SUMMARY AND CONCLUSIONS
The epilepsies are amongst the most common neurological disorders. Many mechanisms have been suggested for the pathophysiological processes related to seizure initiation, amplification and propagation. For the numerous patients with refractory epilepsy, combination therapy is the only alternative. In literature, different types of drug interaction (i.e. addition, synergism and antagonism) have been reported during combined therapy with antiepileptic drugs (AEDs). Therefore, whenever an AED is combined with another drug with or without inherent anticonvulsant activity, there is a need for critical preclinical bioevaluation studies using suitable models to find out the types of drug interaction that may aid in the selection of an ideal agent for better drug combination.

During recent years, accumulating evidence from experimental models suggest a possible involvement of oxidative stress in the pathophysiology of epilepsy. A question emerges whether the combination of an antioxidant with standard AEDs may offer more benefits in terms of seizure protection with minimal adverse drug reactions. The present study thus addressed the interactions between AEDs [i.e. phenytoin (PHT), sodium valproate (SVP)] and N-acetylcysteine (NAC), a potent antioxidant, at three fixed ratio dose combinations (i.e. 1:1, 1:3 and 3:1) in the mouse maximal electroshock (MES) test using isobolographic analysis. Markers of oxidative stress namely reduced glutathione (GSH) and malondialdehyde (MDA) were estimated in the cortex of mice pretreated with either of these drugs alone (ED$_{50}$ doses) or their 3:1 ratio.
Research on epilepsy has largely focused on the impaired inhibitory gamma-aminobutyric acid (GABAergic) and excessive excitatory glutamatergic neurotransmitter systems. The role of other contributing neurotransmitters, including histamine, is less studied and understood. Clobenpropit, a H3 receptor antagonist, has shown effectiveness in different experimental seizure models. Additionally, clobenpropit has been reported to have mild antioxidant properties. Interestingly, NAC, a potent antioxidant has been reported to enhance histamine release from mast cells. Therefore, in the present study, an attempt was also made to find out the influence of histaminergic mechanisms in the effects of PHT, SVP, NAC and their combinations against MES induced seizures.

H3 receptor ligands not only influence the release of histamine and other neurotransmitters like GABA, but they also modulate the activity of histidine decarboxylase (IDC). Therefore, a decarboxylase positive modulator, namely pyridoxine, was taken for interaction studies with clobenpropit in the MES model of seizures. The brain histamine levels were also estimated.

It is well documented that changes in electrolyte homeostasis have a significant influence on neuronal excitability and inhibition. Among the four major ions namely sodium, potassium, calcium and magnesium, the calcium ions play a
critical and pivotal role in various types of neurological disorders including epilepsy. Therefore, in the present study, the effects of PHT, SVP, NAC, clobenpropit, imetit and pyridoxine on intracellular calcium were also investigated.

The findings of the present study may be summarized as below:

1. PHT, SVP and NAC produced anticonvulsant activities in the mouse MES test.

   *NAC seems to possess an inherent anticonvulsant property with therapeutic potential for generalized tonic-clonic seizures.*

2. The experimentally determined ED\(_{50}\) values for the three fixed ratio combinations (i.e. 1:1, 1:3 and 3:1) of PHT and NAC did not differ significantly from their corresponding theoretically calculated ED\(_{50}\) values.

   *All combinations studied between PHT and NAC displayed additive interactions.*

3. The experimentally determined ED\(_{50}\) values for the combinations of SVP and NAC (at the fixed ratios of 1:1, 1:3 and 3:1) were lower than the theoretically calculated ED\(_{50}\) values, however a significant difference was evident only with the 1:3 and 3:1 ratio combinations.

   *The interaction of SVP with NAC at the fixed ratio of 1:1 is additive, while at the fixed ratios of 1:3 and 3:1, these drugs seem to act synergistically.*
The results of the drug interaction studies (as mentioned in points 2 and 3) reveal beneficial pharmacodynamic interactions between AEDs (i.e. PHT, SVP) and NAC for the prevention of electroshock induced seizures.

4. PHT, SVP, NAC (ED$_{50}$ doses) and their 3:1 ratio combinations (ED$_{50 \ exp}$ values) did not produce any significant changes in the grip strength and the spontaneous alternation behavior.

*At optimal dose levels, the adverse effects on neuromuscular function and memory are minimal or rare.*

5. MES induced seizures produced a mild (non-significant) reduction in the cortical GSH and MDA levels.

*Endogenous antioxidant defense mechanisms are activated immediately after seizures.*

6. Compared to the electroshock group, PHT, SVP, NAC (at the ED$_{50}$ doses) and their combinations (at the fixed ratio of 3:1) did not modulate the cortical GSH and MDA levels significantly.

*None of the drug treatments seem to adversely modulate the body's endogenous antioxidant defense mechanisms.*
7. Seizures induced by MES caused a mild elevation (non-significant) in the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels.

Seizures tend to produce mild stress on the liver

8. Pretreatment with the combination of PHT (5 mg/kg) and NAC (54 mg/kg) before electroshock treatment caused a significant elevation in serum ALP activity compared to the normal control group. No such effect was observed in the group that was pretreated with the combination of SVP (110 mg/kg) and NAC (33 mg/kg).

NAC seems to be a better adjunct with SVP than PHT with respect to its effect on serum ALP levels.

9. The serum calcium levels were not modified significantly following combination treatment of AEDs (i.e. PHT, SVP) with NAC.

NAC as an adjunct seems to be devoid of any adverse effects on serum calcium homeostasis.

10. A statistically non-significant decrease in the whole brain histamine concentration was observed following MES induced seizures in mice.

Seizures tend to activate protective histaminergic mechanisms, resulting in histamine release and hence the reduction in brain histamine levels.
11. In the groups that were pretreated with PHT (ED$_{50}$ dose), NAC (ED$_{50}$ dose) or their 3:1 ratio combination (at ED$_{50}$ exp value), a significant decrease in brain histamine content was observed.

Histaminergic mechanisms may play a role in the effects of PHT and NAC against MES induced seizures and these drugs tend to facilitate a state of postictal protection.

12. A significant inhibition of MES induced seizures was seen after the simultaneous use of clobenpropit and pyridoxine.

The combination of clobenpropit with pyridoxine appears to exhibit beneficial interaction for the prevention of electroshock induced seizures.

13. No significant effects were evident on the brain histamine levels following combination treatment with clobenpropit and pyridoxine.

The beneficial pharmacodynamic interaction between clobenpropit and pyridoxine might not be mediated by the histaminergic mechanisms.

14. PHT and SVP produced a significant reduction in the intracellular calcium concentrations in the mouse whole brain synaptosomes. However, NAC, clobenpropit, imetit and pyridoxine did not significantly effect the intracellular calcium concentrations.
Our results not only suggest an inherent anticonvulsant activity of NAC, but also highlight the sensitizing action of NAC on the antiepileptic effects of PHT and SVP against MES induced seizures. Further, the combinations (at the dose ratio tested) produced minimal adverse effects on neuromuscular function, memory and liver function. The serum calcium homeostasis was also not significantly affected. Our results thus hold promise for the use of NAC as an adjunct to PHT and SVP therapy. However, further pharmacological and biochemical investigations are required to understand the basic mechanisms responsible for the beneficial effects.