CHAPTER – 8

SUMMARY AND CONCLUSION

Summary

The present study investigated the antiepileptic activity of aerial parts of *Acalypha fruticosa*, leaves of *Barringtonia racemosa*, bark of *Erythrina stricta* and leaves of *Gossypium herbaceum*.

In the present study, four different extracts were prepared by using solvents of increasing polarity like petroleum ether, chloroform, ethanol and water. Non-polar solvents have low dielectric constants and dissolve non-polar solutes with similar internal pressures through induced dipole interactions. Petroleum ether is a non-polar solvent which solubilises non-polar compounds like steroids and triterpenoids. Chloroform extracts non-polar to intermediately polar compounds such as steroids, triterpenoids, flavonoids. Ethanol dissolves most of the secondary metabolites such as steroids, triterpenoids, flavonoids, tannins, saponins and enhance their release from cellular matrix/cell surface. The polar components like polysaccharides, phenols, aldehydes, ketones, amines, saponins and other oxygen containing compounds dissolve in water due to formation of hydrogen bonding [124]. Thus petroleum ether, chloroform, ethanol and aqueous extracts of the tested plants were prepared to predict which phytoconstituents are responsible for the remarkable antiepileptic activity.
In preliminary phytochemical studies, the chief phytoconstituents present in the different extracts of *Acalypha fruticosa* were alkaloids, steroids, carbohydrates, tannins, flavanoids, saponins, lipids and proteins. The various phytochemicals present in the different extracts of *Barringtonia racemosa* were alkaloids, steroids, carbohydrates, tannins, flavanoids, saponins, lipids and proteins. Different extracts of stem bark of *Erythrina stricta* gave positive test for phytoconstituents such as alkaloids, steroids, carbohydrates, tannins, flavanoids, saponins and proteins. Various extracts of leaves of *Gossypium herbaceum* showed the presence of alkaloids, steroids, carbohydrates, tannins, flavanoids, lipids and proteins.

In acute toxicity studies, all the extracts of *Acalypha fruticosa* and *Barringtonia racemosa* were found to be safe upto 1000 mg/kg, p.o. Hence, 30, 100 and 300 mg/kg, p.o. doses were selected to evaluate antiepileptic activity. In case of *Erythrina stricta* and *Gossypium herbaceum*, all the extracts i.e., petroleum ether, chloroform, ethanol and aqueous extracts were found to be safe upto 600 mg/kg, p.o. So, 10, 30 and 100 mg/kg, p.o. doses were selected for evaluating antiepileptic activity.

The most extensively studied and well established animal seizure models are maximal electroshock, pentylenetetrazole and isoniazid-induced seizures in mice. Another advantage of using the above models is the pharmacological profiles were comparable to the human condition. These models were related to GABAergic neurotransmission [33-34]. Thus maximum electroshock (MES),
pentylenetetrazole (PTZ) and isoniazid (INH)-induced convulsion models were chosen to screen antiepileptic activity of the selected plants.

Ethanol extract of *Acalypha fruticosa* (EAF) exhibited potent antiepileptic activity \((p<0.01)\) in all the three tested models when compared with petroleum ether, chloroform and aqueous extracts. EAF at 300 mg/kg dose significantly inhibited MES-induced THLE to the maximum extent \((69.03\%)\). When compared with all other extracts, EAF significantly afforded maximum protection to the mice against PTZ-induced convulsions at the dose of 300 mg/kg, p.o. with percentage of inhibition of 70.82%. In isoniazid (INH) model, the ethanol extract significantly delayed the latency time in a dose-dependent manner but could not protect the mice from mortality. Results evidenced that out of three tested models, ethanol extract (300 mg/kg) of *Acalypha fruticosa* exhibited maximum antiepileptic activity in PTZ model. The extract might raised the seizure threshold or act as GABA agonist and enhanced GABAergic neurotransmission by increasing GABA levels in brain by facilitating the opening of GABA-activated chloride channels at GABA\(_A\) receptors. These effects might be due to the presence of phytoconstituents such as tannins and flavonoids.

Ethanol extract of *Barringtonia racemosa* exerted remarkable antiepileptic activity when compared to all other extracts in MES, PTZ and INH-induced convulsions. In MES and PTZ models, maximum percentages of inhibition for the ethanol extract (EBR) was found to be
73.11% and 86.62% respectively at the dose of 300 mg/kg, p.o. In INH model, maximum delay in latency of convulsions was observed for EBR at 300 mg/kg, p.o. but none of the extract is able to protect the mice from mortality. Out of all three tested models, ethanol extract of *Barringtonia racemosa* showed maximum antiepileptic activity in PTZ model. It may antagonize the action of PTZ which is a GABA-antagonist. The GABA-agonist action of *Barringtonia racemosa* might be responsible for enhanced GABAergic neurotransmission. Our preliminary phytochemical studies of *Barringtonia racemosa* revealed the presence of flavanoids, triterpenoids and tannins which are potent antioxidants [124]. Hence the presence of antioxidant principles in ethanol extract of leaves of *Barringtonia racemosa* might partially contribute the antiepileptic activity.

