Results and Discussion
Results and Discussion

5.1 Influence of antioxidants on phenytoin induced haematotoxicity and oxidative stress in rats
RESULTS

Effect of antioxidants on phenytoin induced alterations in RBC count

The effect of phenytoin and phenytoin supplemented with different doses of Vit C, Vit E, ALA and NAC on RBC count is summarized in Graph 1. Administration of phenytoin at a dose of 20 mg/Kg for a period of 45 days significantly (p< 0.001) reduced the total RBC count.

Vit C, Vit E and NAC at a dose of 50 mg/Kg significantly increased the total RBC count (p< 0.01), while ALA at all the three doses and the other three antioxidants at higher doses i.e. 100, 200 mg/Kg produced an extremely significant (p< 0.001) increase in total RBC count when compared to those animals treated with phenytoin. All the selected antioxidants at their higher doses increased the RBC count affected by phenytoin and brought the values close to that of normal.

Effect of antioxidants on phenytoin induced alterations in haemoglobin levels

Graph 2 depicts the effect of supplementation of different doses of Vit C, Vit E, ALA and NAC on phenytoin induced alterations in haemoglobin levels of rats. Administration of phenytoin (20 mg/Kg) for a period of 45 days significantly (p< 0.001) reduced the levels of haemoglobin.

Vit C, Vit E, NAC and ALA appreciably increased the haemoglobin levels decreased by phenytoin treatment in a dose dependent fashion. All the antioxidants at a dose of 200 mg/Kg increased the haemoglobin levels significantly (p< 0.001) and brought the values near to that of normal. Antioxidant supplementation with phenytoin was observed to reverse phenytoin induced haemoglobin deficiency.
Graph 1

Effect of antioxidants on phenytoin induced alterations in RBC count

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results - Haematotoxicity

Graph 2

Effect of antioxidants on phenytoin induced alterations in haemoglobin levels

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Effect of antioxidants on phenytoin induced alterations in total leukocyte count

Graph 3 illustrates the effect of phenytoin and phenytoin supplemented with graded doses of Vit C, Vit E, ALA and NAC on total leukocyte count of rats. Administration of phenytoin (20 mg/Kg) for a period of 45 days significantly (p< 0.001) reduced the leukocyte count.

Vit C, Vit E, ALA and NAC supplementation (50, 100 mg/Kg) significantly (p< 0.001) increased the total leukocyte count dose dependently. All the antioxidants at their higher dose (200 mg/Kg) were found to raise the leukocyte count close to that of the control group. Antioxidant supplementation with phenytoin was observed to reverse phenytoin induced leukocytopenia.

Effect of antioxidants on phenytoin induced alterations in platelet count

Graph 4 illustrates the effect of treatment of phenytoin and combination of phenytoin with different doses of Vit C, Vit E, ALA and NAC on platelet count of rats. Phenytoin (20 mg/Kg) treatment for a period of 45 days significantly (p< 0.001) reduced the platelet count.

Supplementation of Vit C, Vit E, ALA and NAC (50 mg/Kg) with phenytoin significantly (p< 0.001) increased the platelet count. All the antioxidants at higher doses (100, 200 mg/Kg) raised the platelet count close to that of the control group. Antioxidant supplementation with phenytoin was observed to reverse the phenytoin induced thrombocytopenia.
Graph 3

Effect of antioxidants on phenytoin induced alterations in total leukocyte count

Values are expressed as mean± SEM of 6 animals.

***\( p < 0.001 \), **\( p < 0.01 \), *(p < 0.05) Vs Control group

+++\( p < 0.001 \), ++\( p < 0.01 \), +(p < 0.05) Vs Phenytoin group
Graph 4

Effect of antioxidants on phenytoin induced alterations in platelet count

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), ** (p< 0.01), * (p< 0.05) Vs Control group

++++ (p< 0.001), +++ (p< 0.01), ++ (p< 0.05) Vs Phenytoin group
Effect of antioxidants on phenytoin induced alterations in packed cell volume

Graph 5 illustrates the effect of treatment of phenytoin and phenytoin supplemented with different doses of Vit C, Vit E, ALA and NAC on packed cell volume of rats. Administration of phenytoin (20 mg/Kg) for a period of 45 days significantly (p< 0.001) reduced the packed cell volume.

The selected antioxidants such as Vit C, Vit E, ALA and NAC showed a dose dependent increase in the packed cell volume. The antioxidants at higher doses (100, 200 mg/Kg) showed a remarkable increase (p< 0.001) in packed cell volume. All the antioxidants significantly improved the packed cell volume affected by phenytoin.

Effect of antioxidants on phenytoin induced alterations in SOD

Graph 6 illustrates the effect of treatment of phenytoin and phenytoin supplemented with different doses of Vit C, Vit E, ALA and NAC on SOD levels. Administration of phenytoin (20 mg/Kg) for 45 days significantly (p< 0.001) reduced the levels of SOD.

Vit C, Vit E, ALA and NAC significantly increased the SOD levels in a dose dependent manner. Vit C, ALA and NAC at the dose of 100 and 200 mg/Kg and Vit E at the dose of 200 mg/Kg showed a very appreciable (p< 0.001) increase in the levels of SOD near to that of control group.

All the antioxidants significantly improved the activity of SOD which was affected by phenytoin treatment.
Results - Haematotoxicity

Graph 5

Effect of antioxidants on phenytoin induced alterations in packed cell volume

Values are expressed as mean±SEM of 6 animals.

*** (p<0.001), ** (p<0.01), * (p<0.05) Vs Control group

+++ (p<0.001), ++ (p<0.01), + (p<0.05) Vs Phenytoin group
Results - Haematotoxicity

Graph 6

Effect of antioxidants on phenytoin induced alterations in SOD

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results - Haematotoxicity

**Effect of antioxidants on phenytoin induced alterations in catalase activity**

Graph 7 shows the effect of phenytoin and phenytoin supplemented with different doses of Vit C, Vit E, ALA and NAC on catalase activity. Administration of phenytoin 20 mg/Kg for 45 days significantly (p< 0.001) reduced the activity of catalase.

Vit E, ALA and NAC at the dose of 50 mg/Kg did not increase the phenytoin reduced catalase levels. Vit C (50mg/Kg) and Vit E (100mg/Kg) moderately (p< 0.01) improved the catalase activity.

Vit C (100, 200 mg/Kg), Vit E (200 mg/Kg), ALA (100, 200 mg/Kg) and NAC (100 and 200 mg/Kg) significantly (p< 0.001) increased the catalase activity; however the values did not reach normal. ALA at the dose of 200 mg/Kg reversed the catalase levels near to normal values.

All the antioxidants at higher doses significantly improved the activity of catalase which was affected by phenytoin treatment.

**Effect of antioxidants on phenytoin induced alterations in GSH**

Graph 8 shows the effect of phenytoin and phenytoin supplemented with different doses of Vit C, Vit E, ALA and NAC on GSH levels. Administration of phenytoin 20 mg/Kg for 45 days significantly (p< 0.001) decreased the levels of GSH.

All the antioxidants at their lower doses (50, 100 mg/Kg) did not significantly reverse the phenytoin depleted GSH levels. At higher doses (100 and 200 mg/Kg) all the antioxidants exhibited a significant (p< 0.01) increase in the levels of GSH but the values did not reach the normal.

**Effect of antioxidants on phenytoin altered Vitamin C levels**

Graph 9 shows the effect of phenytoin and phenytoin supplemented with different doses of Vit C, Vit E, ALA and NAC on Vitamin C levels. Administration of
Results - Haematotoxicity

Phenytoin 20 mg/Kg for a period of 45 days significantly (p< 0.001) decreased the Vitamin C levels.

The antioxidants at all the three doses produced a significant (p< 0.001) increase in Vitamin C levels decreased by phenytoin and at a dose of 100 mg/Kg the values reached normal and at 200 mg/Kg Vitamin C levels were increased more than normal.

**Effect of antioxidants on phenytoin induced alterations in total antioxidant status**

Graph 10 shows the effect of phenytoin and phenytoin supplemented with different doses of Vit C, Vit E, ALA and NAC on total antioxidant status. Administration of phenytoin 20 mg/Kg for a period of 45 days significantly (p< 0.001) decreased the total antioxidant status.

Vit C (200 mg/Kg), NAC (100, 200 mg/Kg), Vit E and ALA at all the three doses (50, 100, 200 mg/Kg) significantly (p< 0.001) increased the phenytoin depleted total antioxidant status, but the values did not reach the normal.

**Effect of antioxidants on phenytoin enhanced lipid peroxidation**

Graph 11 shows the effect of phenytoin and phenytoin supplemented with different doses of Vit C, Vit E, ALA and NAC on lipid peroxidation. Administration of phenytoin (20 mg/Kg) for a period of 45 days significantly (p< 0.001) increased the lipid peroxidation.

Vit C and Vit E at the dose of 50 mg/Kg showed a significant reduction (p< 0.01) in MDA levels; Vit C and Vit E at their higher doses (100 and 200 mg/Kg) and ALA and NAC at all the three doses (50, 100 and 200 mg/Kg) exhibited an extremely significant (p<0.001) reduction in phenytoin induced lipid peroxidation, however the values were not brought down near to normal.
Graph 7

Effect of antioxidants on phenytoin induced alterations in catalase activity

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), ** (p< 0.01), * (p< 0.05) Vs Control group

+++ (p< 0.001), ++ (p< 0.01), + (p< 0.05) Vs Phenytoin group
Results - Haematotoxicity

Graph 8

Effect of antioxidants on phenytoin induced alterations in GSH levels

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results - Haematotoxicity

Graph 9

Effect of antioxidants on phenytoin altered Vitamin C levels

Values are expressed as mean± SEM of 6 animals.

***(p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results - Haematotoxicity

Graph 10

Effect of antioxidants on phenytoin induced alterations in total antioxidant status

Values are expressed as mean± SEM of 6 animals.

***(p < 0.001), **(p < 0.01), *(p < 0.05) Vs Control group

+++(p < 0.001), ++(p < 0.01), +(p < 0.05) Vs Phenytoin group
Results - Haematotoxicity

Graph 11

Effect of antioxidants on phenytoin enhanced lipid peroxidation

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
DISCUSSION

In the present study, administration of phenytoin (20 mg/Kg) caused a significant decrease in haemoglobin content, erythrocytes, leukocytes, platelets and packed cell volume. Supplementation with antioxidants such as Vit C, Vit E, ALA and NAC at graded doses (50, 100 and 200 mg/Kg) for 45 days significantly reversed the phenytoin induced reduction in blood cell count, haemoglobin content and packed cell volume. Phenytoin also significantly decreased the enzymatic antioxidants such as SOD and catalase, non-enzymatic antioxidants like GSH and Vit C along with total antioxidant status and increased the lipid peroxidation in blood. Vit C, Vit E, ALA and NAC at 100 and 200 mg/Kg for 45 days significantly augmented enzymatic, non-enzymatic antioxidants, total antioxidant status and decreased the lipid peroxidation. The above selected antioxidants significantly improved the overall antioxidant defence mechanism, which in turn reversed the haematological disturbances induced by phenytoin.

Effect of phenytoin on antioxidant status

Oxidative stress is the shift in balance between cellular oxidation-reduction reactions in favour of oxidation, leading to cellular damage and is indicated by accumulation of oxidized products of lipids, proteins and nucleic acids (Halliwell and Gutteridge, 1989).

Phenytoin is bioactivated to reactive intermediates that bind irreversibly to macromolecules in neutrophils. Phenytoin was reported to reduce 40% of GSH content in blood (Mannervik, 1985). GSH offers antioxidant defence mechanism and thereby, renders protection against oxidative stress (Jain, et al., 1991). Decreased tissue GSH concentrations are associated with cellular damage (Martensson and Meister, 1991; Suthanthiran, et al., 1990) and depressed immunity (Fischman, et al., 1981; Franklin,
et al., 1990). Phenytoin and its intermediates are proved to generate free radicals and thereby elevate the lipid peroxidation, reduce the antioxidants like GSH, catalase, SOD and total antioxidant capacity (Mays, et al., 1995; Mahle and Dasgupta, 1997). In the present study, phenytoin decreased the enzymatic and non enzymatic antioxidants, total antioxidant status and increased the lipid peroxidation.

**Effect of phenytoin on haematological parameters**

Aromatic AEDs such as phenytoin and carbamazepine are reported to induce haematological disturbances (Thaakur and Puspha, 2007; 2008b). Phenytoin undergoes oxidative metabolism and generates of potentially toxic arene oxide intermediates, which bind covalently to cellular macromolecules and cause cytotoxic damage (Jerina and Daly, 1974; Nebert and Jensen, 1979), bone marrow toxicity (Dosch, et al., 1982) and aplastic anemia. The drug was shown to suppress the T lymphocyte activity (Kikuchi, et al., 1984; Okamoto, et al., 1987; 1988; 1989; Okada, et al., 2001; Abbondazo, et al., 1995) and thereby, depressed immunological function (Brandt and Nilson, 1976; Ishizaka, et al., 1992; Kondo, et al., 1994; Chaudhri, et al., 1989). Haematological abnormalities induced by phenytoin include leukocytosis with atypical lymphocytes, eosinophilia (Choen and Bovasso, 1973), leukopenia (Rawanduzy, et al., 1993) and agranulocytosis (Tsan, et al., 1976; Laurenson, et al., 1994). An immune mechanism with phenytoin dependent antigranulocyte antibody caused leukopenia which resolved on discontinuing the therapy (Laurenson, et al., 1994). Phenytoin was reported to have a direct toxic effect on bone marrow resulting in pancytopenia (Hayashi, et al., 1994) thereby, reduced the packed cell volume as well. Thrombocytopenia was evidenced within 15 days of initiation of phenytoin therapy (Holtzer and Reisner-Keller, 1997; Alehan, et al., 1999).
Online with the above findings, in the present study also phenytoin decreased the haemoglobin, erythrocytes, total leukocytes, thrombocytes and packed cell volume.

**Effect of Vitamin C on phenytoin induced oxidative stress and haematotoxicity**

In the present study, Vit C increased the GSH concentration depleted by phenytoin. Johnston, et al., (1993) reported that Vit C elevated the GSH concentration in blood and RBC and improved the overall antioxidant capacity of blood. Vit C was observed to reduce the plasma oxidised glutathione (GSSG), by which it showed a better index of antioxidant status and protection (Henning, et al., 1991). Vit C supplementation increased the GSH (Lenton, et al., 2000), SOD and catalase activities in lymphocytes (Halliwell and Gutteridge, 1989; Khassaf, et al., 2003). Vit C is proposed to decrease the oxidative stress induced by H$_2$O$_2$ by reducing the generation of free radical species. Vit C reduced oxidative DNA damage (Noroozi, et al., 1998) and single strand breaks (Panayiotidis and Collins, 1997) in human lymphocytes. Vit C supplementation was also observed to reduce 8-oxo deoxyguanosine (oxidized DNA) in WBC (Fraga, et al., 1991). In the present study, Vit C reversed the phenytoin depleted GSH, Vit C, SOD, catalase, total antioxidant status and decreased the lipid peroxide content augmented by phenytoin, thereby protected the erythrocytes, leukocytes and platelets from oxidative stress.

The results of the present study indicated that phenytoin treatment decreased the levels of SOD, CAT, GSH, Vit C and total antioxidant status whereas, it increased the lipid peroxidation. Phenytoin was also observed to cause a decrease in the number of RBCs, WBCs, platelets, haemoglobin content and packed cell volume. Vit C improved the serum enzymatic and non-enzymatic antioxidant levels as well as increased the total
antioxidant capacity and decreased the lipid peroxidation. Vit C also improved the haematological parameters affected by phenytoin.

The present results support the view that Vit C protects against phenytoin induced haematotoxicity and oxidative stress in rats. The effect of Vit C is believed to be related to its intrinsic ability to scavenge free radicals. This investigation reports the protective effect of Vit C against phenytoin induced haematotoxicity.

**Effect of Vit E on phenytoin induced oxidative stress and haematotoxicity**

In the present study, Vit E increased the phenytoin depleted GSH concentration. GSH dependent enzyme, glutathione-S-transferase plays an important role in detoxification of electrophiles and xenobiotics. Vit E by replenishing GSH not only improved the antioxidant status but also enhanced the detoxification process. Vit E together with GSH prevents destructive effects of ROS on PUFAs in biomembranes and thereby reduces lipid peroxidation (Van Haaften, et al., 2003).

Vit E was found to increase the haematological parameters such as RBC, WBC, platelet count, haemoglobin content and packed cell volume, which were critically diminished upon exposure to microwave radiation (Aweda, et al., 2011). Long-term Vit E treatment was found to improve the life span of RBC, increase the haemoglobin content and reduce hemolysis in patients with Glucose-6-Phosphate Dehydrogenase deficiency (Corash, et al., 1980). In streptozotocin induced diabetic rats, Vit E was found to enhance the antioxidant defence, decrease lipid peroxidation (Packer and Landvik, 1990; Radak, et al., 1999), and regulate certain antioxidant enzymes as well as vitamins in the plasma and RBC. GPx and catalase form an integral part of the enzymatic antioxidant defence system responsible for protection against ROS induced damage. Vit C and Vit E supplementation improved GPx activity in the RBC and plasma. One of the most important intracellular antioxidant systems is the glutathione
redox cycle. GSH is an essential compound for maintaining cellular integrity because of its reducing properties and participation in cellular metabolism (Menegola, et al., 1996). Vit E was also proposed to reduce the free radical species generated by $H_2O_2$ and combat oxidative damage. Vit E was reported to elevate SOD levels and total antioxidant status (Knezević, et al., 2000). Oral administration of high doses of Vit E (300 mg/day for 15 days) reduced MDA levels in serum and erythrocytes along with an improvement in haematological values in children with cholestasis and it was concluded that long-term treatment might completely reverse the oxidative damage in the above condition (Lubrano, et al., 1989).

The results of the present study indicated that phenytoin decreased the levels of endogenous enzymatic, non enzymatic antioxidants and total antioxidant status, with concomitant increase in lipid peroxidation. In addition to the above, phenytoin was observed to bring down the blood cell count, haemoglobin content and packed cell volume. Vit E improved the serum enzymatic, nonenzymatic antioxidant levels and total antioxidant capacity along with a decrease in lipid peroxidation, also increased blood cell count, haemoglobin content and packed cell volume. Thus, Vit E (100 and 200 mg/Kg) was found to offer protection against phenytoin induced haematotoxicity and oxidative stress in rats. The protective effect of Vit E is believed to be related to its antioxidant property.

**Effect of ALA on phenytoin induced oxidative stress and haematotoxicity**

ALA regenerates a variety of antioxidants including GSH (Busse, et al., 1992), Vit C and Vit E along with mitochondrial antioxidant coenzyme Q10 (Kagan, et al., 1990; Packer, et al., 1995). ALA is taken up rapidly by the cells and gets reduced to DHLA, which in turn reduces cystine to cysteine and accelerates the biosynthesis of GSH. ALA and DHLA were found to protect RBC membrane (Ghibu, et al., 2009).
Treatment with adriamycin (1 mg/Kg/day i.v.), once a week for a period of 12 weeks, lowered the haematological indices like haemoglobin levels and haematocrit, decreased the activities of endogenous antioxidants like SOD, GPx, Vit A, Vit C and Vit E along with an increase in erythrocyte membrane lipid peroxidation. ALA (35 mg/Kg/day i.p.) was found effective in minimizing the toxic side effects of adriamycin (Malarkodi, et al., 2004).

