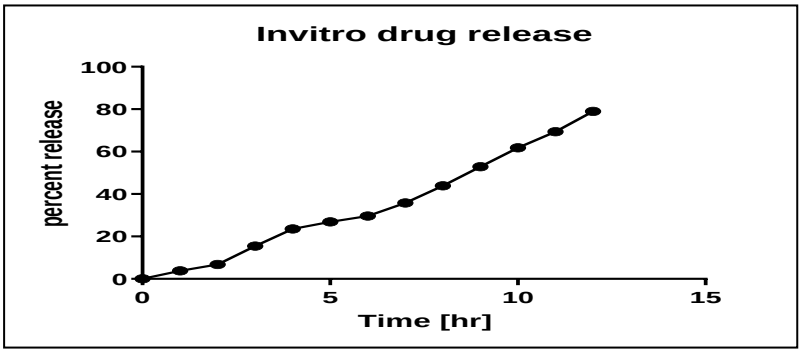

Figure 7. In-vitro agmatine drug release graph (percentage release vs time in hrs.)

3.4.5 FESEM:

The surface morphology of optimized formulation (F7) examined by FESEM was illustrated in (Figure 8). When at ×500 magnification, it could be seen that presence of almost spherical structures of cubosomes.

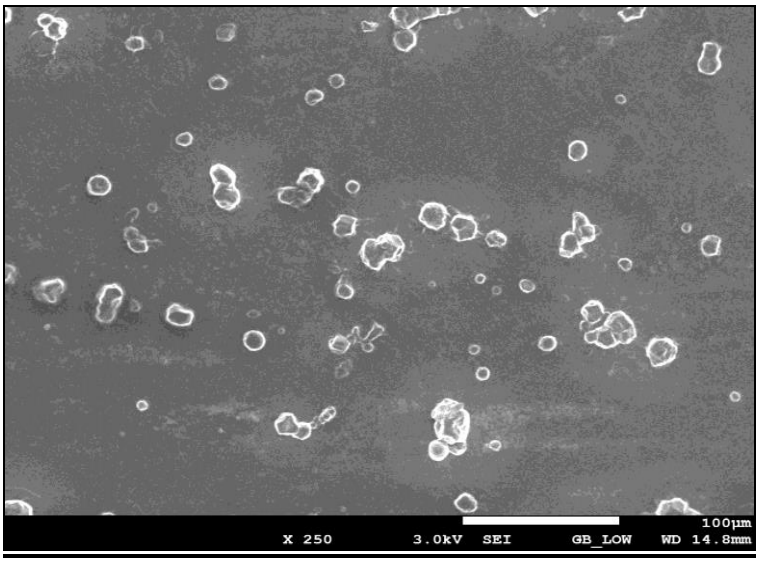

Figure 8. FESEM

3.4.6. Animal Study

Antiepileptic activity in PTZ induced seizures

The Effect of agmatine cubosomes on PTZ induced seizures model has been shown in Table 6. The graphical representation of effect of agmatine loaded cubosomes on onset of convulsion in PTZ induced seizure model is shown in figure 9 and 10.

Table 6. Effect of agmatine cubosomes on PTZ induced seizures model

Groups	**Onset of convulsion (sec)**	**Duration of convulsion (sec)**	**Recover / Mortality**
Saline	0	0	Normal
PTZ control	80±2.45***	69±5.4***	Mortality
Diazepam (standard)(i.p)+ PTZ 45 mg/kg (i.p.)	561±4.03***	25±4.0***	Recovery
8Agmatine+ PTZ 45 mg/kg (i.p.)	492±5.5***	33±3.5***	Recovery
Cubosomes (nasal)+ PTZ 45 mg/kg (i.p.)	611±3.3***	16±5.3***	Recovery

Statistical comparison: One way ANOVA, followed by Tukey's multiple comparisons test.

