Chapter Ten

SUMMARY & CONCLUSION
10.1. INTRODUCTION & PRESENT STATUS

Brain is a delicate organ, isolated from general circulation and characterized by the presence of relatively impermeable endothelial cells with tight junctions, enzymatic activity and the presence of active efflux transporter mechanisms. These formidable obstacles often block the delivery of neurotherapeutic agents to the brain across the blood-brain barrier (BBB). Although several promising molecules have the potential in the in vitro settings but lack of in vivo response is probably because the molecule cannot reach the brain in a sufficient concentration. Delivery of a neurotherapeutic agent across the BBB is a major limitation in the treatment of central nervous system (CNS) disorders and CNS infections (Baratchi et al, 2009).

Nanoconstructs are materials and devices that have a functional organization in at least one dimension on the nanometer (one billionth of a meter) scale, ranging from a few to about 100 nanometers. Nanoconstructs aimed at biologic applications and medicine in general, and neuroscience in particular, are designed fundamentally to interface and interact with cells and their tissues at the molecular level. The potential of nanotechnological applications to biology and medicine arise from the fact that they exhibit bulk mesoscale and macroscale chemical and/or physical properties that are unique to the engineered material or device and not necessarily possessed by the molecules alone. This supports the development of nanoconstructs that can potentially carry out multiple specific functions at once or in a predefined sequence, which is an important property for the clinically successful delivery of neurotherapeutic agents and other molecules to the central nervous system (Silva, 2008).

Epilepsy is a chronic neurological disorder that affects people of all ages. Around 50 million people worldwide have epilepsy. Nearly 90% of the people with epilepsy are found in developing countries. Epilepsy responds to treatment about 70% of the time, yet about three fourths of affected people in developing countries do not get the treatment they need. People with epilepsy and their families can suffer from stigma and discrimination in many parts of the world. Epilepsy is usually treated with medication prescribed by a physician; primary caregivers, neurologists, and neurosurgeons all frequently care for people with epilepsy. In some cases the implantation of a stimulator of the vagus nerve, or a special diet can be helpful. Neurosurgical operations for epilepsy can be palliative, reducing the frequency or severity of seizures; or, in some patients, an operation can be curative. The goal of
pharmacotherapy by using anticonvulsants is to suppress the rapid and excessive firing of neurons that start a seizure. Failing this, an effective anticonvulsant would prevent the spread of the seizure within the brain and offer protection against possible excitotoxic effects that may result in brain damage. In 1857, Sir Charles Locock first used potassium bromide to treat patients with catamenial epilepsy. Over the years new molecules for epilepsy treatment discovered, simultaneously more regulations and requirements were added, increasing the cost of drug development but also leading to improved understanding of potential toxicities of agents. It is likely in large part due to the latter evolution in drug development that present-day AEDs in general are safer and better tolerated by patients. Notwithstanding limitations with the existing AEDs, the ease of use and ready availability of medications, as well as the prompt reversibility of dose-related side effects, will keep AEDs as the mainstay of epilepsy treatment for the foreseeable future. Therefore, new drug therapies with efficacy against drug-resistant seizures, favourable adverse events profiles, especially in regard to neurological and psychiatric effects, and, if possible, low costs to patients and high worldwide availability are clearly needed.

A significant number of people continue to have seizures regularly despite taking medication. Also, AEDs are notorious for their side effects that may vary from gastrointestinal complaints, to problems in cognition, mood and behaviour or severe hypersensitivity reactions due to inherent AED property or unwanted exposure related to routinely used route of administration. It is estimated that in fact in one third of patients the treatment goal as described above cannot be achieved: patients keep getting seizures or experience bothersome side effects.