In mice, it has been demonstrated that ALA protected the bone marrow stem cells against radiation induced injury. Administration of ALA increased the survival rate of irradiated mice from 35% to 90% (Ramakrishnan, et al., 1992).

There are researches on the treatment of AIDS with ALA. AIDS is characterized by HIV infection along with progressive depletion of T-cells, impaired immune system, increased oxidative reactions (Sato, et al., 1995), GSH deficiency (Staal, et al., 1992) and elevated free radical generation (Greenspan and Aruoma, 1994). GSH is necessary for regulating immune response. Oxidative stress is considered to be one of the core mechanisms involved in the depletion of T-helper cells in AIDS (Pace and Leaf, 1995).

ALA was reported to raise the GSH levels as much as 30-70% both in vivo and in vitro, making it beneficial for HIV patients (Han, et al., 1995). In HIV patients, ALA increased the plasma Vit C levels, GSH levels, total blood thiol groups along with T helper lymphocyte levels and decreased the lipid peroxidation products, thus, improved the overall antioxidant status in blood of HIV infected individuals (Fuchs, et al., 1993).

In the present investigation also ALA improved the serum enzymatic, non enzymatic antioxidant levels, total antioxidant capacity and decreased the lipid peroxidation.
ALA also improved the haematological parameters affected by phenytoin. This study supports the view that ALA affords protection against phenytoin induced haematotoxicity and oxidative stress in rats. The effects of ALA are believed to be related to its ability to regenerate antioxidants and scavenge free radicals.

**Effect of NAC on phenytoin induced oxidative stress and haematotoxicity**

NAC is a thiol containing antioxidant, utilized to mitigate various conditions of oxidative stress. NAC maintains adequate intra cellular GSH levels (Moldeus, et al., 1986) and scavenges ROS (Aruoma, et al., 1989), by the virtue of its ability to synthesis GSH. An *in vivo* study reported that NAC reversed lead induced oxidative stress in RBCs (Gurer, et al., 1998).

N-acetylcysteine amide, the amide form of NAC, elevated the GSH content of RBC, platelets and polymorphonuclear (PMN) leukocytes along with a reduction in ROS *in vitro* in blood cells of beta-thalassemic patients. These effects significantly reduced the sensitivity of thalassemic RBC to hemolysis and phagocytosis by macrophages. Intra peritoneal injection of N-acetylcysteine amide to beta-thalassemic mice (150 mg/Kg) reduced the parameters of oxidative stress. Thus NAC was found to be effective in reducing oxidative stress markers in thalassemic cells both *in vitro* and *in vivo* (Amer, et al., 2008).

NAC was found to increase the levels of SOD, catalase, GSH, Vit C and decrease the incidence of lipid peroxidation thus reduces the oxidative stress and maintains the cellular integrity (Thaakur, et al., 2009).

Exposure of RBCs to high concentrations of glucose, results in depletion of Vit E leading to accumulation of Vit E quinine along with increase in lipid peroxidation. The above cascades are believed to induce blood coagulation. Pre treatment of RBCs with
combination of NAC and Vit E reduced membrane lipid peroxidation and the tendency of RBCs to clot (Jain, et al., 1999). Thus, it was evident from the previous studies that NAC efficiently reduced the oxidative stress and improved the haematological parameters.

In the present study also NAC was observed to decrease the oxidative stress induced by phenytoin and ameliorate the haematological parameters which were adversely affected by phenytoin treatment. The effect of NAC against phenytoin induced haematotoxicity is believed to be due to its antioxidant property and its ability to increase the turnover of GSH.

CONCLUSION

Phenytoin was observed to cause serious disturbances in haematological parameters along with decrease in endogenous antioxidants, total antioxidant status and increase in lipid peroxide content. Oxidative stress was considered to play a pivotal role underlying phenytoin induced haematotoxicity. ALA was found to offer superior protection in improving the haematological parameters by its ability to recycle all the other antioxidants such as GSH, Vit C and Vit E. NAC being a precursor of GSH regenerated other antioxidants which in turn improved the Vit C levels and total antioxidant status in blood. Vit C, a water soluble antioxidant showed a pronounced effect in ameliorating haematological parameters. The lipid soluble antioxidant Vit E also exhibited significant improvement in haematological parameters. Though all the antioxidants at all the three doses improved the haematological parameters, at the dose of 50 mg/Kg the values were not brought down to normal values, which might be due to enzyme inducing property of phenytoin. The present study revealed the protective effect of antioxidants against phenytoin induced oxidative stress and haematotoxicity in the order of ALA, NAC, Vit E and Vit C.
5.2 Influence of antioxidants on phenytoin induced behavioural abnormalities in rats
RESULTS

Effect of Vitamin C on phenytoin induced memory impairment

Effect of treatment of phenytoin and supplementation of phenytoin with graded doses of Vit C on memory is depicted in Graph 12. There was no significant difference in the transfer latency of the control, phenytoin and phenytoin with Vit C (50, 100, 200 mg/Kg) treated groups on 0 day of the study. The transfer latencies increased from 34.5±2.23 sec (0 day) to 124.66±1.82 sec (45th day) (p< 0.001) in phenytoin treated animals. Co-administration of Vit C in all the three doses significantly reduced the transfer latency from 15th day till 45th day. The values decreased from 124.66±1.82 sec to 96.33±1.76 sec (p< 0.001), 93.5±1.43 sec (p< 0.001) and 72.33±2.04 sec (p < 0.001) in vit C 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. Vit C at all the three doses produced significant reversal of phenytoin induced memory impairment in a dose dependent fashion but the values did not reach the normal values.

Effect of Vitamin C on phenytoin impaired exploratory activity

There was no significant difference in the exploratory activity of the control, phenytoin treated and phenytoin with Vit C (50, 100, 200 mg/Kg) pre treated groups on 0 day of the study. The exploratory activity was assessed by the number of head dippings into the holes of the hole board apparatus. The number of head dippings decreased from 24.5±1.5 (0 day) to 2.5±0.76 (45th day) (p< 0.001) in phenytoin treated animals. Co-administration of Vit C in all the three doses significantly increased the exploratory movements from 15th day till 45th day. The number of head dippings increased from 2.5±0.76 in the phenytoin treated group to 6.16±0.6, 11±0.96 (p< 0.001) and 14.16±0.94 (p< 0.001) in Vit C 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. Vit C at higher doses (100, 200 mg/Kg) produced significant reversal of phenytoin impaired exploratory
behaviour in a dose dependent manner but the values did not reach the normal level (Graph 13).

**Effect of Vitamin C on phenytoin induced motor in co-ordination**

There was no significant difference in motor coordination of the control, phenytoin treated and phenytoin with Vit C (50, 100, 200 mg/Kg) pre-treated groups on 0 day of the study. Phenytoin (20 mg/Kg, p.o.) significantly impaired the Rota Rod performance of rats from 120 sec (0 day) to 17.83±0.87 sec on 45th day (p< 0.001). Co-administration of Vit C in all the three doses significantly improved the motor coordination from 15th day to 45th day. The values increased from 17.83±0.87 sec in the phenytoin treated group to 56.16±1.13 sec (p< 0.001), 77±0.96 sec (p< 0.001) and 91.16±1.078 sec (p < 0.001) in Vit C 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. Vit C at all the three doses produced significant reversal of phenytoin impaired motor co-ordination in a dose dependent fashion but the values did not reach normal (Graph 14).

**Effect of Vitamin C on phenytoin impaired locomotor activity**

There was no significant difference in spontaneous motor activity of the control, phenytoin and phenytoin with Vit C (50, 100, 200 mg/Kg) pre treated groups on the initial day of the study. Phenytoin 20 mg/Kg, p.o., significantly decreased the spontaneous motor activity count from 311.66±3.73 (0 day) to 86.5±1.408 (45th day) (p< 0.001). Co administration of Vit C in all the three doses significantly improved the spontaneous activity from 15th day till 45th day. The values increased from 86.5±1.408 in the phenytoin treated group to 134.8±1.5 (p< 0.001), 163.83±2.02 (p< 0.001) and 222.33±1.89 (p < 0.001) in Vit C 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. Vit C at all the three doses produced significant reversal of phenytoin impaired locomotor activity in a dose dependent fashion but the values did not reach the normal values (Graph 15).
Results – Behavioural abnormalities

Effect of Vitamin C on phenytoin induced alterations in regional brain lipid peroxidation

Phenytoin showed a significant rise in lipid peroxidation in medulla, pons, midbrain, cerebellum and cortex. Vit C significantly reduced (p< 0.001) the lipid peroxidation in medulla, pons, midbrain, cerebellum and cortex dose dependently but the values did not reach the normal when compared with the control group (Graph 16).

Effect of Vitamin C on phenytoin altered regional brain ACh E activity

Phenytoin showed a significant rise in ACh E activity in medulla, pons, midbrain, cerebellum and cortex. Vit C significantly reduced (p< 0.001) the activity of ACh E in medulla, pons, midbrain, cerebellum and cortex dose dependently, and at higher dose (200 mg/Kg), the values reached near the normal in all the above brain regions, when compared with the control group (Graph 17).

Effect of phenytoin on regional brain histopathology

Figure 2 illustrates the effect of phenytoin on brain tissues. Control group exhibited normal brain architecture (Figure 2.a), while phenytoin treated group illustrated severe congestion and remarkable degeneration of periventricular neurons along with necrosis (Figure 2.b and 2.c).

Effect of Vitamin C on phenytoin induced alterations in brain histopathology

Figure 3 illustrates the effect of phenytoin and Vit C on brain tissues. Phenytoin and 50 mg/Kg Vit C treated group showed decreased degree of congestion and periventricular neuronal degeneration than phenytoin group (Figure 3.a), whereas phenytoin supplemented with 100 mg/Kg Vit C showed no congestion and negligible periventricular neuronal degeneration (Figure 3.b) and phenytoin in combination with 200 mg/Kg Vit C showed normal periventricular neuronal cells without congestion (Figure 3.c).
Results – Behavioural abnormalities

Graph 12

Effect of Vitamin C on phenytoin induced memory impairment

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Graph 13

Effect of Vitamin C on phenytoin impaired exploratory activity

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), ***(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results – Behavioural abnormalities

Graph 14

Effect of Vitamin C on phenytoin induced motor in-coordination

Values are expressed as mean± SEM of 6 animals.

***( p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++( p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results – Behavioural abnormalities

Graph 15

Effect of Vitamin C on phenytoin impaired locomotor activity

Values are expressed as mean± SEM of 6 animals.

*** (p<0.001), ***(p<0.01), *'(p<0.05) Vs Control group

+++(p<0.001), ++*(p<0.01), +*(p<0.05) Vs Phenytoin group
Results – Behavioural abnormalities

Graph 16

Effect of Vitamin C on phenytoin induced alterations in regional brain lipid peroxidation

Values are expressed as mean± SEM of 6 animals.

***( p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++( p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Graph 17

Effect of Vitamin C on phenytoin induced alterations in regional brain acetyl cholinesterase activity

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Figure 2

Effect of phenytoin on regional brain histopathology

Figure 2(a) Control

Normal Cells

Figure 2(b) Phenytoin

Periventricular Congestion

Dead Cells
Results – Behavioural abnormalities

Figure 2(c) Phenytoin

Necrosis in brain

Figure 3

Effect of Vitamin C on phenytoin induced alterations in brain histopathology

Figure 3 (a) Phenytoin + Vit C (50 mg/Kg)

Periventricular Congestion
Results – Behavioural abnormalities

Figure 3 (b) Phenytoin + Vit C (100 mg/Kg)

Figure 3 (c) Phenytoin + Vit C (200 mg/Kg)
Results – Behavioural abnormalities

Effect of Vitamin E on phenytoin induced memory impairment

The effect of treatment of phenytoin and phenytoin along with Vit E on memory is illustrated in Graph 18. There was no significant difference in the transfer latency of the control, phenytoin and phenytoin with Vit E (50, 100, 200 mg/Kg) pre-treated groups on the 0 day of the study. The retention transfer latencies increased from 34.5±2.23 sec (0 day) to 124.66±1.82 sec (45th day) (p< 0.001) in phenytoin treated animals. Co-administration of Vit E in all the three doses significantly reduced the transfer latency from 15th day till 45th day. The values decreased from 124.66±1.82 sec in the phenytoin treated group to 109.55±1.28 sec (p< 0.001), 98±1.39 sec (p< 0.001) and 88.5±0.99 sec (p< 0.001) in Vit E 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. Vit E at all the three doses produced significant reversal of phenytoin induced memory impairment in a dose dependent fashion but the values did not reach near the normal values.

Effect of Vitamin E on phenytoin impaired exploratory activity

There was no significant difference in the exploratory activity of the control, phenytoin treated and phenytoin with Vit E (50, 100, 200 mg/Kg) pre-treated groups on 0 day of the study. The exploratory activity was assessed by the number of head dippings into the holes of the hole board apparatus. The number of head dippings decreased from 24.5±1.5 (0 day) to 2.5±0.76 (45th day) (p< 0.001) in phenytoin treated animals. Co-administration of Vit E in all the three doses significantly increased the exploratory movements from 15th day till 45th day. The number of head dippings increased from 2.5±0.76 in the phenytoin treated group to 9.5±0.76 (p< 0.001), 12.0±0.57 (p< 0.001) and 16.5±0.76 (p< 0.001) in Vit E 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. All three doses of Vit E produced significant reversal of phenytoin impaired exploratory behaviour in a dose dependent manner, yet the values did not reach back to normal (Graph 19).
Results – Behavioural abnormalities

Graph 18

Effect of Vitamin E on phenytoin induced memory impairment

Values are expressed as mean± SEM of 6 animals.

***( p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++( p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Effect of Vitamin E on phenytoin impaired exploratory activity

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Effect of Vitamin E on phenytoin induced motor in co-ordination

There was no significant difference in motor coordination of the control, phenytoin treated, phenytoin with Vit E (50, 100, 200 mg/Kg) pre-treated groups on the 0 day of the study. Phenytoin (20 mg/Kg, p.o.) significantly impaired the Rota Rod performance of rats from 120 sec (0 day) to 17.83±0.87 sec on 45th day (p< 0.001). Co-administration of Vit E in all the three doses significantly improved the motor coordination from 15th day till 45th day. The values increased from 17.83±0.87 sec in the phenytoin treated group to 56.83±1.6 sec (p< 0.001), 78.16±2.4 sec (p< 0.001) and 82.8±1.7 sec (p < 0.001) in Vit E 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. Vit E at all the three doses produced significant reversal of phenytoin induced impairment of motor co-ordination in a dose dependent fashion but the values did not reach that of normal (Graph 20).

Effect of Vitamin E on phenytoin impaired locomotor activity

There was no significant difference in spontaneous motor activity of the control, phenytoin and phenytoin with Vit E (50, 100, 200 mg/Kg) pre-treated groups on the day of study. Phenytoin 20 mg/Kg, p.o., significantly decreased the spontaneous motor activity by reducing the performance of the rats on Actophotometer. The count reduced from 311.66±3.73 (0 day) to 86.5±1.408 (45th day) (p< 0.001). Co-administration of Vit E in all the three doses significantly improved the spontaneous activity from 15th day till 45th day. The values increased from 86.16±1.49 in the phenytoin treated group to 107.8±1.24 (p< 0.001), 157.66±1.22 (p< 0.001) and 216.0±1.52 (p < 0.001) in Vit E 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. Vit E at all the three doses produced significant
reversal of phenytoin impaired locomotor activity in a dose dependent fashion but the values did not reach that of normal values (Graph 21).

Effect of Vitamin E on phenytoin induced regional brain lipid peroxidation

Phenytoin significantly increased the lipid peroxidation in medulla, pons, midbrain, cerebellum and cortex. Vit E significantly reduced the lipid peroxidation induced by phenytoin in medulla, pons, midbrain, cerebellum and cortex dose dependently but the values did not reach the normal values when compared with the control group (Graph 22).

Effect of Vitamin E on phenytoin induced alterations in regional brain AChE activity

Phenytoin showed a significant rise in AChE activity in medulla, pons, midbrain, cerebellum and cortex. Vit E significantly reduced the AChE activity in medulla, pons, midbrain, cerebellum and cortex dose dependently. The values were brought down near the normal values in cerebellum, medulla and pons (Graph 23).

Effect of Vitamin E on phenytoin induced alterations in brain histopathology

Figure 4 shows the effect of Vit E on phenytoin induced histopathological changes in rat brain. Phenytoin in combination with Vit E (50 mg/Kg) showed necrosis in brain (Figure 4a), Vit E (100 mg/Kg) showed congested choroid plexus (Figure 4b). Vit E (200 mg/Kg) treated group showed normal parenchyma with occasional dilated blood vessels (Figure 4c).
Results – Behavioural abnormalities

Graph 20

Effect of Vitamin E on phenytoin induced motor in-coordination

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), ***(p< 0.01), *(p< 0.05) Vs Control group

+++( p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results – Behavioural abnormalities

Graph 21

Effect of Vitamin E on phenytoin impaired locomotor activity

Values are expressed as mean± SEM of 6 animals.

***( p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++( p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results – Behavioural abnormalities

Graph 22

Effect of Vitamin E on phenytoin induced alterations in regional brain lipid peroxidation

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results – Behavioural abnormalities

Effect of Vitamin E on phenytoin induced alterations in regional brain acetyl cholinesterase activity

Graph 23

Values are expressed as mean± SEM of 6 animals.

***( p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results – Behavioural abnormalities

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Figure 4
Effect of Vitamin E on phenytoin induced alterations in brain histopathology

Figure 4 (a)
Phenytoin + Vit E (50 mg/Kg)

Figure 4 (b)
Phenytoin + Vit E (100 mg/Kg)

Figure 4 (c)
Phenytoin + Vit E (200 mg/Kg)
Results – Behavioural abnormalities

Effect of Alpha Lipoic Acid on phenytoin induced memory impairment

The effect of chronic treatment of phenytoin and phenytoin along with ALA on memory is shown in Graph 24. There was no significant difference in the transfer latency of the control, phenytoin and phenytoin with ALA (50, 100, 200 mg/Kg) pre-treated groups on the 0 day of the study. The retention transfer latencies increased from 34.5±2.23 sec (0 day) to 124.66±1.82 sec (45th day) (p< 0.001) in phenytoin treated animals. Co-administration of ALA in all the three doses significantly reduced the transfer latency from 15th day till 45th day. The values decreased from 124.6±1.82 sec in the phenytoin treated group to 93.83±1.7 sec (p< 0.001), 80.66±1.45 sec (p< 0.001) and 73.66±0.76 sec (p< 0.001) in ALA 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. ALA at all the three doses produced significant reversal of phenytoin induced memory impairment in a dose dependent fashion but the values did not reach the normal.