Control Vs Diazepam (Std) (i.p), Control Vs + Agmatine, Control Vs + Agmatine cubosomes (Nasal) are compared with control. (***p<0.0001)

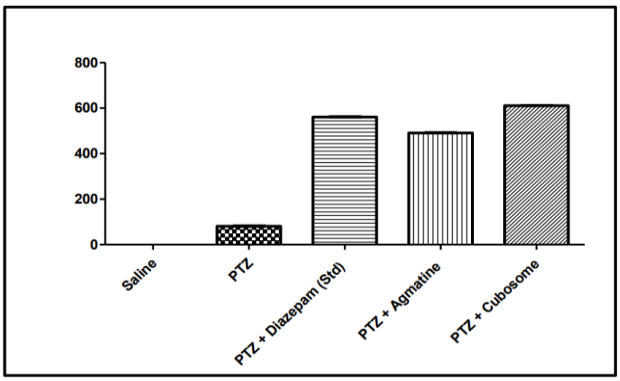

Figure 9. Graphical representation of Effect of agmatine loaded cubosomes of onset of convulsion in PTZ induced seizure model.

***p<0.0001 Control Vs Diazepam (Std) (i.p)

***p<0.0001 Control Vs + Agmatine

***p<0.0001 Control Vs + Agmatine cubosomes (Nasal)

***p<0.0001 Diazepam (Std) vs Cubosomes

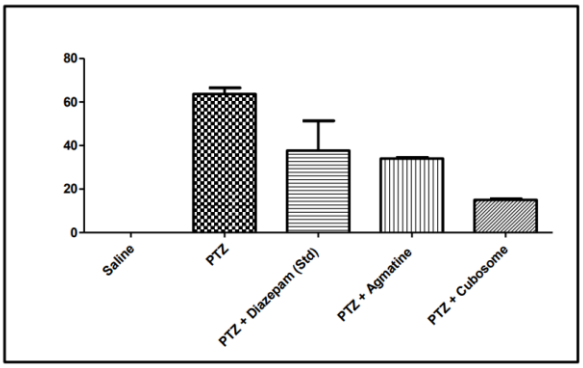

Figure 10. Graphical representation of Effect of agmatine loaded cubosomes duration of convulsion in PTZ induced seizure model

***p<0.0001 Control Vs Diazepam (Std) (i.p)

***p<0.0001 Control Vs + Agmatine

***p<0.0001 Control Vs + Agmatine cubosomes (Nasal)

***p<0.0001 Diazepam (Std) vs Agmatine cubosomes

From Table 6 results demonstrated that in PTZ control group, onset of convulsion (latency period) and duration of convulsion (latency duration) was found to be 80±2.45 sec and

69±5.4 sec respectively. All animals of the control group died after i.p. administration of PTZ injection (45 mg/kg). In Diazepam (standard) (i.p) + PTZ 45 mg/kg (i.p.) group, onset of convulsion and Duration of convulsion were found to be 561±4.03***sec and 25±4.0***sec respectively.

From Table 6 results demonstrated that in agmatine group, onset of convulsion (latency period) and duration of convulsion (latency duration) was found to be 492±5.5*** and 33±3.5***respectively.

From Figure. 10, significantly increased seizure latency ($p<0.0001$***), decreased latency duration ($p<0.0001$***) and show recovery in comparison with control group.

Nasal administration of agmatine cubosomes, onset of convulsion and duration of convulsion were found to be 611±3.3***sec and 16±5.3***sec respectively result demonstrated that increased onset of convulsion, decreased convulsion duration in comparison with Intraperitoneal administration of Diazepam (standard).

4. CONCLUSION

Agmatine cubosomes for intra nasal delivery were prepared using Pluronic F17. These cubosomes will be used to treat epilepsy. The prepared cubosomes were evaluated for various parameters such as particle size, zeta potential, and entrapment study. In all formulations, F7 shows higher entrapment efficiency, zeta potential, and optimum particle size. The formulation F7 is used as optimized batch and further evaluated for FESEM, in vitro release, and an in vivo study in PTZ induced seizures in mice. In comparison to intraperitoneal administration of Diazepam (standard), nasal administration of agmatine cubosomes increased convulsion onset and decreased convulsion duration. Agmatine cubosomes for intra nasal delivery can be a good alternative to conventionally marketed formulations, with possible improvements in the absorption of the drug and subsequent bioavailability. There is further need for evaluation of these prepared cubosomes in terms of efficacy, safety and stability.

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