So far, research in epilepsy has focused largely on GABAergic and glutamatergic neurotransmission as paradigms of inhibitory and excitatory elements of central nervous system activity. However, a growing body of evidence points to the involvement of the sodium–hydrogen exchanger (NHE) in the modulation of seizure activity in neuronal cells. NHE is a ubiquitous transporter present in the plasma membranes of nearly all mammalian cells. This antiporter, by exchanging intracellular H+ for extracellular Na+, plays an essential role in a variety of cell functions, including pH regulation, volume homeostasis, and cell growth. Various investigators have reported the in vitro efficacy of NHE inhibitors in suppressing epileptiform activity elicited by bicuculline, caffeine, or low magnesium. Overwhelming evidence indicates that amiloride (a potassium-sparing diuretic) is a potent and non-selective
acid-sensing ion channels (ASIC—proton-gated channels) blocker that inhibits NHE activity and the drug has been found to suppress seizure activity in various in vivo and in vitro experiments, probably by preventing recovery from neuronal acidification. Thus, intracellular acidification appears to be a primary mechanism for the anticonvulsant action of amiloride.

Intranasal drug delivery is one of the focused delivery option for brain targeting as brain and nose compartments are connected to each other via olfactory/trigeminal route via peripheral circulation. Intranasal drug delivery delivers drug directly to the brain circumventing BBB and reduces drug delivery to the non targeted sites. This may result in reduction of dose, systemic dilution and first pass metabolism of the drug. Direct nose to brain transport results into rapid and/or higher uptake in the brain, which provides an alternative option of self medication in the management of emergencies. It gained importance because of longer residential time at the site of administration and possible enhanced transport mechanism by the mucoadhesive agents. Nanotechnology based nanosized colloidal dispersion e.g. microemulsions/nanoemulsion were already explored as an effective carrier system for the rapid and larger uptake of the drugs like Clonazepam, nimodipine, diazepam, midazolam, sumatriptan and tacrine to the brain. Prolonged residence time of the formulations can be achieved by tailoring the viscosity of the formulation by the addition of various grades and types of mucoadhesive agents. Spilling upon intranasal administration would be an anticipated formulation issue for that mucoadhesive based drug delivery systems. Addition of mucoadhesive agent will not only overcome the spill over problems but helps in retaining the formulations in nasal cavity for longer time.

Relevant Publication Outcomes:

10.2. HYPOTHESIS & RATIONALE
“Existing antiepileptic drugs are associated with dose and unwanted exposure related side effects, which can be mitigated by the use of alternative antiepileptic drugs; however the major obstacle in the delivery of these agents to the brain is constraints
of blood brain barrier. Hence present research work has been designed to develop a nanotechnology formulation design of a nonconventional antiepileptic drug. Intranasal route has been selected to target the drug at the site of action with improved efficacy & compliance.”

Keeping these facts in mind it is therefore important that research on the alternative AEDs, with their safe and effective delivery by implying appropriate route of administration like intranasal route for instant and direct delivery to brain needs to be carried out. After extensive literature survey, it was observed that amiloride (a potassium-sparing diuretic) is reported to be effective in epilepsy. But till date this reported potential antiepileptic drug have only been formulated in liposome and nanoparticles for the improved and effective delivery in the treatment of epilepsy. Hence present hypothesis deals with the novel formulation development of the amiloride for its effective delivery to the brain by implying suitable non-invasive route of administration technique for the treatment of epilepsy.

The objectives of this investigation were to prepare and characterize novel formulations, assess their pharmacokinetic performance for brain drug delivery in mice after i.n. delivery. It was also an objective to assess their role pharmacodynamically in suitable animal models. It was hypothesized that i.n. administration of nanometric formulation will result in selective and effective nose to-brain transport, reduce drug distribution in other parts of body and reduce side effects in the treatment of epilepsy.