Effect of Alpha Lipoic Acid on phenytoin impaired exploratory activity

There was no significant difference in the exploratory activity of the control, phenytoin treated and phenytoin with ALA (50, 100, 200 mg/Kg) pre-treated groups on the 0 day of study. The exploratory activity was assessed by the number of head dippings into the holes of the hole board apparatus. The number of the head dippings decreased from 24.5±1.5 (0 day) to 2.5±0.76 (45th day) (p< 0.001) in phenytoin treated animals. Co-administration of ALA in all the three doses significantly increased the exploratory movements from 15th day till 45th day. The number of head dippings increased from 2.5±0.76 in the phenytoin treated group to 9.5±0.76 (p< 0.001), 13±1.065 (p< 0.001) and 14.83±0.94 (p< 0.001) in ALA 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. ALA at all the three doses produced significant reversal of phenytoin impaired exploratory behaviour in a dose dependent manner but the values did not reach the normal values (Graph 25).
Results – Behavioural abnormalities

Graph 24

Effect of ALA on phenytoin induced memory impairment

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++( p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results – Behavioural abnormalities

Graph 25

Effect of ALA on phenytoin impaired exploratory behaviour

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Effect of Alpha Lipoic Acid on phenytoin induced motor in co-ordination

There was no significant difference in motor coordination of the control, phenytoin treated and phenytoin with ALA (50, 100, 200 mg/Kg) pre-treated groups on the 0 day of the study. Phenytoin (20 mg/Kg, p.o.) significantly impaired the Rota Rod performance of rats from the 120 sec (0 day) to 17.83±0.87 sec on 45th day (p<0.001). Co-administration of ALA in all the three doses significantly improved the motor coordination from 15th day till 45th day. The values increased from 17.83±0.87 sec in the phenytoin treated group to 54.3±1.4 sec (p< 0.001), 87.66±1.68 sec (p< 0.001) and 93±1.48 sec (p< 0.001) in ALA 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. ALA at all the three doses produced significant reversal of phenytoin impaired muscle grip in a dose dependent fashion but the values did not reach the normal (Graph 26).

Effect of Alpha Lipoic Acid on phenytoin impaired locomotor activity

There was no significant difference in spontaneous motor activity of the control, phenytoin and phenytoin with ALA (50, 100, 200 mg/Kg) pre-treated groups on zero day of study. Phenytoin 20 mg/Kg, p.o., significantly decreased the spontaneous motor activity by reducing the performance of the rats on Actophotometer. The count reduced from 311.66±3.7 (0 day) to 86.5±1.408 (45th day) (p< 0.001). Co-administration of ALA in all the three doses significantly improved the spontaneous activity from 15th day till 45th day. The values increased from 86.5±1.408 in the phenytoin treated group to 112.5±1.6 (p< 0.001), 161.33±2.95 (p< 0.001) and 209.5±2.83 (p < 0.001) in Vit C 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. ALA at all the three doses produced significant
reversal of phenytoin impaired locomotor activity in a dose dependent fashion but the values did not reach the normal values (Graph 27).

**Effect of Alpha lipoic Acid on phenytoin induced alterations in regional brain lipid peroxidation**

Phenytoin significantly elevated the lipid peroxidation in medulla, pons, midbrain, cerebellum and cortex. ALA significantly reduced (p< 0.001) the phenytoin induced lipid peroxidation in medulla, pons, midbrain, cerebellum and cortex in a dose dependent fashion, but the values did not reach the normal values when compared with the control group (Graph 28).

**Effect of Alpha Lipoic Acid on phenytoin induced alterations in regional brain ACh E activity**

Phenytoin exhibited a significant increase in ACh E activity in medulla, pons, midbrain, cerebellum and cortex. ALA significantly reduced (p< 0.001) the activity of ACh E in the above brain regions and brought back the values near to normal in cerebellum, medulla, pons, and midbrain when compared with the control group (Graph 29).

**Effect of Alpha Lipoic Acid on phenytoin induced alterations in regional brain histopathology**

Figure 5 shows the influence of ALA on phenytoin induced histopathological changes in rat brain. Phenytoin in combination with 50 mg/Kg ALA showed gliosis and congestion in brain (Figure 5a), 100 mg/Kg and 200 mg/Kg ALA showed normal brain parenchyma (Figure 5b,5c).
Effect of ALA on phenytoin induced motor in-coordination

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results – Behavioural abnormalities

Graph 27

Effect of ALA on phenytoin impaired locomotor activity

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++( p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results – Behavioural abnormalities

Graph 28

Effect of ALA on phenytoin induced alterations in regional brain lipid peroxidation

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results – Behavioural abnormalities

Graph 29

Effect of ALA on phenytoin induced alterations in regional brain acetyl cholinesterase activity

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
**Figure 5**

Effect of ALA on phenytoin induced alterations in brain histopathology

- **Figure 5 (a)**
  Phenytoin + ALA 50 mg/Kg
  Gliosis and congestion in brain

- **Figure 5 (b)**
  Phenytoin + ALA 100 mg/Kg
  Normal cerebral parenchyma

- **Figure 5 (c)**
  Phenytoin + ALA 200 mg/Kg
  Normal Brain Parenchyma
Effect of N Acetyl Cysteine on phenytoin induced memory impairment

The effect of treatment of phenytoin, phenytoin along with NAC on memory is depicted in Graph 30. There was no significant difference in the transfer latency of the control, phenytoin and phenytoin with NAC (50, 100, 200 mg/Kg) pre-treated groups on the 0 day of the study. The retention transfer latencies increased from 34.5±2.23 sec (0 day) to 124.66±1.82 sec (45th day) (p< 0.001) in phenytoin treated animals. Co-administration of NAC in all the three doses significantly reduced the transfer latency from 15th day till 45th day. The values decreased from 124.66±1.82 sec in the phenytoin treated group to 96.5±1.47 sec (p< 0.001), 85.16±1.7 sec (p< 0.001) and 72.0±1.12 sec (p< 0.001) in NAC 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. NAC at all the three doses produced significant reversal of phenytoin induced memory impairment in a dose dependent fashion but the values did not reach the normal values.

Effect of N Acetyl Cysteine on phenytoin impaired exploratory activity

There was no significant difference in the exploratory activity of the control, phenytoin treated and phenytoin with NAC (50, 100, 200 mg/Kg) pre-treated groups on the 0 day of study. The exploratory activity was assessed by the number of head dippings into the holes of the hole board apparatus. The number of head dippings decreased from 24.5±1.5 (0 day) to 2.5±0.76 (45th day) (p< 0.001) in phenytoin treated animals. Co-administration of NAC in all the three doses significantly increased the exploratory movements from 15th day till 45th day. The number of head dippings increased from 2.5±0.76 in the phenytoin treated group to 7.5±0.76 (p< 0.05), 11.166±0.7 (p< 0.001) and 15.33±0.714 (p< 0.001) in NAC 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. NAC at all
the three doses produced significant reversal of phenytoin impaired exploratory behaviour in a dose dependent manner but the values did not reach the normal values (Graph 31).

**Effect of N Acetyl Cysteine on phenytoin induced motor in co-ordination**

There was no significant difference in motor coordination of the control, phenytoin treated and phenytoin with NAC (50, 100, 200 mg/Kg) pre-treated groups on the 0 day of the study. Phenytoin (20 mg/Kg, p.o.) significantly impaired the Rota Rod performance of rats from 120 sec (0 day) to 17.83±0.87 sec on 45th day (p< 0.001). Co-administration of NAC in all the three doses significantly improved the motor coordination from 15th day till 45th day. The values increased from 17.83±0.87 sec in the phenytoin treated group to 55.33±1.54 sec (p< 0.001), 84.83±1.51 sec (p< 0.001) and 91.3±1.86 sec (p< 0.001) in NAC 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. NAC at all the three doses produced significant reversal of phenytoin impaired motor coordination in a dose dependent fashion but the values did not reach the normal (Graph 32).

**Effect of N Acetyl Cysteine on phenytoin impaired locomotor activity**

There was no significant difference in spontaneous motor activity of the control, phenytoin and phenytoin with NAC (50, 100, 200 mg/Kg) pre-treated groups on the day of study. Phenytoin 20 mg/Kg, significantly decreased the spontaneous motor activity. The activity count was decreased from 311.66±3.73 (0 day) to 86.5±1.408 (45th day) (p< 0.001). Co-administration of NAC in all the three doses significantly improved the spontaneous activity from 15th day till 45th day. The values increased from 86.5±1.408 in the phenytoin treated group to 107±1.65 (p< 0.001), 161.5±1.5
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Results – Behavioural abnormalities

(p< 0.001) and 215.5±1.64 (p< 0.001) in NAC 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. NAC at all the three doses produced significant reversal of phenytoin impaired locomotor activity in a dose dependent fashion but the values did not reach the normal values (Graph 33).

**Effect of N Acetyl Cysteine on regional brain MDA levels**

Phenytoin showed a significant rise in lipid peroxidation in medulla, pons, midbrain, cerebellum and cortex. NAC significantly reduced (p< 0.001) the lipid peroxidation in the above brain regions dose dependently, but the values did not reach the normal values except in medulla when compared with the control group (Graph 34).

**Effect of N Acetyl Cysteine on phenytoin altered regional brain ACh E activity**

Phenytoin showed a significant rise in ACh E activity in medulla, pons, midbrain, cerebellum and cortex. NAC significantly reduced (p< 0.001) the activity of ACh E in medulla, pons, midbrain, cerebellum and cortex dose dependently and brought back the values near to the normal in cortex, cerebellum, medulla and pons when compared with the control group (Graph 35).

**Effect of N Acetyl Cysteine on phenytoin induced alterations in brain histopathology**

Figure 6 shows the effect of NAC on phenytoin induced histopathological changes in rat brain. Phenytoin supplementation with NAC (50 mg/Kg) showed periventricular inflammation in brain (Figure 6a), while both the doses of NAC (100 and 200 mg/Kg) showed normal brain parenchyma (Figure 6b, 6c).
Results – Behavioural abnormalities

Graph 30

Effect of NAC on phenytoin induced memory impairment

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), ***(p< 0.01), *(p< 0.05) Vs Control group

+++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results – Behavioural abnormalities

Graph 31

Effect of NAC on phenytoin impaired exploratory behaviour

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), ***(p< 0.01), *(p< 0.05) Vs Control group

+++( p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results – Behavioural abnormalities

Graph 32

Effect of NAC on phenytoin induced motor in-coordination

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Graph 33

Effect of NAC on phenytoin impaired locomotor activity

Values are expressed as mean± SEM of 6 animals.

***( p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results – Behavioural abnormalities

Graph 34

Effect of NAC on phenytoin induced alterations in regional brain lipid peroxidation

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results – Behavioural abnormalities

Graph 35

Effect of NAC on phenytoin induced alterations in regional brain acetyl cholinesterase activity

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results – Behavioural abnormalities

Figure 6
Effect of NAC on phenytoin induced alterations in brain histopathology

Figure 6 (a)
Phenytoin + NAC (50 mg/Kg)

Figure 6 (b)
Phenytoin + NAC (100 mg/Kg)

Figure 6 (c)
Phenytoin + NAC (200 mg/Kg)
DISCUSSION

The results of the present study indicate that phenytoin significantly impaired the motor-coordination, cognitive function, exploratory behaviour and spontaneous motor activity. In addition, it was also observed that phenytoin significantly increased the regional brain lipid peroxidation and ACh E activity along with appreciable degeneration in the brain regions as evidenced by brain histopathological investigations.

Effect of phenytoin on behavioural parameters

Cognitive deficit is one of the major problems associated with epilepsy, both the underlying pathology and drug therapy leads to disturbance in cognitive function (Vermeulen and Aldenkamp, 1995). As cognition is adversely influenced by many factors in epileptic patients, it is logical to evaluate the effect of AEDs on cognitive function in experimental animals without any additional complexities of the disease state. Phenytoin is known to affect the learning and memory (Aldenkamp, et al., 1994; Sudha, et al., 1995; Thaakur and Puspha, 2008a). In the present study, memory function was assessed by elevated plus maze. Elevated plus maze was used initially for evaluation of anxiety, while currently it finds its experimental application as a model for assessment of learning and memory in rodents (Kumar, et al., 2007; Da-Cunha, et al., 2005; Sonkusare, et al., 2005; Blatt and Takahashi, 1998; Sharma and Kulkarni, 1992; Itoh, et al., 1990). It is hypothesized that prolongation of transfer latency (the time taken by the animal to move from open arm to the enclosed arms) indicates the impairment of learning and memory. In the present study, treatment with phenytoin 20 mg/Kg substantially impaired the memory of (prolonged the transfer latency) non-epileptic rats in elevated plus maze paradigm, signifying the risk of this
drug in precipitating cognitive impairment even in healthy individuals as well. Our results are online with previous studies wherein, learning was impaired by most of the conventional AEDs in non-epileptic rats (Shannon and Love, 2007; Thaakur and Puspha, 2008a; Vohora, et al., 2000), non-epileptic pigeons (Poling et al., 1986; Picker and Poling, 1984; Thompson, 1973) and also in healthy volunteers (Meador, et al., 1991; 1993; 1995).

Hole board test helps in assessing the exploratory behaviour/alertness of the rats. Phenytoin significantly reduced the exploratory behaviour/alertness as observed by the decrease in number of head dippings in the holes of the hole board. Phenytoin affected exploratory behaviour (Bala Krishnan, et al., 1998), induced dose related sedation (Reynolds, 1975) and decreased the wakeful state of the animals.

Phenytoin impaired the Rota Rod performance of rats. Long term treatment with phenytoin resulted in muscle weakness and motor in co-ordination, which might be an attributing factor for phenytoin induced ataxia (Bala Krishnan, et al., 1998; Reynolds, 1975, Thaakur and Pushpa, 2008a).

Actophotometer is used to assess the spontaneous motor activity. Phenytoin significantly reduced the spontaneous motor activity, indicating the CNS depressant property of the drug.

Previous investigations reported that chronic administration of phenytoin caused oxidative stress in experimental animals (Bhuller, et al., 2006; Navarova, et al., 2005; Winn, et al., 2003; Sobaniec, et al., 2007, Mahle and Dasgupta, 1997; Ono, et al., 2000; Navarro, et al., 2005). In epileptic patients, long term phenytoin treatment reduced the activities of endogenous antioxidants like SOD, glutathione reductase, GPx, Vit C, Vit E and increased the TBARS. The results of the present study also
illustrated an increase in oxidative stress in the phenytoin treated rats, as indicated by increase in MDA levels in different regions of brain. MDA, an end product of lipid peroxidation (Liu, et al., 1997) is a biochemical marker to measure the extent of lipid peroxidation which indicates the degree of neuronal damage in various brain regions (Thaakur and Pushpa, 2008a). In the present study, phenytoin showed increased lipid peroxidation in different brain regions. Memory is maintained by various groups of neurons present in hippocampus, cerebral cortex, cerebellum and mid brain. The cerebral cortex is the part of the brain involved in many higher level tasks such as language, memory and consciousness. Memory of learned motor sequences (motor subroutines) seems to be stored in the supplementary motor area which is called the "executive centre" of the brain. Cerebellar learning critically involves the cerebellar cortex, while the cerebellar nuclei play a more critical role in long term memory storage (Christian and Thompson, 2005; Du Lac, et al., 1995; Kleim, et al., 2002) and thus memory is consolidated in the cerebellum (Fliessbach, et al., 2007, Guillaumin, et al., 1991). In the present study, phenytoin increased the lipid peroxidation in cerebral cortex, cerebellum, mid brain, pons and medulla oblongata. Increased lipid peroxidation in different brain regions was observed to cause peroxidative injury to the neuronal membranes and macromolecules, alter neurotransmitters, disrupt key neuronal functions and perturb motor function (Markesbery, 1997; McIntosh, et al., 1997). Neuronal damage induced by phenytoin in brain regions was believed to be responsible for memory impairment, motor in co-ordination, sedation, ataxia and loss of muscle grip.

Cholinergic neurons and their projections are widely distributed throughout the CNS with an essential role in regulating many vital functions such as learning, memory, cortical organization of movement and cerebral blood flow (Mesulam, et al., 2002).
This cholinergic activity is in turn regulated by ACh E, which hydrolyses the neurotransmitter acetylcholine (ACh) in the synaptic cleft of cholinergic synapse and neuromuscular junctions (Schmatz, et al., 2009; Soreq and Seidman, 2001). ACh E is an important enzyme for cholinergic neurotransmission and there are strong indications for its essential role in regulating many vital functions as well as neurobehavioral processes (Pari and Murugavel, 2007).

Deutsch, (1971) evaluated the role of the cholinergic synapse in the storage and retrieval of new information. Cognitive decline in aging and dementia is related to a decrease in cholinergic function. The effects of cholinergic antagonists and lesions of cholinergic nuclei are often related to cognitive deficits similar to those observed in aging and dementia (Dawson, et al., 1992; Drachman and Leavitt, 1974; Kopelman, 1986; McEntee and Crook, 1992). Numerous pharmacological studies evaluated the effects of cholinergic antagonists and cholinomimetics on learning and memory performance (Hagan and Morris, 1988; Molchan, et al., 1992; Smith, 1988). The cholinergic muscarinic antagonist scopolamine is most widely used to induce amnesia in experimental subjects. (Drachman and Leavitt, 1974). ACh E inhibitors enhance the availability of ACh in the synaptic cleft and reverse the scopolamine induced memory deficit, indicating that the cognitive deficit is cholinergic in nature. Many studies have shown that there is a relation between the decrease in cognitive functions and markers of the cholinergic system in senile dementia (Perry, et al., 1978). Blockland, (1996) concluded that the decline in the cholinergic system underlies the cognitive deficits of dementia (Blockland, 1996). There are also reports on increased brain ACh E activity in patients with AD (Melo, et al., 2003).
Melo, et al., (2003) studied the involvement of oxidative stress in the enhancement of AChE activity. It was observed that amyloid beta-peptide enhanced ACh E activity mediated via oxidative stress (Melo, et al., 2003).

Phenytoin treatment for 1 month significantly impaired the memory in human beings (Meador, et al., 1991; 1993). In new referrals with epilepsy, patients receiving phenytoin performed consistently poorer on memory tasks than those untreated (Andrewes, et al., 1986). Intellectual dulling and impaired memory has been observed in patients receiving phenytoin (Dodrill, 1988). Investigations on the effect of phenytoin on learning, memory and psychomotor functions showed that both acute and chronic administration of phenytoin significantly impaired learning and memory (Aldenkamp, et al., 1994; Sudha, et al., 1995; Vohora, et al., 2000).

It was reported that phenytoin decreased brain ACh levels (Agarwal and Bhargava, 1964; Domino and Olds, 1972). Phenytoin’s impairing effects on learning and memory are attributed to enhanced ACh E activity in brain. For an optimum antiepileptic therapy, it is desirable to have an absolute seizure control without cognitive impairment. Since central cholinergic system plays an important role in learning and memory (Biegon, et al., 1986; Perry, et al., 1978; Bartus, et al., 1982) and as phenytoin reduced ACh concentration in brain regions, the drug was reported to induce serious memory impairment (Smith, 1991; Agarwal and Bhargava, 1964; Domino and Olds, 1972).

We have measured the ACh E activity in different brain regions as a marker enzyme for cholinergic function. Our results were on par with the previous reports which also revealed that phenytoin at therapeutic doses increased ACh E activity in the brain regions of the rats. The rats also showed poor performance in the elevated plus maze test indicating memory impairment. It was believed that phenytoin via oxidative stress
enhanced the ACh E activity and thereby depleted the levels of ACh in brain regions resulting in subsequent memory impairment.

The histopathological changes in brain were examined by using HE stain in sequential brain sections to confirm the extent of damage induced by phenytoin. Brain sections of phenytoin treated rats showed damaged cells and congestion in periventricular region and cortex, which confirms phenytoin induced apoptosis in cortex and periventricular region. Phenytoin via oxidative stress induced the above damage in rat brain, which in turn resulted in serious behavioural abnormalities.

