Summary & Conclusions
6. SUMMARY AND CONCLUSIONS

The present study was designed to determine whether the antiepileptic drugs with the anti-lipidperoxidative and Ca$^{2+}$ channel antagonistic properties have antiepileptic effects against iron-induced cortical epilepsy in the rat. The drugs studied were: α-tocopherol, verapamil, ethosuximide, flunarizine and valproate.

The iron-induced experimental epilepsy model of focal seizures in the rat is of great interest as it models human post-traumatic epilepsy. The development of epileptiform seizure activity in posttraumatic epilepsy is thought to be initiated by iron-induced neuronal membrane lipid peroxidation. Anticonvulsants were not found to be very effective in preventing trauma-induced seizures. Drugs which have anti-lipidperoxidative actions are likely to be effective against post-traumatic clinical epilepsy. Furthermore, although the involvement of peroxidative mechanisms is indicated in some form of epilepsies, knowledge about the effects of the antiepileptic drugs on antioxidants is poor and controversial.

In the present study, experiments performed on the iron-induced cortical epilepsy in the rat were expected to provide information on: (1) the antiepileptic potential of antiperoxidative drugs against iron-induced epilepsy, and (2) the mechanism of action of these drugs.

The development and progression of epileptiform activity in the iron-induced epileptogenic focus was monitored by electrocorticography. Both ipsilateral and contralateral foci were studied. Biochemical changes accompanying the electrical seizure activity were assessed by measuring TBARS, HNE and the glutathione peroxidase activity, in the ipsilateral and contralateral foci. Histopathological structure of the epileptogenic lesion was assessed by electron microscopy and also by obtaining a few magnetic resonance imaging scans.

Results showed that the epileptic electrophysiological activity began to appear within three days of the intracortical injection of FeCl$_3$. A conspicuous, intense recurrent epileptiform activity consisting of spikes and spike-wave complexes in the ipsilateral focus was observed by the days 8-15 of the intracortical injection of iron (FeCl$_3$). In the homotopic contralateral site also, an
epileptic focus was found to develop in which the epileptiform activity was conspicuous although less intense as compared with that of the ipsilateral focus.

The data obtained during the course of the present study revealed that the increase in the lipid peroxidation (TBA-RS) in the ipsilateral epileptogenic focal tissue at different time points after FeCl₃ injection, correlated with the development and progression of the epileptic electrophysiological activity. Maximum levels of lipid peroxidation products were found on the day 8 after FeCl₃ injection. The levels persisted at significantly high levels through to day 28. Recurrent and well developed epileptic electrophysiological activity that had appeared by day 3 continued to occur thereafter.

The present data also showed some elevation of lipid peroxidation in the homotopic contralateral focus where epileptiform electrocorticographic activity occurred. However, these increases in lipid peroxides were much smaller than those in the ipsilateral focus.

The 4-hydorxynonenal (4-HNE) levels in the ipsilaterlal focus were found to significantly increase at day 8 after FeCl₃ injection and gradually further increased thereafter. Similar significant increase in 4-HNE levels also occurred in the contralateral focus. The increase in levels of 4-HNE observed during this study was concurrent with the observed increase in MDA levels and the build up of the focal epileptic electrographic activity as well. The activity of glutathione peroxidase (GPx), an important antiperoxidant enzyme, was also measured in the epileptogenic focus. The data obtained showed that the activity of GPx in the epileptogenic focus was only slightly elevated. The low elevation of GPx activity was concurrent with the high increase in lipid peroxidation during the iron epilepsy. Thus it can be argued that the high levels of lipid peroxides are accumulated in the epileptic focus because somehow GPx fails to be stimulated sufficiently. The marked deficiency of GPx activity in the epileptogenic focus may, therefore, be an important factor in the pathogenesis of iron-induced experimental epilepsy.
6.1. Effect of α-tocopherol on iron-induced epilepsy

Treatment of epileptic animals with α-tocopherol caused complete suppression of the epileptogenic electrocorticographic activity in the epileptogenic focus together with a significant decline in the levels of lipid peroxides in the ipsilateral and contralateral epileptogenic foci. The drug treatment also increased the activity of glutathione peroxidase in the epileptogenic focus. The protective effect of the antioxidant α-tocopherol was observed at ultrastructural level also. The treatment with α-tocopherol resulted in reappearance of the extensive rough endoplasmic reticulum and myelination. Furthermore there was a decrease in the number of lysosomes, and an improvement in the cytoplasmic and nuclear homogeneity and vacuolation etc. Previous studies had shown that pretreatment with α-tocopherol prevented the iron-induced epileptiform activity in most of the experimental animals. In one study, however, pretreatment with α-tocopherol was found only to delay the onset of iron seizure but was found not to completely suppress them. The present study extends those findings very significantly demonstrating that the drug α-tocopherol can produce its antiepileptic effects even against an active iron-induced epileptogenic focus.