10.3. PROOF OF CONCEPT: PRELIMINARY EVALUATION STUDIES

Razi et al., 2001 (as taken from Central Council for Research in Unani Medicine (CCRUM) Literature source) has reported the use of *Nigella sativa* seeds for anticonvulsant activity, with details of the type of dosage form (roasted seeds poultice), route of administration & the delivery system in form of volatile vapours created during the process of roasting of the dosage form (seed poultice). The vapour form which constitutes the phytoconstituents in nanometric scale created during roasting was able to deliver the active constituents from the seeds to the brain through nasal route. Basis on the above mentioned facts our concept would be stated as: 

*Nanometric formulations can be used for the selected neurotherapeutic agent, amiloride for delivery through nasal route in order to get superior delivery system for seizure control with reduced dose in animal models of epilepsy.*
The present study has explored the nanotechnology (nanometric formulation) for the selected drug amiloride by preparing nanometric novel formulation for administering through nasal route to get superior delivery system for seizure control with low and defined dose in animal models of epilepsy. Intranasal route showed improved brain uptake as compared to Oral route. The conclusion drawn out of prospective pilot studies are given below:

a. Pharmacodynamic effects of amiloride are statistically significant (*p<0.0.5) as compared between various dose levels with respect to previously reported routes of administration i.e., i.n. Vs p.o.. These experiments have provided qualitative background of the observed effects.

b. Based on these studies, amiloride can be used at 0.16 mg/kg dose level (one fourth of reported oral dose of 0.65 mg/kg) for next step of formulation development experiments. The formulation experiments were designed to ensure maximum loading of the active moiety & the proposed doses were the preliminary exploratory dose levels.

10.4. PROTOTYPE FORMULATION DEVELOPMENT
The strategy of applying drugs that are encapsulated into particulate vectors (such as synthetic nanoparticles) or nanosized colloidal dispersions to the olfactory epithelium could potentially improve the direct CNS delivery of drugs—including biologics. If drugs could reach the CNS in sufficient quantity by this route, it could generate interest in previously abandoned drug compounds and enable an entirely novel approach to CNS drug delivery.

Various approaches have been tried for prototype development work for initial proof of concept studies with respect to various pharmaceutical factors for achieving maximum drug loading for achieving ultimate goal of better patient compliance and therapeutic effects.

Brain targeted nanocolloidal carrier systems were formulated using biodegradable polymers using, chitosan, PLGA and PBCA. Nanoemulsion systems were prepared by using various oils and different proportions of surfactants & co-surfactants.

Drug, amiloride was available as amiloride hydrochloride (AMH) salt and the entrapment efficiency (% EE) of drug AMH was very poor in all the formulations.
which could be ascribed to the relatively hydrophilic nature of the drug. To improve its % EE in various formulations, an attempt was made to make this drug less hydrophilic and so its free base form i.e. amiloride free base (AMB) was prepared in laboratory and used further to prepare again these formulations. The AMB showed improved % EE as compared to AMH and thus selected for further formulation development purpose.

Since epileptic disorder requires onset of action with maximum exposure of drug at site of action i.e. brain, hence novel formulations were prepared and compared with specific formulation characteristics such as particle size & drug loading for improved patient compliance with respect to route of administration and disease requirement quick onset and/or sustained action. Nanoemulsion as novel formulation were selected on the basis of the above mentioned selection criteria & extensive development studies were conducted.

10.5. ANALYTICAL METHODS

Amiloride is known for its neuroprotective and anticonvulsant properties in preclinical studies. We have developed a simple UV-spectrophotometric method for estimation of amiloride hydrochloride (AMH) in in-vitro release samples and in assay of developed formulations. Similarly a simple, rapid, selective, sensitive UPLC method was developed for the estimation of amiloride free base (AMB) for its application to biopharmaceutical studies such as in vitro release from nanoparticulate system and in vivo pharmacokinetic study. The method employed was gradient elution using a Waters Acquity BEH C18 (100x2.1 mm, 1.7 mm) UPLC column. The mobile phase consisted of acetonitrile and ammonium acetate, pumped at a flow rate of 0.2 mL/min. The injection volume was 5 µL and amiloride was monitored at 360 nm wavelength with a total run time of 5 min. In solution, brain homogenate as well as in plasma, the method was found to be linear (r ≥ 0.998), precise (CV ≤2.00%) and accurate (recovery ≥ 84.6%) in the selected concentration range of 10–1000 ng/mL. Further, no interference was found at the retention time of amiloride from any plasma components, indicating selectivity of the developed method. For solutions, the limits of detection and quantitation of the method were found to be 0.15 and 0.50 ng/ml, respectively; while in brain homogenate/plasma they were 0.75 and 2.5 ng/mL, respectively. The validated method was successfully applied to quantify amiloride in
dissolution medium as well as oral in vivo pharmacokinetic study of amiloride nano-formulation.