**Effect of Vit C on phenytoin induced behavioural abnormalities**

Vit C is an essential micronutrient required for normal metabolic functioning of the body (Jaffe, 1984; Gey, 1998). It acts as an electron donor and is a reducing agent (Bielski, et al., 1975), is a cofactor for several enzymes involved in the biosynthesis of neurotransmitters (Burri and Jacob, 1997; Tsao, 1997; Landmark, 2006). In addition, it has modulatory action on brain neurotransmitters like cholinergic, serotonergic and dopaminergic system (Castagne, et al., 2004; Cooper, 1961; Lee, et al., 2001), which are involved in learning and memory processes (Myhrer, 2003). Local application of Vit C enhanced the response of neurons to dopamine (Debler, et al., 1988; Heikkila, et al., 1981; Rebec and Pierce, 1994; Tolbert, et al., 1992) and glutamate (Rice, 2000). Glutamate has a critical role in learning and memory processing (Antzoulatos and Byrne, 2004). Vit C was observed to enhance hippocampus evoked potential activity (Laguzzi, et al., 1970) and protect against cognitive impairment (Paleologos, et al., 1998; Naber, et al., 2000). Delwing, et al., (2006) recommended that supplementation with Vit C may be a novel therapeutic strategy for the cognitive dysfunction. The combination of Vit E and Vit C effectively prevented cognitive impairment in post-menopausal women (Cho, et al., 2003; Delwing, et al., 2006; Landmark, 2006;
Monteiro, et al., 2005; Parle and Dhingra, 2003; Reis, et al., 2002). Arzi, et al., (2004) confirmed that chronic oral supplementation with Vit C (300 mg/Kg, 60 days) improved step-down avoidance learning of aged mice. Vit C was observed to prevent memory impairment induced by chemicals like scopolamine (Parle and Dhingra, 2003) and homocysteine (Reis, et al., 2002). In addition, Vit C also reduced the incidence of dementia caused by aging (Arzi, et al., 2004; Landmark 2006; Parle and Dhingra, 2003), hyperprolinemia (Delwing, et al., 2006) and ovariectomy (Monteiro, et al., 2005). Online with these, in the present study also supplementation with Vit C (50, 100 and 200 mg/Kg) for 45 days showed an improvement in phenytoin induced memory impairment in rats.

Vit C is a potent antioxidant (Grunewald, 1993; Levine, et al., 1999; Sanchez-Moreno, et al., 2003) highly concentrated in the CNS (Barabas, et al., 1995; Cooper, 1961). Vit C prevented memory deficit by its antioxidant effect (Castagne, et al., 2004; Cho, et al., 2003; Reis, et al., 2002) and proved to improve tardive dyskinesia induced by antipsychotic drugs (Michael, et al., 2002).

Vit C significantly reduced the ataxia induced by phenytoin on 15th day and the values reached nearer to normal values, whereas on 45th day of the study, though Vit C in all the three doses significantly reversed phenytoin induced ataxia the values did not reach normal. This indicates that there was an increased free radical generation from the 15th day to 45th day leading to severe lipid peroxidation in vital brain regions that regulate motor coordination. As cerebellum maintains motor co-ordination, the enhanced lipid peroxidation in cerebellum was considered as one of the contributing factors for the occurrence of ataxia. Vit C significantly reduced the lipid peroxidation augmented by phenytoin in all the brain regions.
Vit C significantly increased the phenytoin reduced head dippings in a dose dependent manner, but values did not reach normal range. Sleep and wakeful cycle is maintained by reticular activating system in the mid brain and cortex. Vit C supplementation improved the alertness in phenytoin treated rats by significantly reducing phenytoin induced lipid peroxidation in mid brain and cortex.

Shrivastava, et al., (2010) studied the effect of pyritinol against phenytoin induced memory impairment in mice. It was observed that pyritinol through its antioxidant property effectively scavenged the hydroxyl free radicals (Genkova-Papazova, et al., 1994) and protected brain, in particular the cholinergic system from phenytoin enhanced AChE activity (Toledano and Bentura, 1994; Greiner, et al., 1988).

Effect of Vit C was studied on memory impairment and cholinergic dysfunction in the brain of diabetic rats (Hasanein and Shahidi, 2010). Vit C was reported to have a modulatory action on brain cholinergic neurotransmission (Lee, et al., 2001; Siamak, et al., 2008) in diabetic rats. Thus, supplementation with Vit C was proved to regulate brain AChE activity and enhance cholinergic transmission in CNS, together resulting in memory improvement (Lee, et al., 2001). On line with the above reports, in the present study also Vit C brought down the elevated ACh E levels which in turn improved the memory, impaired by phenytoin. The other possible mechanism by which Vit C improved memory was believed to be due to its antioxidant property that combats regional brain lipid peroxidation.

Brain sections of phenytoin treated rats stained by HE showed damaged cells and congestion in periventricular region and cortex. Vit C at the dose of 50 mg/Kg was not much effective in reversing the phenytoin induced alterations in the brain regions as they showed damaged cortical cells and periventricular congestion. The higher
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doses of Vit C (100, 200 mg/Kg) were effective in reversing phenytoin induced damage in rat brain regions as they showed damage to a lesser extent.

**Effect of Vit E on phenytoin induced behavioural abnormalities**

Oxidative stress is a putative factor in the pathogenesis of many CNS disorders. Antioxidants such as Vit E find its application as therapeutic agent in the treatment of such diseases. In addition, Vit E seems to play a specific role in protecting the nervous system against oxidative damage. Vit E has been used in pharmacological doses for the treatment of CNS disorders such as Parkinsons disease, AD and tardive dyskinesia. An investigation showed that the use of 2000 IU of α-tocopheryl acetate is beneficial in the treatment of AD. In other studies, dosages ≥400 IU Vit E/day were found to be beneficial in the treatment of tardive dyskinesia (Vatassery, et al., 1999). Egan, et al., (1992) observed that supplementation with 1600 IU/day of Vit E for 6 weeks showed beneficial effect in patients suffering from tardive dyskinesia.

Morris, et al., (2002) examined the effect of intake of antioxidants like Vit E, Vit C and carotene in cognitive decline associated with increased age in elderly persons. The individuals who consumed Vit E had fewer declines in cognitive functioning suggesting the use of a daily Vit E supplementation to combat the cognitive decline associated with aging. Vit E was proved to be therapeutically efficient against age related cognitive decline and AD (Miller, 2000).

Alpha tocopherol was found to improve neuromuscular function and exploratory activities of mice. These effects were closely correlated with the changes in mitochondrial function suggesting that alpha tocopherol suppressed the production and actions of reactive oxygen and nitrogen species. Young adult mice (28 weeks of age) was supplemented with Vit E in their diet and were subjected to two behavioural
measures, a tightrope test, which evaluates their neuromuscular co-ordination by forcing them to hang by their front legs in the middle of a 60-cm tightrope and try to shuffle over to a column on either side within 30 seconds and a T-maze test, which assesses their exploratory and cognitive activities for every 2 weeks. It was found that successful performance on both the above tests got deteriorated in all mice as they grow old. However, Vit E treated mice possessed a good muscle grip strength and neuromuscular co-ordination despite of aging and the performance was significantly superior to that of the controls at 52 and 76 weeks. A similar pattern was observed in exploratory behaviour in the T-maze test. Vit E treatment improved the exploratory behaviour and cognitive function throughout the period of study. A key mechanism responsible for the above effects was thought to be the direct impact of Vit E against cellular oxidative damage and free radical scavenging activity particularly in brain cell mitochondria. Advancing age is normally associated with rising levels of two biochemical markers of oxidative stress in mitochondria i.e. protein carbonyls and TBARS. Mice that received Vit E had very less increase in both the above measures in brain. Vit E treatment also prevented the normal age-related decrease in the activity of mitochondrial markers of aging that includes cytochrome oxidase, mitochondrial nitric oxide synthase and Mn-SOD (Navarro, et al., 2005).

Passive inhalation of cigarette smoke is reported to enhance AChE activity and lipid peroxidation in brain regions resulting in memory impairment. Thome, et al., (2011) studied the effect of Vit E on cigarette smoke impaired memory, enhanced AChE activity and lipid peroxidation in rats. The results suggested that the rats treated with Vit E significantly reduced the raised activity of ACh E and lipid peroxidation in the brain structures and thus improved memory. Vitamin E was also shown to decrease
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the elevated brain ACh E activity in diabetic rats (Tiwari, et al., 2009). Vit E was thus proved to alleviate memory impairment induced by increased ACh E activity and cholinergic dysfunction. In the present study also Vit E significantly decreased the phenytoin enhanced ACh E activity and improved the memory which was adversely affected by phenytoin.

The current study revealed that Vit E at all the doses was found to reverse the memory impairment, decreased exploratory behaviour, muscular in-coordination, depression, regional brain lipid peroxidation and ACh E activity induced by phenytoin. It was believed that as Vit E decreased the oxidative stress induced by phenytoin in the brain regions, it reversed the behavioural abnormalities significantly. Supplementation of Vit E at low dose along with phenytoin showed necrosis in brain, whereas Vit E at medium dose showed congested choroid plexus and high dose Vit E treated group showed normal parenchyma with occasional dilated blood vessels. Higher dose of Vit E was effective in reversing phenytoin induced neurotoxicity. The beneficial effects of Vit E on improvement of behavioural parameters are attributed to its antioxidant property.

Effect of Alpha Lipoic Acid on phenytoin induced behavioural abnormalities

Oxidative stress plays a crucial role in age related neurodegenerative disorders. Farr, et al., (2003) examined the ability of two antioxidants, ALA and NAC, to reverse the cognitive deficits found in the SAMP8 mouse. Chronic administration of ALA and NAC improved cognition of 12-month-old SAMP8 mice in both the T-maze foot shock avoidance paradigm and the lever press appetitive task. NAC and ALA was found to decrease the levels of protein carbonyls (an index of protein oxidation) and
TBARS (an index of lipid peroxidation), thereby, brought down the oxidative stress. The above study supported the hypothesis that oxidative stress causes cognitive dysfunction and provides evidence for a therapeutic role of antioxidants such as ALA and NAC in dementia.

Thaakur and Himabindu, (2009) studied the effect of ALA on HAL induced tardive dyskinesia and correlated it with oxidative stress by studying total antioxidant status and lipid peroxidation. HAL (1 mg/Kg/i.p.) was observed to induce vacuous chewing movements that represented tardive dyskinesia in rats. ALA supplementation significantly decreased HAL induced tardive dyskinesia and catalepsy. The protective effect of ALA against tardive dyskinesia was attributed to its ability to reduce HAL induced lipid peroxidation, by scavenging ROS and reactive nitrogen species. In the present study also it was found that ALA significantly increased the muscular coordination and muscle strength which was considerably reduced by phenytoin. This improvement in muscle grip was believed to be due to the antioxidant property of ALA and its protective action against oxidative stress in brain regions responsible for muscular coordination.

ALA is proved to increase insulin sensitivity and activity which in turn plays a role in serotonergic activity by increasing the influx of tryptophan into the brain. Increased influx of tryptophan results in an increase in serotonin synthesis. In accordance with the serotonin theory of depression, it is possible to treat depression by increasing insulin activity. Therefore, ALA supplementation is thought to possess anti-depressant activity. In the present study also it was found that ALA significantly increased the locomotor activity which was considerably reduced by phenytoin.
ALA derives its antioxidant capacity from its ability to act as a scavenger of ROS, chelate metals and recycle endogenous antioxidants (Lynch, 2001). ALA scavenges singlet oxygen, H$_2$O$_2$, hydroxyl radical and reactive nitrogen species. ALA and its reduced form DHLA, further scavenge oxygen and peroxyl radicals (Kagan, et al., 1992). ALA also chelates several divalent cations, e.g. manganese, copper, zinc, cadmium and lead. ALA inhibits ascorbate induced production of H$_2$O$_2$ by copper (Ou, et al., 1995). ALA recycles endogenous antioxidants, such as GSH (Ou, et al., 1995) and Vit C (Drake, et al., 2002), which in turn regenerates Vit E. ALA by augmenting GSH, Vit C and Vit E, protects the brain from oxidative stress (Drake, et al., 2002).

ROS are thought to be involved in acute and chronic pathological conditions of brain and neuronal tissue. The metabolic antioxidant ALA is a low molecular weight substance which crosses the blood brain barrier. ALA is taken up and gets reduced in the cells and tissues to DHLA, which is also exported to the extracellular medium, consequently protection is afforded to both intracellular and extracellular environments. Both ALA and DHLA were considered to be potent antioxidants to regenerate other antioxidants like Vit C and Vit E and GSH through redox cycling. Thus, it would seem an ideal substance in the treatment of oxidative brain and neuronal disorders involving free radical processes. ALA reveals protection against cerebral ischemic reperfusion, excitotoxic amino acid brain injury, mitochondrial dysfunction, diabetes and diabetic neuropathy, inborn errors of metabolism and other causes of acute or chronic damage to brain or neuronal tissue. Antioxidant properties of ALA achieve its possible therapeutic roles in a variety of brain and neuronal tissue pathologies (Packer, et al., 1997). Acetyl-L-carnitine along with ALA was observed
to reduce age associated mitochondrial ultra structural decay showing improved brain function (Aliev, et al., 2009).

Acute dose of lindane was observed to cause significant reduction in catalase activity, total protein and elevation in cholesterol contents. Pre-treatment by a combination of antioxidants such as Vit E, Vit C, ALA and stilbene resveratrol (125 mg/Kg, ip) significantly augmented the catalase activity and total protein and protected the brain regions. The study suggested the neuroprotective efficacy of combination of antioxidants against lindane induced neurotoxicity (Bano and Bhatt, 2010).

ALA modulated ACh E activity, increased choline acetyl transferase activity, improved cholinergic transmission and thus reversed the memory impairment induced by many factors such as aging and epilepsy (Arivazhagan, et al., 2006). Antioxidant property of ALA was believed to combat oxidative stress induced memory impairments by improving the cholinergic transmission. The present study showed that ALA by its antioxidant property, brought down the phenytoin elevated ACh E activity, therefore, preserved the cholinergic transmission and improved memory.

In the present study, ALA decreased phenytoin induced lipid peroxidation and thus reversed the behavioural abnormalities induced by phenytoin. ALA (50 mg/Kg) supplementation showed gliosis and congestion in brain, whereas ALA (100 and 200 mg/Kg) treated group showed normal brain parenchyma. ALA at higher doses (100 and 200 mg/Kg) exhibited a dose dependent protective effect on phenytoin induced behavioural abnormalities and neurotoxicity. The neuroprotective potential of ALA is attributed to its antioxidant property and its ability to regenerate endogenous antioxidants.
Effect of N Acetyl Cysteine on phenytoin induced behavioural abnormalities

NAC was observed to decrease phenytoin induced lipid peroxidation in brain regions and improve the phenytoin affected cognitive function, muscular coordination, locomotion and exploratory activity in a dose dependent fashion.

Mitochondrial oxidative damage is implicated in brain aging and in age related neurodegenerative diseases. NAC was reported to prevent apoptotic death in neuronal cells and protect synaptic mitochondrial proteins from oxidative damage in aged mice. Administration of thiolic antioxidants such as NAC retarded age-related memory loss along with a significant reduction of lipid peroxide and protein carbonyl content in the mitochondria (Martínez, et al., 2000).

Reduction in ATP synthesis and increased generation of ROS are the possible causes of nigrostriatal cell death. Since sulfhydryl groups are essential in oxidative phosphorylation required for ATP synthesis, thiolic antioxidants contribute to the preservation of nigrostriatal cells against oxidative damage. Thus, NAC was believed to provide a neuroprotective therapeutic strategy against Parkinson's disease (Martínez, et al., 1999). In the present investigation, NAC improved the muscular coordination which was affected by phenytoin treatment.

Oxidative stress is reported in depressed patients and in animals subjected to stress. NAC, a cysteine prodrug with powerful antioxidant activity, was found to possess significant antidepressant like activity in forced swimming induced depression without affecting the exploratory behaviour (Ferreira, et al., 2008). NAC was found to be safe and effective against depressive symptoms in bipolar disorder (Berk, et al., 2008). The present investigation also reports that NAC improved the alertness as well as spontaneous motor activity and reversed the phenytoin induced depression.
NAC was found to selectively inhibit acute fatigue of rodent skeletal muscles stimulated at low tetanic frequencies. NAC pre-treatment was found to improve performance of human limb muscle during fatiguing exercise, suggesting that oxidative stress plays a crucial role in the fatigue process and recommended an antioxidant NAC therapy against fatigue (Reid, et al., 1994). Our results are on par with the above findings. In the present study, it was observed that phenytoin treatment adversely affected the muscle grip of rats and NAC effectively ameliorated the muscle grip and improved the Rota Rod performance in those rats affected by phenytoin.

Jayalakshmi, et al., (2005) characterized the biochemical changes in primary cultured hippocampal neurons during hypoxic exposure and studied the protective effect of NAC on hypoxia-induced cytotoxicity. It was observed that there was a significant decrease in mitochondrial membrane potential, appreciable increase in ROS and single-strand DNA breaks in cells exposed to hypoxia along with a significant fall in GPx, glutathione reductase and GSH levels followed by a significant elevation in the intracellular calcium level. Supplementation with NAC resulted in a significant cytoprotection, fall in ROS generation followed by inhibition of DNA strand breaks. The study indicated that NAC has significant neuroprotective effect during hypoxia in primary hippocampal culture.

Jayalakshmi, et al., (2007) also explored the effect of supplementation of NAC on hypobaric hypoxia induced deficits on spatial working, reference memory functions and oxidative stress in rats. Spatial working and reference memory were tested immediately after the termination of hypobaric hypoxia and then the rats were sacrificed for the estimation of oxidative stress markers in hippocampus. Rats displayed significant deficits in spatial working memory, increase in oxidative stress along with decrease in antioxidant status after hypoxic exposure. Supplementation with NAC in hypoxia exposed group improved the spatial memory performance and
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decreased the oxidative stress. These findings indicated that NAC supplementation reverses the memory deficit and oxidative stress caused by hypoxic exposure in rats.

The effect of NAC on memory, ACh E activity and lipid peroxidation in different brain structures of cadmium exposed rats was studied. Exposure to cadmium increased the TBARS levels in hippocampus, cerebellum and hypothalamus along with increased serum urea and creatinine levels. NAC significantly decreased the TBARS as well as oxidative stress and improved memory along with reduction in serum urea and creatinine levels (Goncalves, et al., 2010).

Nehru and Kanwar, (2004) investigated the effects of exposure of NAC on lead induced oxidative damage and lipid peroxidation in brain regions of the rat. Lipid peroxidation was increased following lead exposure and the antioxidant capacity of the cell in terms of the activity of antioxidant enzymes such as SOD and catalase was diminished. Following NAC treatment, lead induced lipid peroxidation was decreased along with improved catalase activity in the cerebral region and enhanced SOD activity in cerebellar region. Thus, NAC supplementation following lead exposure was found to enhance the reductive status of brain regions by arresting the lipid peroxidative damage and oxidative stress.

