CHAPTER - 6

Summary
Phenytoin is one of the most effective and widely used antiepileptic drugs. Phenytoin induced toxicity is considered to be associated with oxidative stress. Intervention of antioxidants on phenytoin induced toxicity and oxidative stress is considered worthwhile to improve the quality of antiepileptic treatment. The present investigation revealed the adverse effects of phenytoin and evaluated the influence of antioxidants such as Vit C, Vit E, ALA and NAC on phenytoin induced oxidative stress and adverse effects.

The present study exemplified that administration of phenytoin (20 mg/Kg) for 45 days significantly decreased the enzymatic (SOD, catalase) and non enzymatic (GSH, Vit C) antioxidants along with total antioxidant status whereas, increased the lipid peroxidation. Phenytoin therapy was also observed to significantly reduce the total RBC, WBC and platelet count, haemoglobin content as well as packed cell volume. The potentially toxic arene oxide metabolites of phenytoin are believed to be responsible for the induction of oxidative stress and bone marrow toxicity resulting in anaemia and other haematological disorders.

Supplementation of Vit C reversed the phenytoin depleted GSH, Vit C, SOD, catalase, total antioxidant status and decreased the extent of lipid peroxidation augmented by phenytoin thereby, ameliorated the antioxidant defence against oxidative stress. Thus, Vit C protected the erythrocytes, leucocytes and platelets from oxidative damage and restored the blood cell count along with haemoglobin content near to normal values. Vit C at higher doses (100 and 200 mg/Kg) was found to be more effective against phenytoin induced oxidative stress and haematotoxicity. Vit E (100 and 200 mg/Kg) revealed protection against phenytoin induced haematotoxicity and oxidative stress, this effect is believed to be related to its intrinsic ability to scavenge free radicals. Supplementation of ALA (100 and 200 mg/Kg) along with
Phenytoin up-regulated the levels of enzymatic and non-enzymatic antioxidants, total antioxidant status, blood cell count, haemoglobin percentage and packed cell volume whereas it decreased the lipid peroxide content. The protective effect of ALA against phenytoin induced haematotoxicity is believed to be due to its antioxidant as well as endogenous antioxidant regenerating potential. In the present investigation, co-administration of NAC (100 and 200 mg/Kg) with phenytoin was observed to improve the enzymatic and non-enzymatic antioxidant status, reduce the oxidative stress and ameliorate the haematological parameters adversely affected by long term phenytoin administration. All the above antioxidants at their higher doses (100 and 200 mg/Kg) offered protection against phenytoin induced oxidative damage and haematotoxicity.