10.6. PREPARATION, OPTIMIZATION, CHARACTERISATION AND IN-VITRO PERMEATION STUDIES OF NANOEMULSION FORMULATIONS

After performing solubility study in different oils, it was found that amiloride free base exhibited maximum solubility in the oleic acid (37.0 ± 0.5 mg/mL). Therefore oleic acid was chosen as the oil phase. The other advantage with the use of oleic acid was that it has also been reported as a powerful enhancer for biomembrane delivery, as it increases the fluidity of the intercellular lipid barriers in the stratum corneum by forming separate domains which interfere with the continuity of the multilamellar stratum corneum and induce highly permeable pathways in the stratum corneum. Similarly based on solubility of AMB in various surfactant and co-surfactant, their screening was done. Pseudoternary phase diagrams were constructed separately for each Smix ratio, through those o/w nanoemulsion regions was identified and nanoemulsion formulations were optimized.

Normal nanoemulsion could be limited in use during clinical practices, due to its less adherence or retention in nasal cavity due to spill over during administration. To overcome such limitation we have tried to prepare mucoadhesive formulations. Before preparing different mucoadhesive formulations, mucoadhesive strength studies were performed for various polymers i.e. chitosan, sodium alginate, and carbopol. Out of these polymers chitosan were selected because of natural origin as well as with significant higher mucoadhesive strength, which can synergize adhesion and drug permeation. The mucoadhesive nanoemulsions were prepared by first preparing a nanoemulsion of the drug using minimum volume of external phase and then adding the required volume of concentrated polymer solution to it such that the required final concentration was achieved.

The prepared nanoemulsions/ mucoadhesive nanoemulsions were characterized for globule size, zeta potential, transparency, % assay, viscosity, pH, and TEM. It was found that the addition of chitosan to NE tends to change the zetapotential from negative mode to positive side respectively. The globule size and zeta potential were fairly reproducible. The pH of the formulations was found to be within the range of nasal cavity secretions (4-6). The viscosities of the nanoemulsions were in the range of 50-150 cps and exhibited Newtonian flow.
The formulations were selected on the basis of various parameters like globule size, zeta potential, transparency, % assay, viscosity, pH and taken for the next step of in-vitro permeation studies using goat nasal mucosa. In-vitro permeation studies through goat nasal mucosa were performed using an automated Transdermal Diffusion Cell Sampling System (SFDC6, LOGAN Inst, NJ, USA). Various diffusion study parameters like flux, permeability coefficient were calculated. The results were recorded and formulation ANE1 with Flux, 10.61 μg/cm²/min & Permeability Coefficient Pb x 10³(cm²/min), 3.21 was selected. The formulation with highest permeation characteristics, ANE 1 was selected for mucoadhesive nanoemulsion preparation and evaluated for three different level of chitosan concentration (i.e. AMNE 0.25%, AMNE 0.50%, and AMNE 1.0%). Out of the prepared mucoadhesive nanoemulsion formulations, one formulation was evaluated for the permeability parameters across nasal mucosa. Results indicate that chitosan increases the permeability of the amiloride linear manner along with shortening of initial drug release time (drug release lag phase). In order to elucidate the disposition of nanoemulsions in the nasal mucosa, we examined cross-sections of the nasal mucosa by CLSM. The confocal images of different cross-sections of the nasal mucosa post washing with buffer solution exposed to the curcumin loaded Nanoemulsions. Qualitative assessment of confocal images revealed intense blue colored fluorescent areas located in between and inside the mucosal cells. Due to the mucoadhesive nature, it was observed that mucoadhesive formulation AMNE showed more blue colored intense areas as compared to non-mucoadhesive nanoemulsion. The results showed that flux and permeability coefficients for amiloride nanoemulsion are in the order of ANE1 > ANE45 > ANE57 > ANE 45. Incorporation of mucoadhesive agent Chitosan at various concentration increases the permeability of the formulation as concentration goes increase which clearly confirms the permeation improvement with NE systems in the presence of mucoadhesive agent.