Hypercholesterolemia is found to deplete the GSH content of cerebral tissues (Gokkusu and Mostafazadeh, 2003; De La Cruz, et al., 2000). GSH provides major protection in oxidative injury by participating in the cellular defence systems against oxidative damage (Ross, 1988). GSH scavenges ROS and protects protein thiol groups from oxidation. NAC being a GSH precursor and antioxidant directly scavenges hydrogen peroxide, hydroxyl free radicals and hypochloric acid \textit{in vitro} (Aruoma, et al., 1989). It was observed that a low dose of NAC has a protective effect against cholesterol induced lipid peroxidation. The antioxidant activity of NAC primarily involves two mechanisms (1) NAC acts as a free radical scavenger via
sulphhydryl group and (2) NAC acts as a precursor of GSH to facilitate intracellular GSH synthesis. NAC administration decreased the lipid peroxidation products and enhanced the GSH content in the brain of hypercholesterolemic rats. The neuroprotective potential of NAC is contributed by its antioxidant property and its ability to increase the intracellular GSH (Sevgi, et al., 2008).

Aluminium is a potent neurotoxin involved in the initiation and progression of various cognitive disorders like AD by inducing oxidative stress and increased amyloid beta levels in vivo. Prakash and Kumar, (2009) investigated the effect of NAC on chronic aluminium exposure induced cognitive dysfunction mediated via oxidative stress. Chronic administration of aluminium chloride (100 mg/Kg) resulted in poor retention of memory in Morris water maze, elevated plus maze task paradigms and caused marked oxidative damage. It also caused a significant increase in the ACh E activity. Pre-treatment with NAC (50 and 100 mg/Kg, i.p.) significantly improved memory retention in tasks, attenuated oxidative damage and ACh E activity in aluminium treated rats. The study suggested a neuroprotective effect of NAC against aluminium induced cognitive dysfunction and oxidative damage. The results of the present study also coincided with the above study. NAC decreased the regional brain ACh E activity and reversed the phenytoin induced memory impairment.

The present study also revealed the protective effect of NAC against phenytoin induced histopathological damages in brain regions. Though low dose of NAC (50 mg/Kg) showed periventricular inflammation in brain, higher doses (100 and 200 mg/Kg) showed normal brain parenchyma evidencing the degree of protection offered by the above antioxidant against phenytoin induced brain damage. In the present study, NAC offered protection against phenytoin induced behavioural abnormalities and oxidative stress. The neuroprotective potential of NAC is due to its antioxidant property and its ability to increase the intracellular GSH.
CONCLUSION

The results of the present investigation suggest that Vit C, Vit E, ALA and NAC decreased phenytoin induced oxidative stress, lipid peroxidation, ACh E activity and behavioural disturbances. It is proved that long term treatment with phenytoin causes serious behavioural abnormalities which is believed to be due to increased oxidative stress induced by the drug as evidenced by increased regional brain lipid peroxidation and lesions in brain histopathology. The order of protection offered by the antioxidants against phenytoin induced oxidative stress and behavioural abnormalities was found to be ALA, NAC, Vit C and Vit E. All the antioxidants at a dose of 100, 200 mg/Kg were effective in reducing the oxidative stress and thereby decreased the behavioural abnormalities caused by phenytoin. 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 50mg/Kg. ALA and NAC were found to be more effective when compared with the other two antioxidants in alleviating the behavioural abnormalities and neurotoxicity induced by phenytoin therapy. This investigation reports the beneficial effect of antioxidants on phenytoin induced oxidative stress and neurotoxicity.
5.3 Influence of antioxidants on phenytoin induced hepatotoxicity in rats
RESULTS

Effect of antioxidants on phenytoin induced alterations in SGOT levels

Graph 36 summarizes the effect of phenytoin and phenytoin supplemented with different doses of Vit C, Vit E, ALA and NAC on SGOT levels. Administration of phenytoin 20 mg/Kg for a period of 45 days significantly increased the levels of SGOT.

Vit C (100, 200 mg/Kg), Vit E (50,100, 200 mg/Kg), ALA (50,100 mg/Kg) and NAC (50,100 mg/Kg) significantly (p< 0.001) decreased the elevated SGOT levels when compared with phenytoin treated animals but the values did not reach that of normal.

ALA and NAC at their higher dose (200 mg/Kg) dropped off the levels of SGOT near to that of normal control animals.

All the antioxidants appreciably decreased the SGOT which was amplified by phenytoin treatment in rats. ALA and NAC were found to exhibit an excellent reduction in SGOT levels among the four antioxidants studied.

Effect of antioxidants on phenytoin induced alterations in SGPT levels

Graph 37 summarizes the effect of phenytoin and phenytoin supplemented with different doses of Vit C, Vit E, ALA and NAC on SGPT levels. Administration of phenytoin 20 mg/Kg for a period of 45 days significantly increased the levels of SGPT.

Vit C (50,100 mg/Kg), Vit E (50,100, 200 mg/Kg), ALA (50,100 mg/Kg) and NAC (50 mg/Kg) significantly (p< 0.001) decreased the levels of SGPT when compared with phenytoin treated animals but the values did not reach that of normal.
Vit C (200 mg/Kg), ALA (200 mg/Kg) and NAC (100, 200 mg/Kg) decreased the levels of SGPT near to that of normal control.

All the antioxidants appreciably decreased the SGPT in a dose dependent fashion which was increased by phenytoin treatment in rats. Vit C, ALA and NAC were found to reveal an excellent reduction in SGPT levels.

**Effect of antioxidants on phenytoin induced alterations in Total Bilirubin levels**

Graph 38 summarizes the effect of phenytoin and phenytoin supplemented with different doses of Vit C, Vit E, ALA and NAC on bilirubin levels. Administration of phenytoin 20 mg/Kg for a period of 45 days significantly increased the levels of total bilirubin.

Vit C, Vit E, ALA and NAC at 50 mg/Kg showed no significant decrease in the levels of total bilirubin elevated by phenytoin, whereas at 100 mg/Kg all the antioxidants significantly (p< 0.001) reduced the levels of total bilirubin and at 200 mg/Kg the values were brought near that of normal values.

All the antioxidants considerably decreased the total bilirubin in a dose dependent fashion which was increased by phenytoin treatment in rats. ALA and NAC revealed an excellent reduction in total bilirubin levels.

**Effect of antioxidants on phenytoin induced alterations in ALP levels**

Graph 39 summarizes the effect of phenytoin and phenytoin supplemented with different doses of Vit C, Vit E, ALA and NAC on ALP levels. Administration of phenytoin 20 mg/Kg for a period of 45 days significantly increased the levels of ALP.
Vit E (100, 200 mg/Kg) significantly (p < 0.001) decreased the levels of ALP when compared with phenytoin treated animals but the values did not reach that of normal.

Vit C, ALA and NAC at 50, 100 mg/Kg significantly (p < 0.001) brought down the levels of ALP but not closer to the normal values, whereas the antioxidants at their higher dose (200 mg/Kg) decreased the levels of ALP near to that of normal control.

All the antioxidants appreciably decreased the ALP in a dose dependent fashion which was increased by long term phenytoin treatment in rats. ALA and NAC were found to reveal an excellent reduction in elevated ALP levels.

**Effect of antioxidants on phenytoin induced alterations in Albumin levels**

Graph 40 summarizes the effect of phenytoin and phenytoin supplemented with different doses of Vit C, Vit E, ALA and NAC on albumin levels. Administration of phenytoin 20 mg/Kg for a period of 45 days significantly decreased the levels of albumin.

All the antioxidants at 50 mg/Kg showed no significant increase in the levels of albumin, whereas at 100 and 200 mg/Kg the antioxidants augmented the levels of albumin in a dose dependent fashion.

Vit C, ALA and NAC at 200 mg/Kg significantly (p < 0.001) increased the levels of albumin raised the levels of albumin near to that of normal control.

All the antioxidants appreciably increased the albumin in a dose dependent fashion which was decreased by phenytoin treatment. Among the four antioxidants used, the effect of Vit C, ALA and NAC was most prominent in terms of improving the albumin levels.
Graph 36

Effect of antioxidants on phenytoin induced alterations in SGOT levels

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), ** (p< 0.01), * (p< 0.05) Vs Control group

+++ (p< 0.001), ++ (p< 0.01), + (p< 0.05) Vs Phenytoin group
Graph 37

Effect of antioxidants on phenytoin induced alterations in SGPT levels

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), ** (p< 0.01), * (p< 0.05) Vs Control group

+++ (p< 0.001), ++ (p< 0.01), + (p< 0.05) Vs Phenytoin group
Results - Hepatotoxicity

Graph 38

Effect of antioxidants on phenytoin induced alterations in Total Bilirubin levels

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Graph 39

Effect of antioxidants on phenytoin induced alterations in ALP levels

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Graph 40

Effect of antioxidants on phenytoin induced alterations in Albumin levels

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Effect of antioxidants on phenytoin induced alterations in Total protein levels

Graph 41 summarizes the effect of phenytoin and phenytoin supplemented with different doses of Vit C, Vit E, ALA and NAC on total protein levels. Administration of phenytoin 20 mg/Kg for a period of 45 days significantly decreased the levels of total protein.

Vit C and Vit E at 50 mg/Kg showed no significant increase in the levels of total protein, whereas ALA and NAC at 50 mg/Kg slightly increased the levels of total protein (p< 0.05). All the antioxidants at 100 and 200 mg/Kg significantly (p< 0.001) augmented the levels of total protein and at 200 mg/Kg the values were brought near to the normal values. The effect of ALA and NAC was more prominent among the four antioxidants tested.

Effect of antioxidants on phenytoin enhanced liver lipid peroxidation

Graph 42 summarizes the effect of phenytoin and phenytoin supplemented with different doses of Vit C, Vit E, ALA and NAC on liver lipid peroxidation. Administration of phenytoin 20 mg/Kg for a period of 45 days significantly increased the lipid peroxide contents in liver.

All the antioxidants at all the three doses (50, 100, 200 mg/Kg) significantly (p< 0.001) reduced the liver lipid peroxidation in a dose dependent manner but the values did not reach the normal. ALA and NAC (200 mg/Kg) were found to decrease the same more appreciably when compared to the Vit C and Vit E.
Effect of antioxidants on phenytoin induced alterations in

**Total protein levels**

Values are expressed as mean± SEM of 6 animals.

- *** (p< 0.001), ** (p< 0.01), * (p< 0.05) Vs Control group
- +++ (p< 0.001), ++ (p< 0.01), + (p< 0.05) Vs Phenytoin group
Results - Hepatotoxicity

Graph 42

Effect of antioxidants on phenytoin enhanced liver lipid peroxidation

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), ***(p< 0.01), *(p< 0.05) Vs Control group

+++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Effect of antioxidants on phenytoin induced alterations in body weight, absolute and relative liver weight

At the end of 45 days of treatment with phenytoin, there was a statistically significant (p< 0.001) decrease in body weight and an increase in the absolute and relative liver weights when compared to the control group. All the antioxidants at all the three doses except Vit C (50 mg/Kg) increased the body weight and decreased the absolute and relative liver weights significantly (p< 0.001) when compared to phenytoin group (Table 1-4).

Effect of antioxidants on phenytoin induced alterations in hepatic architecture

The gross macroscopy of liver is shown in Figure 7. Figure 7a shows normal liver of control group and Figure 7b illustrates hepatic necrosis induced by phenytoin treatment.

Effect of phenytoin on liver histopathology

Figure 8 explains the light microscopic examination of the livers revealing that the liver of the control group exhibited normal hepatic architecture (Figure 8 a) and the liver of the phenytoin treated animals exhibited severe congestion, periportal inflammation revealing centrilocular congestion, fatty degeneration and hepatocellular necrosis (Figure 8 a and 8b).

Effect of Vitamin C on phenytoin induced alterations in liver histopathology

Phenytoin plus Vit C 50 mg/Kg treated rats showed less congestion and mild periportal inflammation than phenytoin group (Figure 9a). Phenytoin plus Vit C 100 mg/Kg treated rats showed normal hepatocytes with mild centrilocular congestion.
Results - Hepatotoxicity

(Figure 9b). Phenytoin plus Vit C 200 mg/Kg treated rats exhibited normal arrangement of the hepatocytes (Figure 9c).

**Effect of Vitamin E on phenytoin induced alterations in liver histopathology**

Figure 10 shows the effect of Vit E on phenytoin induced histopathological changes in rat liver. Phenytoin + Vit E (50 mg/Kg) treated group showed interlobular fibrosis and congestion in liver (Figure 10a), whereas Vit E (100 mg/Kg) showed hepatic necrosis (Figure 10b). Vit E (200 mg/Kg) treated group showed normal hepatic parenchyma (Figure 10c).

**Effect of Alpha Lipoic Acid on phenytoin induced alterations in liver histopathology**

Figure 11 shows the effect of ALA on phenytoin induced histopathological changes in rat liver. Phenytoin + ALA (50 mg/Kg) treated group showed hepatic necrosis and congestion in liver (Figure 11a), ALA (100 mg/Kg) showed hepatic necrosis (Figure 11b). ALA (200 mg/Kg) treated group showed normal hepatic parenchyma (Figure 11c).

**Effect of N Acetyl Cysteine on phenytoin induced alterations in liver histopathology**

Figure 12 shows the effect of NAC on phenytoin induced histopathological changes in rat liver. Phenytoin + NAC (50 mg/Kg) treated group showed hepatic necrosis (Figure 12a), whereas NAC (100 mg/Kg and 200 mg/Kg) showed normal hepatic architecture (Figure 12b) and normal periportal area respectively (Figure 12c).
### Table 1

**Effect of phenytoin and phenytoin + Vitamin C on body weight, absolute and relative liver weight**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight in gram</th>
<th>Absolute Liver weight (g)</th>
<th>Relative liver weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (g)</td>
<td>Final (g)</td>
<td>% Change</td>
</tr>
<tr>
<td>Control</td>
<td>225±0.00</td>
<td>268.3±2.1</td>
<td>↑19.2±0.93***</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>228.3±4.4</td>
<td>201.6±1.0</td>
<td>↓11.3±0.64***</td>
</tr>
<tr>
<td>Phenytoin+Vit C 50 mg/Kg</td>
<td>225±0.00</td>
<td>205±1.8</td>
<td>↓8.8±0.8***</td>
</tr>
<tr>
<td>Phenytoin+Vit C 100 mg/Kg</td>
<td>225±0.00</td>
<td>206.6±1.6</td>
<td>↓8.1±0.7***,**,*</td>
</tr>
<tr>
<td>Phenytoin+Vit C 200 mg/Kg</td>
<td>224.2±0.8</td>
<td>214.2±1.5</td>
<td>↓4.3±0.7***,**,*</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group.
Table 2

Effect of phenytoin and phenytoin + Vitamin E on body weight, absolute and relative liver weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight in gram</th>
<th>Absolute liver weight (g)</th>
<th>Relative liver weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (g)</td>
<td>Final (g)</td>
<td>% change</td>
</tr>
<tr>
<td>Control</td>
<td>225</td>
<td>268.3±2.1</td>
<td>↑19.2±0.93***</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>228.3±4.4</td>
<td>201.6±1.0</td>
<td>↓11.3±0.64***</td>
</tr>
<tr>
<td>Phenytoin+Vit E 50 mg/Kg</td>
<td>227.5±2.1</td>
<td>217.5±2.8</td>
<td>↓3.6±0.7***,+++</td>
</tr>
<tr>
<td>Phenytoin+Vit E 100mg/Kg</td>
<td>215.8±3.7</td>
<td>212.5±3.5</td>
<td>↓1.5±0.5***,+++</td>
</tr>
<tr>
<td>Phenytoin+Vit E 200mg/Kg</td>
<td>225±1.29</td>
<td>219.2±2.3</td>
<td>↓2.2±1.1***,+++</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group.
Table 3

Effect of phenytoin and phenytoin + Alpha Lipoic Acid on body weight, absolute and relative liver weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight in gram</th>
<th>Absolute liver weight (g)</th>
<th>Relative liver weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (g)</td>
<td>Final (g)</td>
<td>% Change</td>
</tr>
<tr>
<td>Control</td>
<td>225</td>
<td>268.3±2.1</td>
<td>↑19.2±0.93***</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>228.3±4.4</td>
<td>201.6±1.0</td>
<td>↓11.3±0.6***</td>
</tr>
<tr>
<td>Phenytoin+ALA 50 mg/Kg</td>
<td>222.5±2.1</td>
<td>211.6±2.4</td>
<td>↓4.8±1.0***,+++</td>
</tr>
<tr>
<td>Phenytoin+ALA 100 mg/Kg</td>
<td>221.6±3.3</td>
<td>216.6±3.0</td>
<td>↓2.1±0.9***,+++</td>
</tr>
<tr>
<td>Phenytoin+ALA 200 mg/Kg</td>
<td>229.2±1.5</td>
<td>225±1.29</td>
<td>↓1.8±0.7***,+++</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group.
### Table 4

**Effect of phenytoin and phenytoin + N Acetyl Cysteine on body weight, absolute and relative liver weight**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight in gram</th>
<th>Absolute liver weight (g)</th>
<th>Relative liver weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (g)</td>
<td>Final (g)</td>
<td>% change</td>
</tr>
<tr>
<td>Control</td>
<td>225</td>
<td>268.3±2.1</td>
<td>↑19.2±0.93***</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>228.3±4.4</td>
<td>201.6±1.0</td>
<td>↓11.3±0.64***</td>
</tr>
<tr>
<td>Phenytoin+ NAC 50 mg/Kg</td>
<td>220±1.82</td>
<td>212.5±2.1</td>
<td>↓3.4±0.5***,<strong>,</strong></td>
</tr>
<tr>
<td>Phenytoin+ NAC 100 mg/Kg</td>
<td>225.8±4.2</td>
<td>221.7±4.2</td>
<td>↓1.8±0.4***,<strong>,</strong></td>
</tr>
<tr>
<td>Phenytoin+ NAC 200 mg/Kg</td>
<td>221.7±1.6</td>
<td>217.5±3.0</td>
<td>↓1.9±0.9***,<strong>,</strong></td>
</tr>
</tbody>
</table>

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), ** (p< 0.01), * (p< 0.05) Vs Control group

+++ (p< 0.001), ++ (p< 0.01), + (p< 0.05) Vs Phenytoin group.
Figure 7
Gross macroscopy of Liver

Figure 7(a)
Control

Figure 7(b)
Phenytoin

Normal liver

Hepatic necrosis
Figure 8

Effect of phenytoin on liver histopathology

- **Normal hepatic architecture**
- **Fatty degeneration**
- **Hepatic Necrosis**
- **Periportal inflammation**
- **Centrilobular Congestion**

**Figure 8(a)** Control

**Figure 8(b)** Phenytoin

**Figure 8(c)** Phenytoin
Results - Hepatotoxicity

Figure 9

Effect of Vitamin C on phenytoin induced alterations in liver histopathology

Figure 9 (a)
Phenytoin + Vit C
(50 mg/Kg)

Figure 9 (b)
Phenytoin + Vit C
(100 mg/Kg)

Figure 9 (c)
Phenytoin + Vit C
(200 mg/Kg)
Results - Hepatotoxicity

Figure 10

Effect of Vitamin E on phenytoin induced alterations in liver histopathology

Figure 10 (a)
Phenytoin + Vit E
(50 mg/Kg)

Figure 10 (b)
Phenytoin + Vit E
(100 mg/Kg)

Figure 10 (c)
Phenytoin + Vit E
(200 mg/Kg)

Congestion
Interlobular fibrosis
Hepatic Necrosis
Normal hepatic parenchyma
Figure 11

Effect of ALA on phenytoin induced alterations in liver histopathology

Figure 11 (a)
Phenytoin + ALA
(50 mg/Kg)

Figure 11 (b)
Phenytoin + ALA
(100 mg/Kg)

Figure 11 (c)
Phenytoin + ALA
(200 mg/Kg)
Results - Hepatotoxicity

Figure 12

Effect of NAC on phenytoin induced alterations in liver histopathology

Figure 12 (a)
Phenytoin + NAC
(50 mg/Kg)

Figure 12 (b)
Phenytoin + NAC
(100 mg/Kg)

Figure 12 (c)
Phenytoin + NAC
(200 mg/Kg)
DISCUSSION

Effect of phenytoin on liver

Administration of phenytoin at a dose of 20 mg/Kg for 45 days showed severe hepatic damage and oxidative stress associated with marked increase in the activity of serum aminotransferases, bilirubin, ALP and a decrease in albumin and total protein. The body weight of the rats was decreased whereas the relative liver weight was increased in phenytoin treated rats. Phenytoin also increased lipid peroxidation in liver.