Phenytoin was also observed to induce oxidative stress in brain regions resulting in serious behavioural abnormalities. The present study revealed that phenytoin increased the degree of lipid peroxidation in cerebral cortex, cerebellum, mid brain, pons and medulla oblongata. Phenytoin induced neuronal damage and enhanced ACh E activity in the above brain regions is considered to be responsible for memory impairment. Phenytoin induced damage to cerebellum resulted in postural imbalance, ataxia and motor co-ordination. Phenytoin also decreased the spontaneous motor activity and exploratory behaviour in rats, which describes the CNS depressant action of the drug. The histopathological changes in brain sections illustrated the extent of damage induced by phenytoin. Brain sections of phenytoin treated rats showed damaged cells in addition to congestion in periventricular region and cortex, which confirmed phenytoin induced apoptosis in cortex and periventricular region. Oxidative stress and damage in brain regions were considered to be the underlying factors responsible for phenytoin induced behavioural abnormalities.
Vit C (50, 100, 200 mg/Kg) supplementation with phenytoin decreased the lipid peroxidation in different brain regions. The higher doses of Vit C (100, 200 mg/Kg) were effective in reversing phenytoin induced damages in rat brain regions. Vit C appreciably reversed the behavioural abnormalities by offering protection against oxidative stress induced by phenytoin in rat brain regions. Vit C also significantly reduced the phenytoin enhanced regional brain ACh E activity in a dose dependent fashion thereby, improved the cholinergic transmission in CNS. This was believed to be one of the mechanisms through which Vit C ameliorated memory. The histopathological investigations revealed that Vit C at higher doses (100, 200 mg/Kg) was effective in reversing phenytoin induced damage in rat brain regions. Vit E at all the doses decreased the regional brain lipid peroxidation, which in turn protected the brain regions from oxidative damage and thus, reversed the phenytoin induced behavioural abnormalities. Vit E also exhibited a significant dose dependent reduction in regional brain ACh E activity. Though Vit E at doses 50 and 100 mg/Kg showed necrosis in brain and congested choroid plexus respectively, at higher dose Vit E (200 mg/Kg) showed normal parenchyma with occasional dilated blood vessels. Hence, higher dose of Vit E (200 mg/Kg) was found to be more effective in reversing phenytoin induced neurotoxicity when compared to lower doses. In the present study, it was found that ALA significantly increased the muscular coordination, muscle strength and locomotor activity which were considerably reduced by phenytoin. Both ALA and especially DHLA are potent antioxidants and regenerate other antioxidants like Vit C and Vit E through redox cycling and raise intracellular GSH levels. Thus, ALA is considered as an ideal antioxidant in the treatment of oxidative brain damage and neural disorders involving free radicals. ALA decreased phenytoin induced lipid peroxidation and thus alleviated the behavioural abnormalities induced by long term
Phenytoin administration. ALA dose dependently reduced the regional brain AChE activity and thereby, preserved ACh in CNS and prevented phenytoin induced memory impairment. ALA (50 mg/Kg) supplementation showed gliosis and congestion in brain, whereas ALA (100 and 200 mg/Kg) treated groups showed normal brain parenchyma. ALA produced a dose dependent improvement on phenytoin induced behavioural abnormalities and neurotoxicity. NAC was observed to decrease phenytoin induced lipid peroxidation as well as AChE activity in brain and improved the phenytoin affected cognitive function, muscular coordination, locomotor and exploratory activity in a dose dependent fashion. Though low dose of NAC (50 mg/Kg) showed periventricular inflammation in brain, at a dose of 100 and 200 mg/Kg showed normal brain parenchyma evidencing the degree of protection offered by the above antioxidant against phenytoin induced brain damage. NAC and ALA (100 and 200 mg/Kg) at their higher doses were found to be more effective when compared with the other two antioxidants in alleviating the behavioural abnormalities and neurotoxicity induced by phenytoin therapy.

Administration of phenytoin at a dose of 20mg/Kg for 45 days showed severe hepatic damage, increased liver lipid peroxidation, markedly increased the levels of serum aminotransferases, ALP, bilirubin, along with a decrease in albumin and total protein. In addition to the above, body weight of the rats was decreased whereas the relative liver weight was increased in phenytoin treated rats. The histopathological investigation revealed that livers of the phenytoin treated animals exhibited severe congestion, periportal inflammation, fatty degeneration and hepatocellular necrosis. Phenytoin and its metabolites by depleting the endogenous antioxidant levels and enhancing lipid peroxidation in hepatocytes resulted in severe hepatotoxicity.
Co-administration of Vit C (100 and 200 mg/Kg) along with phenytoin for 45 days significantly decreased the phenytoin augmented SGOT, SGPT, bilirubin and ALP, whereas increased albumin and total protein levels depleted by phenytoin. Vit C supplementation also significantly up regulated the enzymatic, non-enzymatic antioxidants reduced by phenytoin and decreased the phenytoin enhanced liver lipid peroxidation. Vit C reversed phenytoin induced periportal inflammation, sinusoidal congestion, haemorrhage and hepatic necrosis. Thus, Vit C was reported to alleviate the liver damage precipitated by phenytoin. The present study also reveals the hepatoprotective nature of Vit E against phenytoin induced hepatic damage. The antioxidant was observed to significantly reduce the markers of hepatotoxicity such as serum transaminases, ALP and bilirubin augmented by phenytoin, whereas increased the levels of albumin and total protein depleted by phenytoin. Vit E also improved the liver histopathological damages induced by phenytoin. On virtue of its free radical scavenging property, Vit E alleviated lipid peroxidation and oxidative stress, thus rendered significant protection against phenytoin induced hepatotoxicity. The present study also showed that supplementation with ALA decreased the biochemical markers of hepatotoxicity and improved the hepatic histopathological damages induced by phenytoin. ALA exerted significant protection against phenytoin induced hepatic damage by its ability to attenuate the lipid peroxidation and augment the antioxidant defence mechanism by recycling endogenous antioxidants. Supplementation with NAC along with phenytoin was observed to decrease the markers of hepatotoxicity and increase the albumin and total protein which were affected by phenytoin. NAC improved the liver histopathological damages induced by phenytoin. NAC was proved to replenish the depleted GSH in liver, which describes its hepatoprotective potential. All the antioxidants offered protection against hepatotoxicity induced by
Phenytoin. The degree of protection offered by NAC and ALA was more appreciable than Vit C and Vit E.