Nanoemulsions and mucoadhesive nanoemulsions were successfully prepared by titration method. NEs of amiloride had very small globule size (~ 50nm) and negative zeta potential. While MNEs had a bit bigger globule size (~ 100nm) and positive zeta potential. The spherical surface of NE and MNE was confirmed from TEM. pH of NEs and MNEs was compatible with nasal fluid and viscosity of NEs and MNEs was suitable for nasal administration. In vitro release of NE and MNE system in nasal mucosal membrane demonstrated prompt and effective release with
more than 75% of drug release in 4 h. The NE and MNE were further subjected to stability studies according to ICH guidelines.

**10.7. STABILITY STUDY OF NANOEMULSION (NE)**

Amiloride loaded NEs were evaluated for stability study by performing physical and chemical stability of the optimized formulation. The optimized formulations have been subjected to accelerated stability study for the assessment of physical stability. Chemical stability of the formulation was assessed by long term stability study.

In order to assess the thermodynamic stability, the accelerated stability studies were done by subjecting the formulations for centrifugation, freeze-thaw cycle and heating cooling cycle. Before and after each treatment, GS, ZP and %T (in case of NE) of the formulations were determined and recorded. The parameters after accelerated stability conditions were found to be non-significant which clearly indicates that the prepared ANE systems were thermodynamically stable.

In long term stability study, the ANEs were packed in the screw capped borosil vials. ANEs were kept at room temperature (25°C / 60% RH) and accelerated temperature (40°C / 75% RH) conditions. During the storage period, ANE systems were assessed for their GS, ZP, assay, pH and %T. Over the time period there was minimal increment in GS and ZP and change in assay, %T, pH. However, the changes observed were non-significant when no visual indications of physical instability of ANEs were seen. Irrespective of the storage conditions, the ANE system remained stable for 3 months duration at 40°C / 75% RH and 25°C / 60% RH respectively.

It was concluded from the results of the accelerated stability studies, performed by subjecting the formulations for centrifugation, freeze-thaw cycle and heating cooling cycle that the prepared ANE systems were thermodynamically stable. From the result of stability study, it was concluded that the nanoemulsion when stored at 40 ± 2°C / 75 ± 5% RH for 3 months showed that stability was not altered by changes, such as increase or decrease in the GS, ZP, reduction or degradation in the % assay and changes in %T. Hence, we can conclusively say that nanoemulsion of amiloride were stable and can be stored at 25 ± 2°C / 60 ± 5% RH for 6 months retaining its original formulation characteristics.
Relevant Publication Outcomes:


International Presentations:

“Preparation and characterization of nanoemulsion of amiloride for nose to brain delivery” Workshop on Biomaterials and Their Interactions with Biological and Model Membranes 2011, held on 19-23 September 2011 in Salou, Spain.

10.8. RADIOLABELLING, BIODISTRIBUTION, PHARMACOKINETICS & PHARMACODYNAMIC STUDIES

The labelling of drug solution (DS: Amiloride solution = AS), Nanoemulsion (NE: Amiloride nanoemulsion = ANE) and mucoadhesive nanoemulsion (MNE: Amiloride mucoadhesive nanoemulsion = AMNE) was performed by direct labelling using technetium as per the reported method with some modifications. Labelling efficiency & stability were determined as in solution and as well in-vitro with respect to incubation time, pH, and amount of reducing agent SnCl₂.

The optimum quantity of stannous chloride for high labelling efficiency and low free and reduced/hydrolyzed ⁹⁹ᵐ⁻Tc, was found to be 100 μg for NE, MNE formulations and drug solutions respectively. The incubation time was optimized at 30 min for NE and MNE formulations. AS require incubation of 15 min. The pH of all the formulations was kept at around 6.5. The labelling efficiency for AS, ANE and AMNE was found to be 98.18%, 99.82% and 95.34 % respectively. The radiolabeled complex showed high stability in mice serum with radiolabelling efficiencies measured, greater than 90%.