The mechanism responsible for AED induced hepatotoxicity has been attributed to the accumulation of arene oxides due to a defective detoxification by the epoxide hydrolase (Bavdekar, et al., 2004). Arene oxide metabolites of phenytoin are proved to be involved in the pathogenesis of drug induced hepatotoxicity where, a heritable defect in response to arene oxides predisposes some patients to phenytoin induced hepatotoxicity (Spielberg, et al., 1981). Phenytoin is metabolized to arene oxides by CYP450 enzymes. These arene oxides covalently bind with sulphhydryl groups and other hepatic components to produce acute hepatic necrosis (Roy, et al., 1993). Oxidative stress is a potential mechanism responsible for AED induced hepatotoxicity (Santos, et al., 2008a).

Mitochondria are the major targets in drug induced liver injury (Kass, 2006; Boelsterli and Lim, 2007) and mitochondrial dysfunction is generally accompanied by oxidative stress, a key regulator of mitochondria-mediated cell death (Roy, et al., 1993; Jaeschke, 2007). Oxidative stress is induced by arene oxide metabolites of phenytoin in humans and rats (Bavdekar, et al., 2004; Shear and Spielberg, 1988; Roy and Snodgrass, 1988; George and Farrell, 1994; Madden, et al., 1996; Kalapos, et al.,...
Discussion - Hepatotoxicity

2002; Zaccara, et al., 2007). Santos, et al., (2008a) demonstrated aromatic AED induced depletion of mitochondrial antioxidant defence (GSH/GSSG ratio) in rat liver. Arene oxides are non radical oxidants and do not require free radicals as intermediates to oxidize thiols (Jones, 2008). Oxidative stress occurs as a consequence of the disruption of thiol redox circuits, which are controlled by GSH. These findings converge on the potential role of mitochondrial toxicity and oxidative stress in the etiology of aromatic AED induced hepatotoxicity in human beings.

SGOT, SGPT, ALP and bilirubin are the most sensitive markers employed in the diagnosis of hepatic damage. The above markers are actually located in hepatocellular cytoplasm and are liberated into the circulation in response to hepatocellular damage (Sallie, et al., 1991; Ncibi, et al., 2008; Gokcimen, et al., 2007; Eraslan, et al., 2009). A low serum albumin indicates poor liver function and reduction in albumin levels are generally suggestive of liver disease (Suna, et al., 2010).

In the present study, phenytoin treated rats showed a significant increase in the levels of SGOT, SGPT, bilirubin and ALP followed by a significant decrease in the levels of albumin and total protein indicating the hepatotoxic nature of phenytoin. Phenytoin is observed to alter protein and free amino acid metabolism and their synthesis in the liver. The body weight of phenytoin treated rats was decreased whereas the relative liver weight was increased.

Phenytoin induced periportal inflammation, hemorrhage, sinusoidal congestion and hepatic necrosis in rat liver was evidenced by histopathological investigation. These changes were consistent with the changes in various biochemical parameters that were
observed and liver damage was considered to arise from the toxic effects of phenytoin via oxidative stress.

**Effect of Vitamin C on phenytoin induced hepatotoxicity**

Antioxidant vitamins have a number of biological activities, including immune stimulation, alteration of metabolic activities of carcinogens and prevention of genetic changes by inhibiting the DNA damage induced by reactive oxygen metabolites (Verma, et al., 2007). Vit C is a low molecular weight antioxidant which rejuvenates Vit E, suggesting that a major function of ascorbic acid is to recycle the tocopheroyl radical and GSH (Serbecic and Beutelspacher, 2005). Vit C inhibits *in vitro* lipid peroxidation (Ayse, et al., 2008) and is found to be effective in reversing hepatic damage induced by pesticides like malathion (Suna, et al., 2010) and dichlorvos (Ayse, et al., 2008). Vit C prevented nonalcoholic fatty liver disease (NAFLD) in choline deficient diet fed rats (Oliveira, et al., 2003) and protected against sodium nitrite induced lipid peroxidation and hepatocellular damage (Krishnamoorthy and Sangeetha, 2008). Vit C acted against the hepatotoxic effect of repeated high dose acetaminophen. The hepatoprotective effect of Vit C was mediated via free radical scavenging and inhibition of free radical generation (Adeneye and Olagunju, 2008). Vit C protected the lipids and lipoproteins in hepatocellular membranes against oxidative damage caused by toxic free radicals and prevented hepatocellular damage (Parola, et al., 1992).

Phenytoin was observed to reduce enzymatic and non-enzymatic antioxidants such as SOD, catalase, GSH and increased lipid peroxidation. Phenytoin also increased the generation of super oxide anion (Liu, et al., 1997) and reduced 40% of GSH content
GSH is an antioxidant which plays an important role in defence mechanism of living cells (Jain, et al., 1991) and decreased GSH concentrations are associated with cellular damage (Martensson and Meister, 1991; Suthanthiran, et al., 1990) and depressed immunity (Fischman, et al., 1981; Franklin, et al., 1990). Phenytoin and its intermediates produce free radicals thereby, elevate the lipid peroxides and reduce the antioxidants like GSH, catalase and SOD (Mays, et al., 1995; Mahle and Dasgupta, 1997). Free radicals and lipid peroxides cause acute lethal damage to hepatocytes (Poli, 1993). Oxidative stress is an imbalance between the production of oxidants and the respective defence system of an organism. MDA content is a direct indicator of the extent of lipid peroxidation which cause marked alteration in the structural integrity and function of cell membranes. An imbalance between antioxidant defence mechanisms and lipid peroxidation processes results in cell and tissue damage (Gutteridge and Halliwell, 1990). Cells are protected from oxygen derived radical injury by naturally occurring free radical scavengers and antioxidant pathways, including Vit C, Vit E, SOD, catalase and GPx however, overwhelming of these protective mechanisms makes the host tissues susceptible to damage by oxygen radicals and disturb the cell membrane function (Imoly and Linn, 1988). In the present study, administration of phenytoin for 45 days significantly reduced the enzymatic and non-enzymatic antioxidants like SOD, catalase, reduced GSH, Vit C, total antioxidant status and markedly increased the lipid peroxidation, indicating the role of free radicals in phenytoin induced hepatic damage.

Vit C was reported to increase the levels of GSH, SOD and catalase; improve the overall antioxidant protection capacity of blood (Johnston, et al., 1993; Lenton, et al., 2000) and reduce the oxidised glutathione (GSSG) in plasma which showed a better
index of antioxidant status and protection against oxidants (Henning, et al., 1991). Vit C is also proposed to reduce oxidative stress from H$_2$O$_2$ potentially by reducing the free radical species generated from H$_2$O$_2$. Vit C was reported to reduce the oxidative DNA damage (Noroozi, et al., 1998) and single strand breaks (Panayiotidis and Collins, 1997). In the present study, Vit C restored the phenytoin depleted GSH, Vit C, SOD, catalase and decreased the lipid peroxide products thereby, protected the hepatocytes from oxidative stress induced by phenytoin.

Supplementation of Vit C at higher doses (100 and 200 mg/Kg) along with phenytoin reversed periportal inflammation, sinusoidal congestion, haemorrhage and hepatic necrosis induced by phenytoin, while Vit C at the dose of 50 mg/Kg exhibited lower degree of inflammation and necrosis when compared to phenytoin treated rats. Thus, the present investigation reports the protective effect of Vit C on hepatotoxicity induced by long term phenytoin exposure.

Co-administration of Vit C 100 and 200 mg/Kg along with phenytoin for 45 days significantly decreased the phenytoin augmented SGOT, SGPT, bilirubin, ALP and increased the levels of albumin and total protein. Vit C supplementation increased the body weight and decreased the relative liver weight unlike in phenytoin treated rats. Vit C 100 and 200 mg/Kg supplementation along with phenytoin for 45 days significantly augmented the phenytoin reduced enzymatic, non-enzymatic antioxidants and decreased phenytoin enhanced liver lipid peroxidation. Vit C at higher doses (100, 200 mg/Kg) exhibited a significant protective effect against phenytoin induced hepatic damage.
Effect of Vitamin E on phenytoin induced hepatotoxicity

The hepatoprotective effect of orally administered *Nigella sativa* oil (100 mg/Kg/day) and Vit E (10 mg/Kg/day) was studied, on malathion induced hepatotoxicity in workers involved in the formulation of pesticides and in male albino rats subjected to oral administration of malathion (50 mg/Kg/per day) for 60 days. Liver function tests such as ALT, AST, ALP, albumin, globulin, albumin/globulin ratio, total proteins, and antioxidant enzymes such as CAT, SOD, GPx along with the degree of lipid peroxidation were analyzed in both human and experimental animals. The results of both human and animal study revealed that, exposure to malathion produced significant increases in AST, ALT and lipid peroxides along with decrease in albumin, albumin/globulin ratio, total protein and antioxidant enzymes. *Nigella sativa* oil and Vit E administration showed significant improvement of liver function tests, lipid peroxides and antioxidant enzymes impaired by malathion. Thus, dietary supplementation of *Nigella sativa* oil or Vit E may represent a potential therapeutic strategy in reducing malathion induced hepatotoxicity (Mahmood, et al., 2010).

Exposure to unleaded gasoline vapors (UGV) for 6 hrs/day, 5 days/week for 20 weeks was reported to cause hepatotoxicity in rats. Hepatoprotective effect of Vit A (400 IU/Kg/day) and Vit E (200 IU/Kg/day) against UGV induced toxicity was assessed in rats of either sex with concomitant exposure to UGV in the last two weeks of the experiment. Exposure to UGV caused significant increase in the activities of serum ALT, AST, ALP and bilirubin in rats. Though both the vitamins were found to possess significant hepatoprotective effect, Vit E was observed to be more potent than Vit A (Uboha, et al., 2009).
The influence of CCl$_4$ on the activity of SOD, GPx, glutathione reductase and on the content of MDA and GSH was monitored in plasma and whole blood of rabbits. The administration of CCl$_4$ decreased 50% of the activity of GPx and glutathione reductase. These changes were accompanied with the increase in MDA concentration and decrease in GSH concentration. Oxidative stress caused by CCl$_4$ was characterised by the development of ROS, especially superoxide radical anion (Pawlowska, et al., 2007). Effect of Vit E (100 mg/Kg) on CCl$_4$ induced liver damage was studied in rats. Serum levels of SGOT, SGPT, LDH, ALP, GGT, total and conjugated bilirubin were significantly increased in animals treated with CCl$_4$, but returned to normal values after the administration of Vit E. Liver Vit E levels were found to be significantly lower in the CCl$_4$ group and the same was significantly increased by co-administration of Vit E. On histological examination, Vit E administered animals showed incomplete, but significant, protection against hepatic necrosis and cirrhosis induced by CCl$_4$. Vit E showed protective effect against CCl$_4$ induced chronic liver damage and cirrhosis (Naziroglu, et al., 1999).

The effect of pre-treatment with Vit E on membrane lipid alterations produced by the acute intoxication with CCl$_4$ was studied. The phospholipid and protein ratio determined in plasma membranes of CCl$_4$ treated rats were almost three fold higher than the control. Sphingomyelin and phosphatidyl choline increased, while phosphatidyl ethanolamine decreased in the hepatic plasma membranes isolated from the CCl$_4$ treated group. Animals pre-treated with a daily dose of Vit E (200 IU/Kg) for 7 days showed a lower increase in the phospholipid: protein ratio (two-fold) and slightly altered the changes in sphingomyelin, phosphatidyl choline and phosphatidyl ethanolamine increased by CCl$_4$. Rats pre-treated with a higher dose of Vit E (400
IU/Kg) for the same period showed normal lipid composition in the plasma membrane. The protective action of Vit E against CCl₄ induced plasma membrane damage was considered to be associated with its antioxidant properties (Martinez, et al., 1984).

A study investigated the effect of Vit C and Vit E on the hepatotoxicity induced by Rifampicin. Rifampicin was found to increase the hepatic markers such as AST, ALT and total bilirubin, while the Vit E and Vit C improved the hepatic function. Vit E showed remarkable hepatoprotection than Vit C (Awodele, et al., 2010).

Vit E (100 mg/Kg) was also observed to exhibit a very significant hepatoprotective effect against hepatotoxicity induced by combination of two hepatotoxic drugs Isoniazid (INH) and Rifampicin in rabbits (Tayal, et al., 2007).

Protective effect of glycine and Vit E was studied on a model of ethanol induced acute liver injury during the early phase of liver regeneration after partial hepatectomy in rats. Ethanol caused a decrease in serum albumin along with increase in cholesterol, AST and ALT, while glycine and Vit E reversed the ethanol induced alterations in the above parameters. Glycine and Vit E also decreased the levels of TBARS and increased the SOD activity, suggesting protective effect of glycine or Vit E against ethanol induced liver injury mediated via oxidative stress (Judith, et al., 2009).

All the above studies confirmed the hepatoprotective nature of Vit E and the present study also revealed the hepatoprotective nature of Vit E against phenytoin induced hepatic damage. The antioxidant was observed to significantly reduce the levels of SGOT, SGPT and bilirubin, the markers of hepatotoxicity elevated by phenytoin and
increase the levels of albumin and total protein depleted by phenytoin. Vit E (200 mg/Kg) also improved the hepatic histopathological damages induced by phenytoin. Vit E exerted significant protection against phenytoin induced hepatotoxicity by its ability to decrease the lipid peroxidation through its free radical scavenging activity.

**Effect of Alpha Lipoic Acid on phenytoin induced hepatotoxicity**

ALA is used to treat liver poisoning induced by alcohol, mushrooms and heavy metals. The antioxidant abilities of ALA and its role in GSH recycling have encouraged its use in liver damage. Pari and Murugavel, (2004) investigated the hepatoprotective effect of ALA against chloroquine induced liver toxicity in rats. Pre-treatment with ALA at the dose of 10, 30 and 100 mg/Kg/day for 7 days before a single oral administration of chloroquine (970 mg/Kg/day) reversed the chloroquine induced liver enzymes and lipid peroxides. A significant decrease in plasma antioxidants such as GSH, Vit C and Vit E was observed in chloroquine treated rats, while ALA significantly improved the levels of the above endogenous plasma antioxidants decreased by chloroquine. ALA at its higher dose (100 mg/Kg/day) exhibited an appreciable effect than its lower doses (10 and 30 mg/Kg/day). The results of the study revealed the hepatoprotective effect of ALA against chloroquine induced hepatotoxicity. It was also observed that ALA possessed a superior hepatoprotective effect than silymarin, a reference drug (Pari and Murugavel, 2004).

Tamoxifen (TAM) citrate, a drug of choice in the treatment of breast cancer, causes liver damage to the extent of hepatic carcinogenesis. Hesham, (2007) elucidated the effects of ALA against TAM induced liver damage, oxidative stress and DNA
fragmentation. TAM treatment without schedules of ALA elicited a significant decline in antioxidant enzymes such as GPx, SOD and catalase, liver GSH and tumour necrosis factor-α (TNF-α) with concomitant significant elevations in TBARS, levels of liver enzymes such as SGPT, SGOT and serum LDH. The prophylactic administration of ALA to TAM intoxicated rats produced significant increase in all the antioxidant enzymes and GSH, with significant decrease in the levels of liver TBARS, transaminases and LDH. ALA, as a curative agent though showed protection against TAM intoxication, it was not very effective when compared to the prophylactic therapy. In addition, it was noted that TAM intoxicated rats exhibited high degree of DNA fragmentation which was partially inhibited by ALA treatment. ALA by scavenging the free radicals, prevented DNA fragmentation, alleviated liver injury and offered protection against oxidative stress induced by TAM intoxication. The study suggested the use of ALA in the prophylactic treatment of TAM induced liver injury than its use as curative agent (Hesham, 2007).

The effects of ALA and DHLA were studied by Foo, et al., (2011), against thioacetamid induced liver fibrosis in rats. It was found that co-administration of ALA to rats chronically treated with thioacetamide inhibited the development of liver cirrhosis, as indicated by reductions in cirrhosis incidence, hepatic fibrosis and AST, ALT activities. It was found that ALA and DHLA inhibited ROS generation and exhibited beneficial role in the treatment of chronic liver disease induced by thioacetamide (Foo, et al., 2011).

Liu, et al., (2010) explored the effect of ALA (10 mg/Kg/day) and Vit C (25 mg/Kg/day) on arsenic (50 mg/l water) induced oxidative stress. It was observed that the combination of both the antioxidants significantly decreased the TBARS level in
brain and liver and thereby attenuated oxidative stress and protected against arsenic toxicity.

Valdecantos, et al., (2010) analysed the effects of three antioxidants on hepatic mitochondrial function and antioxidant status. Isolated rat liver mitochondria were incubated with Vit C, resveratrol and ALA, and the activity of antioxidant enzymes (Mn-SOD and GPx), ROS generation and respiratory parameters were measured. Vit C enhanced the mitochondrial function by decreasing the ROS generation, by stimulating the activity of Mn-SOD as well as GPx. Resveratrol induced a significant increase in Mn-SOD activity and a decrease in ROS generation, ALA also enhanced the GPx activity. All the antioxidants improved the antioxidant defence system in hepatic mitochondria (Valdecantos, et al., 2010).

Influence of ALA treatment in malathion (100 mg/Kg) induced toxicity was studied. The activities of SGOT, SGPT, ALP and acid phosphatase, and the levels of creatinine, urea and uric acid were increased, while total protein and albumin levels were significantly decreased in rats exposed to malathion. Moreover, administration of malathion for one month resulted in damage of liver and kidney structures. Administration of ALA before malathion exposure prevented severe alterations in hemato-biochemical parameters and disruptions of liver and kidney structures. This study demonstrated that pre-treatment with ALA significantly attenuated the physiological and histopathological alterations induced by malathion. Also, the study identified new areas of research for development of better therapeutic agents for liver, kidney and other major organ dysfunctions and diseases (Al-Attar, 2010).
The potential protective effect of ALA and aminoguanidine against combination of INH and Rifampicin induced hepatotoxicity was investigated. Administration of INH and Rifampicin combination (50 mg/Kg each for 14 days) resulted in an elevation of serum hepatic marker enzymes and a significant increase in lipid profile. Combination treatment increased lipid peroxidation products, decreased GSH content, SOD, catalase and myeloperoxidase activities. Furthermore, liver total nitrite level was significantly increased in INH and Rifampicin treated rats. Co-administration of either ALA or aminoguanidine significantly reversed INH and Rifampicin combination induced alterations in hepatic marker enzymes. These effects were directly linked to a greater decrease in the combination induced elevation in lipid peroxidation products and total nitrite levels. Furthermore, co-administration of ALA and aminoguanidine restored SOD, catalase and myeloperoxidase activities and elevated the GSH levels. Additionally, such beneficial effect of ALA was linked to a marked lipid-lowering effect. Histopathological examination also revealed hepatoprotective nature of ALA and aminoguanidine (Saad, et al., 2010).