Ethanol extract of *Erythrina stricta* showed significant antiepileptic activity in all three tested models when compared with petroleum, chloroform and aqueous extracts. In both MES and PTZ models, the onset time was delayed and duration of convulsions was reduced in groups which received ethanol extract of *Erythrina stricta*. EES (100 mg/kg, p.o.) exhibited maximum inhibition in both MES and PTZ models with percentages of inhibition of 70.20% and 71.41% respectively. In INH model, all the extracts of *Erythrina stricta* significantly delayed the latency of convulsions (p<0.01) in dose-dependent manner. Maximum delay in latency of convulsions was observed with EES at the dose of 100 mg/kg, p.o. and all the extracts could not protect the mice from mortality. Out of three models,
maximum antiepileptic activity was observed in PTZ model. This might be due to raised seizure threshold or enhanced GABAergic neurotransmission by increasing GABA levels in brain. Further, presence of phytoconstituents like flavanoids and tannins in the ethanol extract may be responsible for antiepileptic activity [148-149].

At 100 mg/kg, p.o. dose ethanol extract of leaves of *Gossypium herbaceum* remarkably protected MES-induced THLE with percentage inhibition of 73.04% (p<0.01) when compared with all other extracts. In PTZ model EGH significantly protected the mice from convulsions induced by PTZ in a dose-dependent manner (p<0.01) when compared with petroleum, chloroform and aqueous extracts of *Gossypium herbaceum*. In isoniazid (INH) model also EGH exhibited maximum delay in the latency time. Although EGH exhibited significant antiepileptic activity in all the three models but maximum activity was observed in PTZ model. Our preliminary phytochemical studies on leaves revealed the presence of phenolic compounds such as flavanoids, tannins which is good agreement with earlier reports on phytochemical studies on leaves of *Gossypium herbaceum* [26]. Earlier reports suggested that many flavonoids could act as benzodiazepine-like molecules in the central nervous system and modulate GABA-generated chloride currents in animal models of anxiety, sedation and convulsion [186-187]. The presence of flavonoids in ethanol extract may partially responsible for the antiepileptic activity.

Our study demonstrated that the presence of either flavonoids (chloroform extract of all plants) or tannins (aqueous extract of all
plants) alone exhibited significant but not maximum antiepileptic activity, whereas ethanol extract of all plants contain both flavonoids and tannins and showed maximal activity in all the three models. Studies carried out till date suggests that flavonoids inhibit voltage gated sodium channels, activate Ca+ activated K+ channels, stimulate GABAergic inhibition, interact with opioid receptors, inhibit NMDA receptors and exhibit antioxidant actions via modulation of nitric oxide and xanthine oxidase pathways and by leukocytic immobilization, one or more of these mechanisms are involved in suppression of epileptic seizures [127]. Hence, the ethanol extract of all the plants showed maximum antiepileptic activity when compared to all other extracts.

Conclusion

Herbal medicines play a key role in the management of neurological disorders. Many a number of plants and plant derived products stated to exhibit antiepileptic activity in Indian system of medicine. But still there are many plants whose traditional uses are not yet scientifically exploited. Therefore the present work was undertaken. Ethanol extract of aerial parts of Acalypha fruticosa, leaves of Barringtonia racemosa, bark of Erythrina stricta and leaves of Gossypium herbaceum exhibited promising antiepileptic activity in three tested experimental models, but remarkable antiepileptic activity was observed in PTZ model when compared to other two models. This
might be due to raised seizure threshold or enhanced GABAergic neurotransmission by increasing GABA levels in brain.

Our results also evidenced that the ethanol extract of tested plants contain steroids, phenolic compounds such as flavanoids and tannins. Hence the presence of above phytoconstituents may partially contribute the significant antiepileptic activity of ethanol extracts of tested plants. Present study proves that tested plants i.e., *Acalypha fruticosa*, *Barringtonia racemosa*, *Erythrina stricta* and *Gossypium herbaceum* were effective in the treatment of epilepsy.

Among the tested plants, *Gossypium herbaceum* exhibited potent antiepileptic activity in all three tested models.