Direct radiolabelling was found to be useful tool to study biodistribution. Radiolabelling of nanoemulsion, mucoadhesive nanoemulsion and solution preparations of amiloride were successfully performed and the results indicated good stability and bonding strength of the radiolabeled complex. Hence, these formulations were found stable and suitable to study biodistribution and to study gamma scintigraphy imaging of these formulations on animals.
For biodistribution studies, particularly drug levels in blood and brain, three mice were used at each time point for each formulation. The mice were divided into three groups. Group I, group II and group III were administered $^{99m}$Tc-AS, $^{99m}$Tc-ANE and $^{99m}$Tc-AMNE respectively. Drug Solution was used for comparative evaluation. All groups received 44.4-53.28 MBq/kg of radioactivity incorporated in 10 μL of $^{99m}$Tc-AS, $^{99m}$Tc-ANE, and $^{99m}$Tc-AMNE, administered via intranasal route. Before nasal administration of the formulations, the mice were partially anesthetized with diethyl ether. 4/5 μL of formulation was administered in each nostril using micropipette (10 μL). The mice were held from the back in slanted position during nasal administration. The mice were sacrificed at different time intervals of 0.5, 1, 2, 4, 6 and 8 h and blood was collected via cardiac puncture. Brain were dissected washed twice with normal saline, made free from any adhering tissues, dried between adsorbent paper-folds, placed in pre-weighed plastic tubes, and weighed. The radioactivity present in each tissue/blood sample was determined using shielded well-type gamma scintillation counter along with 3 samples of standard solution representing 100% of the administered dose. The radioactivity in each organ/blood sample was determined as fraction of administered dose per gram of the tissue (%A/g).

Biodistribution studies of $^{99m}$Tc-Amiloride formulations following intravenous administration (ANE) and intranasal administrations (AS, ANE and AMNE) on Swiss albino mice were performed and the radioactivity was estimated at different intervals up to 8 h. The brain–blood ratio of the drug at all sampling time points for different formulations was also calculated and recorded. The Amiloride concentration in brain following the intranasal (i.n.) administration of AMNE were found to be significantly higher at all the time points compared to both ANE (i.n.) and ANE (i.v.). While the brain concentration of amiloride after i.n. administration of ANE was comparable to that of i.v. administration of ANE at all the time points. The brain/blood ratios of 0.62, 0.76, 0.80, and 0.22 of AS (i.n), ANE (i.n), AMNE (i.n) and ANE (i.v), respectively, at 0.5 h were indicative of direct nose to brain transport bypassing the blood–brain barrier hence; it proved the superiority of nose to brain delivery of amiloride by nanosized colloidal dispersion like nanoemulsion. Various Pharmacokinetic parameters were calculated using Kinetica software. Finding of
lower $T_{\text{max}}$ values for brain (0.5 h) when compared to blood (1 h) may also be attributed to preferential nose to brain transport following i.n. administration. When the $C_{\text{max}}$ and AUC of brain concentration of AS (i.n.), ANE (i.n.) and AMNE (i.n.) were compared, the $C_{\text{max}}$ (0.71% g) and $\text{AUC}_{0-t}$ (2.65 h %/g) of AMNE were found to be significantly higher because the addition of mucoadhesive agent decreased the mucociliary clearance, which under normal circumstances rapidly clears the instilled formulation. AMNE showed the highest DTE% (80) and DTP% (72.4) amongst the three tested formulations, followed by the ANE i.n. and then by AS i.n.. Statistically significant difference in DTP% of AMNE as compared to AS and ANE showed the benefit of mucoadhesive nanoemulsion formulation. The scintigrams taken by Gamma camera clearly demonstrate the accumulation of formulations in brain administered following intranasal administration of AMNE as compared to intravenous administration of ANE. Dynamic imaging clearly showed retention of AMNE till 30 min in the head region of the animal showing drug was accumulated in brain region and systemic exposure was less. Significant quantity of amiloride was quickly and effectively delivered to the brain by intranasal administration of formulated mucoadhesive nanoemulsion of amiloride.