In the present study, supplementation with ALA decreased the markers of hepatotoxicity such as SGOT, SGPT, ALP, bilirubin, and increased the levels of albumin and total protein. ALA improved the histopathological changes and hepatic damage induced by phenytoin. ALA exerted significant protection against phenytoin induced toxicity by its ability to decrease the lipid peroxidation and thus oxidative stress through its free radical scavenging activity, which improved the levels of antioxidant defence system.
Effect of N Acetyl Cysteine on phenytoin induced hepatotoxicity

Ozaras, et al., (2003) investigated the effect of NAC (1 g/Kg) on intragastrically ethanol fed rats. Ethanol elevated the levels of liver enzymes such as ALT and AST, increased serum and tissue levels of MDA indicating augmented lipid peroxidation. On the other hand, decreased the serum GPx level along with serum and liver SOD activity signifying that ethanol induced liver damage was associated with oxidative stress. Co-administration of NAC was observed to attenuate the hepatic damage.

Methanol is oxidized in vivo to formaldehyde and then to formate which involves the generation of free radicals. Dobrzynska, et al., (2000) studied the effect of NAC on liver cell membranes of rats intoxicated with methanol. Methanol administration caused an increase in lipid peroxidation products which resulted in the liver cell membrane damage and a leak of SGOT and SGPT into the blood. Ingestion of NAC with methanol prevented these methanol induced damages. The study suggested that NAC being an antioxidant protects free radical induced hepatocellular damage following methanol intoxication.

Raza, et al., (2003) compared the efficacy of NAC with the antioxidant potential of aminoguanidine against azathioprine induced hepatotoxicity in rats. Aminoguanidine (100 mg/Kg; i.p.) and NAC (100 mg/Kg; i.p.) was administered for 7 days after which single dose of Azathioprine (15 mg/Kg,i.p.) was administered. Azathioprine increased the serum hepatic aminotransferases, liver lipid peroxides and decreased the levels of GSH content in rats which were not pre-treated with aminoguanidine and NAC. Pretreatment with NAC decreased both the aminotransferases increased by azathioprine followed by a significant decline in the contents of lipid peroxides and elevated the
GSH levels. Pre-treatment with aminoguanidine significantly prevented the increase in levels of SGOT and SGPT after azathioprine administration but did not reverse the azathioprine induced alterations in levels of lipid peroxides and GSH levels. These observations indicate that NAC exhibited a protective mechanism by improving the levels of GSH. The protective effect of aminoguanidine against the enzyme leakage seems to be through the restoration of liver cell membrane integrity. The study reported a significant protection offered by NAC against the toxic effects of azathioprine. The antioxidant potential of NAC was observed to be superior when compared to aminoguanidine (Raza, et al., 2003).

Narasimhanaidu, et al., (2005) studied the efficacy of NAC on marker enzymes, lipid peroxidation and antioxidants in CCl₄ induced hepatotoxicity in rats. Subcutaneous administration of CCl₄ (3 ml/Kg/week) in albino Wistar rats for a period of three months significantly increased the activities of marker enzymes in plasma such as SGOT, GGT and ALP, increased the levels of TBARS and hydroperoxides in plasma and tissues (liver and kidney). A significant decrease in the levels of plasma antioxidants (GSH, Vit C and Vit E) was also noted along with decrease in the concentration of GSH and the activities of SOD, catalase and GPx. Co-administration of NAC (150 mg/Kg) orally along with CCl₄ for a period of three months decreased the activities of marker enzymes, lipid peroxides and improved the antioxidant status. Histopathological observations of the liver also showed the protective effect of NAC against CCl₄ induced hepatotoxicity in rats. Thus, the study reported the protective effect of NAC in CCl₄ induced hepatotoxicity in rats which was considered to be due to its antioxidant potential.
Kilciksiz, et al., (2011) investigated the potential radioprotective effects of NAC on gamma irradiation (single dose, 6 Gy) induced nitrosative stress in rat liver. Liver tissue of each animal was harvested and utilized for 3-nitrotyrosine detection. Irradiation significantly increased the 3-nitrotyrosine levels and the prophylactic use of NAC reduced the nitrosative damage during radiotherapy.

Priya, et al., (2011) evaluated the hepatoprotective and antioxidant properties of NAC on dimethylnitrosamine induced hepatotoxicity in rats. A single intraperitoneal dose of dimethylnitrosamine (5 mg/Kg) caused a significant increase in the levels of the serum marker enzymes SGOT, SGPT, ALP, LDH and GGT indicating hepatocellular damage. Elevation in the status of lipid peroxidation along with fall in the activities of the enzymatic (SOD, catalase) and non-enzymatic antioxidants (Vit C, Vit E) in the liver tissue further confirms oxidative stress and hepatocellular damage induced by dimethylnitrosamine administration. Oral administration of NAC (50 mg/Kg) for 7 days significantly prevented the above alterations in the status of the marker enzymes of hepatotoxicity and antioxidant parameters and restored them towards normal, which was further substantiated by the histopathological studies of the liver tissue. These results suggested that NAC offered hepatoprotection by reversing dimethylnitrosamine induced oxidative stress, this protective effect was attributed to its antioxidant and free radical scavenging properties.

In the present study, it was observed that the phenytoin treatment increased significantly the levels of SGOT, SGPT and bilirubin, the markers of hepatotoxicity and decreased the levels of albumin and total protein. Supplementation with NAC decreased the markers of hepatotoxicity and increased the albumin and total protein. NAC exerted significant protection against phenytoin induced toxicity by its ability to
decrease the lipid peroxidation and thus oxidative stress through its free radical scavenging activity, which improved the levels of antioxidant defence system. NAC also has alleviated the hepatic histopathological damages induced by phenytoin. This study revealed the hepatoprotective potential of NAC against phenytoin induced liver damage.

CONCLUSION

The results of the present investigation revealed the protective effect of antioxidants against phenytoin induced oxidative stress and hepatotoxicity. The antioxidants also reversed the histopathological damages induced by phenytoin in liver. The order of protection offered by antioxidants against phenytoin induced hepatotoxicity was NAC, ALA, Vit C and Vit E. The degree of protection offered by NAC and ALA was more appreciable than Vit C and Vit E. All the antioxidants at a dose of 100 and 200 mg/Kg were effective in reducing the oxidative stress and hepatic damage. The enzyme inducing property of phenytoin might possibly explain the relative inefficiency of the selected antioxidants at 50 mg/Kg. This investigation reports the beneficial effect of antioxidants on phenytoin induced oxidative stress and hepatotoxicity.
Results and Discussion

5.4 Influence of antioxidants on phenytoin induced metabolic disorders in rats
RESULTS

Effect of antioxidants on phenytoin induced hyperglycemia

Graph 43 summarizes the effect of long term treatment of phenytoin and phenytoin supplemented with graded doses of Vit C, Vit E, ALA and NAC on blood glucose levels. Administration of phenytoin 20 mg/Kg for a period of 45 days significantly increased the blood glucose levels.

All the antioxidants at all the three doses (50, 100, 200 mg/Kg) significantly (p<0.001) decreased the blood glucose levels which were increased by long term phenytoin treatment in rats.

ALA and NAC at their higher dose 200 mg/Kg exhibited an excellent reduction in phenytoin induced hyperglycemia.

Effect of antioxidants on phenytoin induced alterations in total cholesterol levels

Graph 44 summarizes the effect of phenytoin and phenytoin supplemented with graded doses of Vit C, Vit E, ALA and NAC on serum TC levels. Administration of phenytoin 20 mg/Kg for a period of 45 days significantly increased the levels of serum TC.

Vit C, ALA and NAC at all the three doses (50,100, 200 mg/Kg) significantly (p<0.001) decreased the TC levels in a dose dependent fashion when compared with phenytoin treated animals but the values did not reach that of normal.
Vit E (50, 100 mg/Kg) showed no significant reduction in TC levels, whereas at the dose of 200 mg/Kg Vit E significantly (p< 0.001) reduced the TC levels.

All the selected antioxidants substantially decreased the TC levels increased by phenytoin treatment in rats. Though Vit E (200 mg/Kg) exhibited a significant reduction in the levels of TC, the effect was observed to be inferior to other antioxidants.

**Effect of antioxidants on phenytoin induced alterations in triglyceride levels**

Graph 45 summarizes the effect of phenytoin and phenytoin supplemented with graded doses of Vit C, Vit E, ALA and NAC on TG levels. Phenytoin 20 mg/Kg for a period of 45 days significantly increased the levels of TG.

Vit C (100, 200 mg/Kg), ALA (50,100, 200 mg/Kg) and NAC (50,100, 200 mg/Kg) significantly (p< 0.001) decreased the TG levels in a dose dependent fashion when compared with phenytoin treated animals but the values did not reach that of normal.

Vit E at all the three doses showed no significant reduction in the levels of triglycerides elevated by phenytoin.

All the selected antioxidants except Vit E considerably decreased the TG levels increased by long term phenytoin treatment in rats.

**Effect of antioxidants on phenytoin induced alterations in LDL levels**

Graph 46 summarizes the effect of phenytoin and phenytoin supplemented with graded doses of Vit C, Vit E, ALA and NAC on LDL levels. Administration of phenytoin 20 mg/Kg for a period of 45 days significantly increased the levels of LDL.
Vit C, ALA and NAC at all the three doses (50, 100, 200 mg/Kg) significantly (p<0.001) decreased the LDL levels in a dose dependent fashion when compared with phenytoin treated animals but the values did not reach that of normal.

Vit E (50 mg/Kg) showed no significant reduction in LDL levels, whereas at 100 mg/Kg (p< 0.05) and 200 mg/Kg (p< 0.001) significantly reduced the LDL levels in a dose dependent fashion. Though there was a significant reduction, the effect was observed to be inferior to other antioxidants.

All the selected antioxidants except Vit E considerably decreased the LDL levels increased by long term phenytoin treatment.

**Effect of antioxidants on phenytoin induced alterations in VLDL levels**

Graph 47 summarizes the effect of long term treatment of phenytoin and phenytoin supplemented with graded doses of Vit C, Vit E, ALA and NAC on VLDL levels. Administration of phenytoin 20 mg/Kg for a period of 45 days significantly increased the levels of VLDL.

Vit C (100, 200 mg/Kg), ALA (50, 100, 200 mg/Kg) and NAC (50, 100, 200 mg/Kg) significantly (p<0.001) decreased the VLDL levels in a dose dependent fashion when compared with phenytoin treated animals but the values did not reach that of normal.

Vit E (50, 100 mg/Kg) showed no significant reduction in levels of VLDL, whereas at the dose of 200 mg/Kg showed significant reduction (p< 0.05) in VLDL levels.

All the selected antioxidants except Vit E considerably reversed the VLDL levels increased by long term phenytoin treatment in rats.
Results - Metabolic disorder

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Graph 43

Effect of antioxidants on phenytoin induced hyperglycemia

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), ***(p< 0.01), *(p< 0.05) Vs Control group

+++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
**Results - Metabolic disorder**

Graph 44

**Effect of antioxidants on phenytoin induced alterations in Total Cholesterol levels**

Values are expressed as mean± SEM of 6 animals.

***( p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group**

**+++( p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group**
Results - Metabolic disorder

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Graph 45

Effect of antioxidants on phenytoin induced alterations in Triglyceride levels

![Graph showing effect of antioxidants on triglyceride levels](image)

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), ** (p< 0.01), *(p< 0.05) Vs Control group

+++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Graph 46

Effect of antioxidants on phenytoin induced alterations in LDL levels

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), ***(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenyoitn group
Results - Metabolic disorder

Graph 47

Effect of antioxidants on phenytoin induced alterations in VLDL levels

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++ (p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
Results

Effect of antioxidants on phenytoin induced alterations in HDL levels

Graph 48 summarizes the effect of phenytoin and phenytoin supplemented with graded doses of Vit C, Vit E, ALA and NAC on HDL levels. Administration of phenytoin 20 mg/Kg for a period of 45 days significantly decreased the levels of HDL.

Vit C and NAC at all the three doses (50, 100, 200 mg/Kg) significantly (p< 0.001) increased the HDL levels in a dose dependent fashion when compared with phenytoin treated animals, whereas ALA at higher doses (100 and 200 mg/Kg) exhibited significant increase in HDL (p< 0.001), but the values did not reach that of normal. Vit E at all three doses showed no significant increase in the levels of HDL.

All the selected antioxidants except Vit E considerably increased the HDL levels decreased by long term phenytoin treatment in rats.
Effect of antioxidants on phenytoin induced alterations in HDL levels

Values are expressed as mean± SEM of 6 animals.

*** (p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++( p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group
DISCUSSION

Effect of phenytoin on blood glucose level

Phenytoin is known to induce hyperglycemia primarily by an inhibitory effect on insulin release. Administration of phenytoin (100 mg, p.o., thrice a day) in patients with DM caused diabetic ketoacidosis (Carter, et al., 1981), whereas non diabetic patients developed hyperglycemia (Rubeaan and Ryan, 1991) along with increased insulin requirements. This clinical picture suggested an insulin resistant state, in which dose reduction of phenytoin resulted in decrease in blood glucose levels back to normal. In vitro studies of phenytoin in a primary culture system of adipocytes, assessed both insulin receptor binding and post binding function. The study showed that phenytoin had no effect on maximum insulin binding, while there was a 57% reduction in maximum methylglucose transport in the presence of phenytoin. This suggested that phenytoin administration resulted in insulin insensitivity by inducing a post binding defect in insulin action (Rubeaan and Ryan, 1991).

The diabetogenic activity of 5,5-diphenyl-2-thiohydantoin (DPTH) was assessed in both normal and streptozotocin induced diabetic rats. DPTH increased the liver weight and liver lipid content in both normal and diabetic rats. In vitro, infusion of DPTH inhibited pancreatic insulin secretion which could be correlated to a significant elevation in blood glucose concentration following DPTH administration (Mackerer, et al., 1977). Oxidative stress induced by ROS or reactive nitrogen species activates serine kinase resulting in the activation of multiple stress sensitive serine/threonine kinase signalling cascades such as inhibitor of nuclear factor kappa β kinase (IKK- β) and others. These kinases phosphorylate multiple targets, such as the insulin receptor (IR) and insulin receptor substrate (IRS) proteins (including IRS-1 and IRS-2). Increased phosphorylation of IR or IRS proteins on discrete serine or threonine sites
Discussion - Metabolic disorder

decreased the extent of insulin stimulated tyrosine phosphorylation (Paz, et al., 1997; Birnbaum, 2001), resulting in reduced insulin action (insulin resistance).

Abnormally high levels of free radicals with a decline in antioxidant defence mechanisms damage cellular organelles and enzymes, increase lipid peroxidation and enhance the development of insulin resistance resulting in DM (Maritim, et al., 2003).

It was reported that a patient treated with phenytoin developed pancreatic symptoms and pancreatitis which was confirmed by pancreatic oedema and elevated pancreatic enzymes (Sepulveda, et al., 1999; Yamashina, et al., 1991).

It was also observed that long term anticonvulsant drug therapy with carbamazepine and phenytoin had a contributory role in the development of chronic pancreatitis (Pezzilli, et al., 1992). Oxygen radicals mediate an important step in the initiation of acute pancreatitis (Schoenberg, et al., 1995). Oxidative stress was also proposed as the root cause underlying the β-cell dysfunction, impaired glucose tolerance, insulin resistance and NIDDM, which was implicated in the progression of complications, including microvascular and macrovascular dysfunction. The ROS initiates a chain reaction, increases the generation of inflammatory mediators and modifies the chemical nature of lipoproteins, resulting in atherogenesis (Wright, et al., 2006). On line with this, in the present study also phenytoin induced hyperglycemia which was proposed to be due to oxidative stress.

**Effect of phenytoin on serum lipid level**

Long term phenytoin therapy was observed to be associated with an increase in serum TG and cholesterol levels. The elevation in serum TG concentration was comparatively greater than that of serum cholesterol and the changes reflected the
effect of phenytoin on hepatic lipid metabolism (Luoma, et al., 1979). Hypercholesterolemia and hypertriglyceridemia are independent risk factors that alone or in combination accelerate the development of atherosclerosis and progression of atherosclerotic lesions (McKenney, 2001). The crucial event in the development of atherosclerosis is the accumulation of lipids within the arterial wall. In addition, it has been demonstrated that increased intracellular generation of ROS plays an important role in atherosclerosis (Chisolm and Steinberg, 2000). ROS reacts with a variety of biomolecules including lipids, carbohydrates, proteins, nucleic acids and macromolecules of connective tissues, thereby interfering with cell function. A lot of oxygenated compounds, particularly aldehydes such as MDA are produced during the attack of free radicals on membrane lipoproteins and PUFAs. SOD, GPx and non-enzymatic antioxidants play an important role in alleviating tissue damage due to the formation of free radicals. It was observed that serum MDA is higher in hyperlipidemic subjects (Minhajuddin, et al., 2005; Yang, et al., 2006), suggesting ROS exerted cytotoxic effects in the early clinical stage of the disease. Enhanced lipid peroxidation and decreased antioxidant enzyme activity represent early events in the development of hyperlipidemia in human beings. Further hypertriglyceridemia and hypercholesterolemia are associated with oxidative modification of LDL, protein glycation, glucose auto-oxidation, thus leading to excess production of lipid peroxides and oxidative stress in hyperlipidemic subjects (Keevil, et al., 2007).

SOD and GPx are the first line cellular defence against oxidative injury which is involved in the disposal of superoxide anions and hydrogen peroxide. Thus, insufficient detoxification of ROS by antioxidant enzymes leads to imbalance between antioxidant and oxidant systems. Apart from endogenous antioxidant systems, HDL also has a protective effect against hyperlipidemia by reversing
cholesterol transport, inhibiting the oxidation of LDL and by neutralizing the atherogenic effects of oxidized LDL (Parthasarathy, et al., 1990). Low serum total antioxidant status and elevated lipid peroxides in subjects with higher serum TC, LDL-C and lower HDL-C levels are likely to increase the rate of LDL oxidation, leading to the development of atherosclerosis (Thomas, 2000).

Serum cholesterol (TC, HDL-C, and LDL-C) and TG levels of epileptic patients receiving chronic antiepileptic monotherapy were compared with age and sex matched controls. Patients on phenytoin showed significantly higher LDL-C values and non-significant differences in TC, HDL-C and TG values (Nikolaos, et al., 2004).

Long term treatment with phenytoin especially in males showed significant increase in lipid levels which is considered as risk factor for atherosclerosis (Zeithlofer, et al., 1993).