Phenytoin is known to induce hyperglycemia primarily by an inhibitory effect on insulin release. Phenytoin induced free radical generation and oxidative stress was believed to be the basic reason for the incidence of insulin resistance. Administration of phenytoin was also found to cause pancreatitis. In the present study also phenytoin was observed to induce hyperglycemia. In addition to the above carbohydrate metabolic disorder, phenytoin therapy was also observed to be associated with hyperlipidemia. Long term phenytoin treatment was reported to increase the serum TG and cholesterol levels. The changes were most likely to reflect phenytoin’s effects on hepatic lipid metabolism. Phenytoin is a potent inducer of CYP450 enzymes, involved in cholesterol synthesis. The present investigation reported that phenytoin treatment significantly increased the TC, TG, LDL, VLDL and decreased the HDL levels.

Vit C was found to exert a dose dependent anti-hyperglycemic and anti-hyperlipidemic effect against phenytoin induced metabolic disorders. Vit E supplementation reduced the blood glucose level which was increased by phenytoin, whereas it showed negligible resistance against phenytoin induced hyperlipidemia. The present research explored the anti-hyperglycemic and anti-hyperlipidemic effect of ALA against phenytoin induced increase in blood glucose and lipids. The effect was observed to be dose dependent in which 100 and 200 mg/Kg of ALA had more significant activity. Co-administration of NAC with phenytoin restored the disturbed glucose and lipid profile. NAC dose dependently decreased the blood glucose and lipids elevated by phenytoin treatment.
Investigation of pharmacokinetic and pharmacodynamic interaction revealed that the serum phenytoin concentration did not differ between phenytoin treated group and groups subjected to combination of antioxidants and phenytoin. This finding suggests that antioxidant supplementation did not reverse the phenytoin induced toxicity by reducing serum phenytoin levels, thereby confirming that there was no incidence of pharmacokinetic interaction between phenytoin and antioxidants. Both phenytoin alone treated group and groups subjected to combination of phenytoin and antioxidants showed the same degree of protection against MES induced convulsions. This investigation suggests that antioxidant supplementation with phenytoin did not reduce the therapeutic effect of phenytoin, illustrating that there was no pharmacodynamic interaction between phenytoin and the selected antioxidants.

CONCLUSION

In the present investigation, phenytoin was observed to induce haematotoxicity, behavioural abnormalities, hepatotoxicity and metabolic disorders. The toxicities induced by phenytoin were mediated via oxidative stress as the drug caused a rise in lipid peroxidation (blood, liver and brain) and fall in SOD, catalase and GSH levels in blood. All the antioxidants were effective in reversing phenytoin induced toxicities except Vit E, which was not as effective as the other antioxidants against hyperlipidemia induced by phenytoin. The degree of protection offered by ALA and NAC was more appreciable than Vit C and Vit E. The selected antioxidants effectively reduced the toxicities induced by phenytoin without interfering with the bioavailability of the drug and its therapeutic effect.

Though all the antioxidants at all the three doses improved the toxicities induced by phenytoin, at the dose of 50 mg/Kg the values were not reversed back to normal
values. Enzyme inducing property of phenytoin was believed to increase the clearance and decrease the bioavailability of co-administered drugs and supplements. This might possibly account for the ineffectiveness of all the above antioxidants at the dose of 50 mg/Kg.

The degree of protection offered by the antioxidants against phenytoin induced toxicities was observed to be in the order of ALA, NAC, Vit C and Vit E, exception being NAC which conferred a superior protection against phenytoin induced hepatotoxicity. Even though the physicochemical properties of the above selected antioxidants are different, they exerted protection by their antioxidant potential.

This investigation recommends antioxidant supplementation against phenytoin induced toxicities without affecting the potential therapeutic outcomes of phenytoin. Antioxidant supplementation is suggested to render an excellent antiepileptic therapy devoid of toxicity which may substantially improve the quality of life in epileptic patients undergoing long term phenytoin treatment.

**FUTURE SCOPE OF THE WORK**

The present study evaluated the degree of protection offered by antioxidants against phenytoin induced toxicities in rats and the results were found to be satisfactory. This investigation may find an excellent scope in framing a superior antiepileptic treatment strategy if extended on epileptic patients undergoing long term phenytoin mono therapy.