We found an improved anticonvulsant action with mucoadhesive nanoemulsion formulation administered through intranasal route as compared to other routes (mentioned in chapter 3) in Chemical induced seizure model (Pentylenetetrazole model: PTZ), & Electrical induced seizure (Increased current electroshock model: ICES). While pre-treatment with amiloride alone at high dose (0.65 mg/kg) through other route (p.o.) significantly affect seizure threshold in the ICES test or latencies to myoclonic jerks and clonic generalized seizures in the PTZ test, but that can be achieved with a significant lower dose of amiloride (0.16 mg/kg) loaded formulations (ANE/AMNE) when administered through intranasal route.

From these observations, it can be hypothesized that nanometric formulations of amiloride when administered through intranasal route may enter the brain to a greater extent than free amiloride when administered through other routes i.e., per oral. This is in line with the earlier reports that suggested enhanced anticonvulsant action of drugs like Clonazepam, Lamotrigine, Midazolam and Diazepam when administered through intranasal route as nanoemulsion/mucoadhesive nanoemulsion formulations. The augmented action of nanoemulsion based formulation may be
attributed to greater ability of the nanometric carrier to cross the BBB. Further mucoadhesive nanoemulsion could be better adhered and retained in nasal cavity as compared to non-mucoadhesive formulations leading to better patient compliance.

10.9. SAFETY ASSESSMENT STUDIES

In order to confirm the safety of the optimised and selected nanoemulsion formulations, in vivo toxicity evaluation of ANE and AMNE was carried out in adult Wistar rats for 14 days sub acute toxicity assessment.

There were no mortalities of rats observed in any of the groups during the 14-day treatment period with intranasal administration of developed formulation (ANE/AMNE). Clinical examination of the rats’ brain tissues prior to and after administration of each (ANE/AMNE) formulation for 14 days revealed no signs of irritation or tissue damage for all the rats as compared to the vehicle control groups. Macroscopic examination of the brain tissues exposed to the polymeric (ANE/AMNE) formulation, vehicle also did not show any change in the morphology or tissue microstructure. As compared to vehicle control, the (ANE/AMNE) formulation treated groups showed no visible sign of inflammation or necrosis demonstrating the safety of formulations. Histological images showed the dissected nasal mucosa, no nasociliary damage was observed and the nasal membrane remained intact when treated with formulations, thus substantiating the safety of the excipients used in the formulations. From the neurotoxicity studies it can be concluded that amiloride loaded mucoadhesive nanof ormulations does not cause any neurotoxicity or motor coordination impairment. In the short-term (14 days) toxicity studies, repeated intranasal administration of the amiloride loaded nanof ormulations to rats caused no significant inflammation, or tissue toxicity.

The efficacy & safety studies have been performed on the animal models & henceforth the anticipated use of these novel formulations will require the extensive preclinical & clinical safety & efficacy studies as per regulatory guidelines.
10.10. CONCLUSIONS

The research work has achieved the objective of making a nanoconstruct of amiloride for anticonvulsant activity with better delivery and efficacy when administered through intranasal route.

The optimized formulations were designed in nanometric scale with mucoadhesive properties which synergize & improve the pharmacokinetic requirements especially to disease requirement. The route of administration via nose demonstrated & authenticated the concept of nose to brain targeting in safe and effective manner.

To conclude, mucoadhesive delivery systems demonstrated their potential in effective brain targeting through intranasal route. Hence, these studies suggest potential application of intranasal amiloride loaded nanoemulsion formulations in treating epilepsy disorder possibly by reducing dose when given intranasally.

FUTURE STUDIES

Safety and use of intranasal formulations developed in this investigation can be realized after animal studies on atleast two more animal species followed by clinical studies with focus on toxicological evaluation on chronic use along with the dose titration studies.