The present results have clearly shown that prolonged treatment with vitamin E completely suppresses the iron-induced seizures. The finding from the present work that α-tocopherol is also effective against the epileptic activity of the contralateral epileptic focus is of interest. From this, it would appear that the vitamin E on its own could be effective against spontaneous seizures. Our data also indicate that the anti-lipidperoxidative effects of α-tocopherol will partly be due to the drug's stimulation of the enzyme glutathione peroxidase. In conclusion, the data derived from the present study show that post treatment of iron-induced epileptic animals with α-tocopherol is efficacious in suppressing epileptic electrocorticographic activity; decreasing lipid peroxidation; and elevating glutathione peroxidase activity in the epileptogenic focus. The data also revealed that these antiepileptic effects were also manifest against contralateral focus epileptiform activity indicating thereby that at least to some extent α-tocopherol
(vitamin E) has antiepileptic effects also against epileptiform activity arising due to causes other than metal-tissue interaction.

6.2. Effect of verapamil on iron-induced epilepsy

Verapamil when given intraperitoneally suppressed the epileptiform electrocorticographic activity, decreased the lipid peroxidation and elevated the glutathione peroxidase activity in the ipsilateral iron-induced epileptogenic focus. In the contralateral focus also the drug showed similar effects. The anti-lipidperoxidative effects of verapamil in the iron epileptic focus appear similar to its effects in other tissues. In conclusion the present data have demonstrated significant antiepileptic effects of verapamil in iron focus and these effects are mediated by reduction of lipid peroxidation and the elevation of glutathione peroxidase activity. The present data further confirm that intraperitoneally administered verapamil does show antiepileptic effects.

6.3. Effect of flunarizine on iron-induced epilepsy

The present results showed that on treatment with flunarizine, levels of TBA dropped to almost control levels. The contralateral epileptogenic focus too showed a decrease in lipid peroxidation. Levels of 4-HNE were also significantly reduced by flunarizine. Flunarizine enhanced the activity of glutathione peroxidase both in the ipsilateral and the contralateral foci. The drug also suppressed the electrographic seizure activity with a marked change. The frequency and amplitude of the electrocorticogram decreased both in the contralateral and the ipsilateral foci and this change was apparent from the first day of treatment itself. On the whole, flunarizine appears to show a higher potency to reduce epileptogenesis. In conclusion the results demonstrate that flunarizine has strong antiepileptic effects in iron-induced epileptogenic focus, and the drug's antiepileptic effects may be due to its anti-lipidperoxidative and calcium channel blocking properties.
6.4. **Effect of ethosuximide on iron-induced epilepsy**

The data derived from the present experiments showed that the drug ethosuximide decreased the lipid peroxidation levels (measured by TBA-RS) in the ipsilateral focus. The levels came down to control levels. In the contralateral focus too a similar decrease in lipid peroxide levels was conspicuous. The levels of 4-HNE also declined in both the ipsilateral and contralateral foci. The decrease in levels occurred progressively. As the lipid peroxide levels fell the levels of GPx were found appreciably elevated as compared to those of iron-induced epileptic animals. The antiepileptic action of the drug in this model is associated with alterations in lipid peroxidation mechanisms. Ethosuximide also greatly suppressed the electrographic seizure activity. The mechanism of action of the drug is presumed to be related to its action on Ca²⁺ currents particularly thalamic currents. The drug's effects on lipid peroxidation and antioxidative enzymes have not been studied so far. The present data demonstrate that ethosuximide also has anti-lipidperoxidative properties. In this respect the drug would appear to be similar to other Ca²⁺ channel blocking agents. The present data thus provide additional new information about the mechanism of action of ethosuximide that the drug is an anti-lipidperoxidant.

6.5. **Effect of valproate on iron-induced epilepsy**

The results of the present study show that valproate reduced the epileptiform activity in ECoG and it acts as an anti-peroxidant as the drug lowered the levels of TBA-RS markedly in the ipsilateral and in the contralateral foci. 4-HNE, the other parameter studied for assessing levels of lipid peroxidation, was also found to be decreased by the drug. The drug also induced an increase in the levels of GPx in both the ipsilateral and contralateral foci. The present data thus show that valproate exerts anti-lipidperoxidative action in the iron-induced epileptic tissue.
6.6. **Structure of the lesion**

The electron microscopic data obtained in the present study demonstrated that the antiperoxidant antiepileptic drugs α-tocopherol, verapamil, ethosuximide, flunarizine and valproate reversed the deleterious structural changes in the lesion induced by iron. The most prominent change seen in the drug-treated animals was the reappearance of rough endoplasmic reticulum (RER) and Golgi bodies in large numbers. Free ribosomes also became conspicuous. The cytoplasm regained its homogeneity and the vacuolization decreased. The lysosomes showed a reduction in their number. The mitochondria also showed a return to normal size except in the ethosuximide treated animals that still showed swollen mitochondria. The chromatin became dispersed and the dense chromatin apparent in epileptic animals was no longer visible. The nucleolus too showed some activity.