Dewan, et al., (2008) conducted a case control study to evaluate the effect of phenytoin on serum lipids and liver function in epileptic children. Seventy nine children receiving at least 6 months of phenytoin monotherapy were compared with age matched healthy controls. The mean TC in children on phenytoin therapy was significantly higher and a periodic monitoring of serum lipids was suggested. Phenytoin is a potent inducer of CYP450 enzymes, which are also involved in cholesterol synthesis. Mintzer, et al., (2009) studied the effect of phenytoin on cholesterol and other serological markers of vascular risk. 34 epileptic patients under phenytoin monotherapy were recruited and were subjected to a change of treatment with one of the non-enzyme inducing anticonvulsants lamotrigine or levetiracetam. Fasting blood samples were obtained both before and 6 weeks after the switch (from phenytoin to lamotrigine or levetiracetam) to measure serum lipid fractions,
lipoprotein (a), C-reactive protein and homocysteine. In the epileptic patients, change from phenytoin produced significant decline in TC, atherogenic cholesterol, TG, C-reactive protein and homocysteine levels, suggesting that phenytoin substantially increased the risk for cardiovascular and cerebrovascular disease by inducing hyperlipidemia, indirectly by enzyme induction (Mintzer, et al., 2009).

The CYP_{450} enzyme system is involved in the synthesis of cholesterol (Nebert and Russel, 2002). A commonly expressed member of the CYP_{450} superfamily, CYP_{51}, encodes lanosterol 14 alpha-demethylase, the first step in the conversion of lanosterol into cholesterol in mammals (Gibbons, 2002). Enzyme inducing AEDs like phenytoin and carbamazepine therefore, were observed to increase cholesterol production and elevate TC levels compared with normal controls (Eiris, et al., 2000; Nikolaos, et al., 2004). Specific studies have shown increase in most of the various lipid fractions, including LDL and TG associated with AED therapy. Online with this, in the present study also phenytoin significantly increased the TC, TG, LDL and VLDL, while decreased the HDL levels. The elevated cholesterol was considered to be due to enzyme inducing property of phenytoin.

In the present study, administration of phenytoin (20 mg/Kg) for 45 days induced hyperglycemia and hyperlipidemia. Phenytoin induced carbohydrate and lipid metabolic disorders were believed to be due to the drug induced oxidative stress.

**Effect of Vitamin C on phenytoin induced metabolic disorder**

Vit C was reported to reduce total lipids, LDL C and increase HDL-C (Ginter, 1975; Dobson, et al., 1984; Horsery, et al., 1981; Erden, et al., 1985). Vit C was observed to stimulate plasma lipoprotein lipase and cholesterol 7-alpha-hydroxylase which are
involved in the catabolism of TG (Gordon, et al., 1977) and synthesis of bile acids from cholesterol (Turley, et al., 1976) respectively.

Das, et al., (2006b) investigated the effect of ascorbic acid on the progress of hypercholesterolemia induced atherosclerosis in rabbits fed with 100 mg cholesterol per day. Ascorbic acid (150 mg/Kg/day) was highly effective in reducing the total surface area covered by atherosclerotic plaque and the degree of atherogenecity, suggesting the use of ascorbic acid in the prevention of hypercholesterolemia induced atherosclerosis (Das, et al., 2006b).

The possible mechanisms by which ascorbic acid affects the development of atherosclerosis and the onset of acute coronary events include altered cholesterol metabolism mediated by Vit C dependent conversion of cholesterol to bile acids along with its effect on TG levels via modulation of lipoprotein lipase activity. A particularly intriguing probable mechanism for the anti-atherogenic effect of Vit C is prevention of atherogenic oxidative modification of LDL. A number of in vitro studies have demonstrated that ascorbic acid strongly inhibits LDL oxidation by a variety of mechanisms. The potential effects of ascorbic acid on platelet function and endothelium derived relaxation factor metabolism are considered to prevent the development of atherosclerotic lesion. The antioxidant and metabolic functions of Vit C contribute to the possible reduction in risk of cardiovascular diseases (Lynch, et al., 1996).

Vit C supplementation in hypercholesterolemic humans and animals resulted in a significant reduction in plasma cholesterol concentrations. Vit C reduced the risk of atherosclerosis not only by decreasing plasma cholesterol and triglyceride
concentrations but also by maintaining the integrity of the vascular wall (Turley, et al., 1976).

Vit C was observed to increase the activity of the microsomal enzyme cholesterol-7 α-hydroxylase and bile acid synthesis. In Vit C deficiency states such as oxidative stress, the activity of the cholesterol 7-alpha-hydroxylating system containing CYP_{450} is depressed in the liver of guinea pigs. Deceleration of the above rate limiting reaction of cholesterol transformation to bile acid results in cholesterol accumulation in the liver, plasma and arteries. This in turn was found to increase TC: HDL C ratio, prolongation of plasma cholesterol half-life, increased cholesterol: bile acids ratio in the gall bladder bile, cholesterol gallstone formation and atheromatous changes on coronary arteries of guinea pigs. Administration of Vit C (500-1000 mg/day), in most of the hypercholesterolemic persons with a low Vit C status, reduced the total cholesterol concentration in blood plasma. It was suggested that, irrespective of the type of hypercholesterolemic therapy (dietary and/or pharmacological), an adequate Vit C supplementation should be ensured in doses capable of creating maximal steady state levels of ascorbate in human tissues (Ginter, et al., 1982; Ness, et al., 1996), to achieve a better control in lipid profile.

Vit C is structurally similar to glucose and therefore is effective in prevention of non-enzymatic glycosylation of proteins (Afkhami-Ardekani, et al., 2003). In addition, Vit C acts as a regulator of catabolism of cholesterol to bile acid in guinea pig and has been demonstrated to be an important factor in lipid regulation (Simom, 1992). There are many reports stating that basal Vit C levels are decreased in diabetic patients (Cunningham, et al., 1991; Dyer, et al., 1997) and oxidative stress is increased in diabetes (Ting, et al., 1996; Evans, et al., 2003; Tousoulis, et al., 2003). Patients with
Diabetes are observed to have decreased HDL C and increased TG (Battisi, et al., 2003). Ascorbic acid at a dose of 2 g/day improved blood glucose regulation and reduced the serum cholesterol as well as TG in NIDDM patients (Errikson and Kahvakka, 1995). Sargeant, et al., (2000) found an inverse relationship between plasma Vit C and glycated haemoglobin levels. Afkhami-Ardekani and Shojaoddiny-Ardekani, (2007) evaluated the effects of Vit C supplementation on blood sugar, insulin, serum lipids and glycated haemoglobin in patients with NIDDM. Consumption of 1000 mg Vit C significantly altered the serum levels of fasting blood sugar, TG, LDL, glycated haemoglobin and insulin. LDL particles are small and dense in NIDDM and are susceptible to oxidation. Alpha tocopherol is a lipid soluble antioxidant and protects LDL particles from oxidative attack. Vit C is required for regeneration of alpha tocopherol and thus prevents LDL oxidation (Mullan, et al., 2002). Paolisso, et al., (1995) carried out a placebo controlled study on 40 elderly type 2 diabetic patients in which supplementation with 500 mg Vit C twice daily for 4 months reduced the plasma levels of blood glucose, LDL, TC and TG significantly.

Oxidative stress is known to impair insulin action (Chen, et al., 2006; Paolisso, et al., 1994b; Paolisso and Guigliano, 1996) and Vit C plays a role in ameliorating insulin resistance because of its antioxidant function (Paolisso, et al., 1994a). Reducing free radical damage in pancreatic β-cells (Papaccio, 1991; Suarez-Pinzon, et al., 1996) may be another mechanism by which Vit C protects against diabetes mellitus. Vit C was found to be important for insulin secretion (Wells, et al., 1995; Dou, et al., 1997) and thus reduced the blood glucose level. Our results are on line with these findings; in the present study also Vit C reversed the phenytoin induced hyperglycemia.
Complications associated with diabetes are mediated through free radical damage resulting from auto-oxidation of glucose and glycosylation of structural proteins. The antioxidant property of Vit C is observed to potentially reduce the above complications. (Lyons, 1995). Vit C being an antioxidant probably reduces insulin resistance by improving the function of insulin receptors and by lowering oxidative stress. Thus, Vit C is believed to reduce hyperglycemia and hyperlipidemia.

In the present study, phenytoin was observed to induce hyperlipidemia mediated through oxidative stress and enzyme induction (CYP450). Phenytoin also was found to induce hyperglycemia and even this was believed to be due to oxidative stress caused by the drug. Vit C exerted a dose dependent antihyperglycemic and antihyperlipidemic effect against phenytoin induced carbohydrate and lipid metabolic disorders. The protective effect of Vit C against phenytoin induced metabolic disorders was considered to be due to its antioxidant property. In addition, Vit C plays a significant role in regulating the catabolism of both cholesterol and TG, by conversion of cholesterol to bile acid, which in turn reduces the serum cholesterol levels and activation of lipoprotein lipase that reduces serum TG levels, elevated by phenytoin.

**Effect of Vitamin E on phenytoin induced metabolic disorder**

Vit E was reported to exhibit an inhibitory effect on glucose auto-oxidation process which interrupted glycosylation (Ceriello, et al., 1988; 1991) thereby, reduced the formation of glycosylated haemoglobin. Glucose undergoes auto-oxidation and generates hydroxyl radicals, also reacts and forms complex with proteins in cases of long standing hyperglycemia and finally results in the formation of AGEs. Accumulation of AGEs results in renal damage and atherosclerosis associated with
DM. Vit E being a reducing agent, inhibits the auto-oxidation of glucose and thereby, prevents the generation of hydroxyl radicals and accumulation of AGEs resulting in reduced incidence of AGEs associated complications in DM. Vit E also was observed to improve glycemic control by protecting β-cells of islets of langerhans and by enhancing insulin action (Ceriello, et al., 1988; Beales, et al., 1994).

Karasu, et al., (1997) evaluated the effects of Vit E in streptozotocin induced diabetic rats. Streptozotocin increased plasma glucose, cholesterol and TG concentrations markedly along with a significant increase in lipid peroxides. Vit E supplementation in Streptozotocin induced diabetic rats eliminated accumulation of lipid peroxides and returned plasma blood glucose towards normal levels (Karasu, et al., 1997). Garg, et al., (2005) also studied the effect of Vit E on Streptozotocin (50 mg/Kg) induced diabetes. Streptozotocin increased plasma glucose and MDA levels along with decrease in the antioxidant defence. Supplementation of Vit E (200 mg/Kg., ip) for 5 weeks reduced the plasma glucose and MDA levels along with a significant increase in Vit E, Vit C and red blood cell glutathione levels. The activities of antioxidant enzymes like catalase, GPx and glutathione reductase were also concomitantly restored near to normal levels in diabetic rats. The study demonstrated that Vit E supplementation augments the antioxidant defence mechanism against hyperglycemia in DM.

Torres, et al., (2003) evaluated the effect of Vit E (200 mg/day for 4 weeks) on hypercholesterolemia and atherosclerosis in male hypercholesterolemic New Zealand White rabbits, fed with cholesterol rich diet. Vit E was observed to have no preventive effect on the development of atherosclerotic plaque or any modification in the lesion type induced by the hypercholesterolemic diet. Vit E was not reported to have
antihyperlipidemic effect while a protective defence against hyperglycemia was observed.

In the present investigation, Vit E reduced the phenytoin induced hyperglycemia by protecting β-cells of islets of langerhans and by enhancing insulin action. All the above actions of Vit E were considered to be accomplished by its antioxidant potential. Though Vit E reduced the phenytoin elevated lipids its lipid lowering activity was inferior to other antioxidants.

**Effect of ALA on phenytoin induced metabolic disorder**

Midaoui, et al., (2003) investigated the effect of dietary supplementation of ALA on mitochondrial superoxide and formation of AGE, which in turn is associated with insulin resistance in chronically glucose fed rats. ALA supplementation was observed to prevent hyperglycemia, AGE formation and associated complications in DM (Midaoui, et al., 2003).

ALA has the potential to prevent DM and chronic hyperglycemia induced complications such as neuropathy. DHLA was observed to protect rat pancreatic islet cells from ROS induced destruction (Heller, et al., 1997). *In vitro* studies reported that ALA stimulated glucose uptake by muscle cells similar to insulin’s mechanism of glucose reuptake (Estrada, et al., 1996). Intravenous administration of 1,000 mg of ALA in NIDDM patients proved a 50% improvement in insulin stimulated glucose uptake (Jacob, et al., 1995). The effect of ALA on both lean and obese NIDDM patients was studied on the parameters like insulin sensitivity, glucose effectiveness, serum lactate and pyruvate levels along with oral glucose tolerance load. Treatment
with 600 mg ALA twice daily for four weeks increased insulin sensitivity and prevented hyperglycemia (Konrad, et al., 1999).

In a placebo controlled, multi centre pilot study, 74 patients with NIDDM were randomized to receive 600 mg ALA or placebo orally for four weeks. At the end of the trial, it was found that patients who received ALA experienced significant improvement in insulin-stimulated glucose disposal compared to those on placebo, suggesting oral administration of ALA improved insulin sensitivity in NIDDM patients (Jacob, et al., 1999).

Hyperglycemia increases oxidative stress and contributes to the increased incidence of atherosclerosis and cardiovascular complications in diabetic patients. Yi and Maeda, (2006) evaluated the effect of ALA on atherosclerosis in apolipoprotein E deficient streptozotocin induced diabetic mice. ALA remarkably reduced plasma TC levels, atherosclerotic lesions and prevented general deterioration of health caused by diabetes. These protective effects of ALA were accompanied by a reduction of plasma glucose and an accelerated recovery of insulin producing cells in the pancreas, which revealed the protection offered by ALA over pancreatic β cells from damage. The study recommended the protective effect of dietary ALA against cardiovascular complications of DM (Yi and Maeda, 2006).

Elevated blood TG is a significant contributing factor for obesity associated with other related health disorders, including NIDDM, NAFLD and cardiovascular diseases. Five-week-old Zucker Diabetic Fatty rats were fed a diet containing ALA (200 mg/Kg/day) for 5 weeks. ALA counteracted the rise in blood and liver TG by inhibiting liver lipogenic gene expression (e.g. glycerol-3-phosphate acyltransferase-1 and diacylglycerol O-acyltransferase-2), lowering hepatic TG secretion as well as by
stimulating the clearance of TG rich lipoproteins. Livers from ALA treated rats exhibited elevated glycogen content, signifying the conversion of dietary carbohydrates into glycogen rather than becoming lipogenic substrate. ALA was proved to have potential clinical applications in the treatment or prevention of hypertriglyceridemia and diabetic dyslipidemia (Butler, et al., 2009).

ALA reduced body weight and prevented the development of DM in diabetes prone obese rats by reducing TG accumulation in non-adipose tissues. Adenosine monophosphate activated protein kinase (AMPK) is a major regulator of cellular energy metabolism and is reported that AMPK activity is decreased in obese rats. Administration of ALA to obese rats increased insulin stimulated glucose disposal in whole body and in skeletal muscle. ALA also increased fatty acid oxidation and activated AMPK in skeletal muscle. The study suggested that ALA improved insulin sensitivity through activation of AMPK and resulted in reduced TG accumulation in skeletal muscle (Lee, et al., 2005).

Protective effect of ALA on hypercholesterolemic rabbits was investigated. ALA decreased plasma TC and LDL levels along with lipid peroxides. Histomorphometric intimal lesion analysis of the aorta showed less atheromatous plaque formation in ALA supplemented group. This study revealed a dual lipid lowering and anti-atherosclerotic properties of ALA (Amom, et al., 2008).

Yenjerla, et al., (2006) evaluated the role of ALA against cyclophosphamide induced hyperlipidemic cardiomyopathy. Cyclophosphamide (200 mg/Kg) administration resulted in abnormal elevation of serum lipids such as LDL-C and VLDL-C along with a marked fall in HDL-C. Supplementation with ALA (25 mg/Kg, for 10 days)
was observed to reverse the abnormalities in the lipid levels and activities of lipid metabolizing enzymes altered by cyclophosphamide.

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. The protective effect of ALA against phenytoin induced metabolic disorders is attributed to its antioxidant property.

**Effect of NAC on phenytoin induced metabolic disorder**

ROS is involved in the destruction of pancreatic β cells and development of IDDM. Activation of NF kappa B by ROS is the key cellular signal in initiating a cascade of events leading to β cell death. Enhancement of pancreatic GSH, a key regulator of NF kappa-light-chain-enhancer of activated B cells, is considered to protect against IDDM. Weanling CD1 mice were subjected to alloxan (50 mg/Kg i.v.) induced IDDM, in which alloxan generated ROS and activated NF kappa B in pancreatic nuclear extracts. Supplementation with NAC (500 mg/Kg), a GSH precursor, inhibited alloxan induced NF kappa B activation and reduced hyperglycemia. Thus, NF kappa B activation by ROS may initiate a sequence of events leading to IDDM. Inhibition of NF kappa B activation by NAC attenuated the severity of IDDM (Ho, et al., 1999).

NAC is a hydrophilic cysteine containing compound naturally formed in Allium plants such as garlic and onion, intake of which was found to effectively decrease high saturated fat induced triacylglycerol and cholesterol accumulation in mice livers. Moreover, it has been shown to protect the liver against high fat induced oxidative
damage (Lin, et al., 2004). NAC reduced the cholesterol levels in plasma and liver in mice consuming a high saturated fat diet (Lin, et al., 2004). Krieger, et al., (2006) demonstrated a reduction in plasma lipid fractions by means of NAC supplementation in hypercholesterolemic LDL receptor knockout mice. The potential mechanism accounting for the lipid-lowering effects of NAC was believed to be related to its antioxidant properties. The maintenance of the normal structure of lipoprotein receptors is indispensable for their function, which improves the cellular uptake of serum lipids from the blood. ROS produced during oxidative stress react with lipoproteins, diminishing the cellular uptake of lipids from the blood (Diniz, et al., 2006; Schaffer, 2003; Brizzi, et al., 2003). Thus, the antioxidant action of NAC might contribute to elevated cellular lipid uptake, resulting in the reduction of serum cholesterol levels. According to Lin and Yin, (2008) the lipid lowering action of NAC in mice consuming a high fat diet is attributed partially to the suppression of mRNA expression of three lipogenic related enzymes namely malic enzyme, fatty acid synthase and 3-hydroxy-3-methylglutaryl coenzyme A reductase (Lin and Yin, 2008).

The putative hypolipidemic effect of NAC was studied in a mouse model of diet induced hypercholesterolemia. Higher serum levels of TC, LDL-C and peroxide content were recorded in groups fed with high cholesterol diet. NAC reduced the lipid levels along with a significant decrease in serum lipid peroxides. Co-administration of NAC restored the disturbed lipid profile and ameliorated the serum antioxidant defence mechanism (Korou, et al., 2010).

In the present study, it was observed that NAC dose dependently decreased the levels of blood glucose increased by phenytoin. NAC also significantly reduced the TC, TG, LDL-C and VLDL-C and increased the levels of HDL altered by phenytoin. The
protective effect of NAC against phenytoin induced metabolic disorders is believed to be due to its antioxidant property.

CONCLUSION

Oxidation of lipids and propagation of free radicals contribute to the pathogenesis of atherosclerosis. Antioxidant supplementation is proposed to circumvent the damage caused by oxygen free radicals.

The present study revealed the protective effect of antioxidants against phenytoin induced metabolic disorder in the order of ALA, NAC, Vit C and Vit E. The antioxidants were more effective at their higher doses (100, 200 mg/Kg). Vit E was effective only against phenytoin induced hyperglycemia. Though Vit E (200 mg/Kg) reduced the lipid profile its lipid lowering effect was observed to be inferior to other antioxidants. As discussed earlier, all the selected antioxidants at a dose of 50 mg/Kg failed to confer protection against phenytoin induced toxicities. It is proved that long term treatment with phenytoin caused carbohydrate and lipid metabolic disorder which was considered to be due to oxidative stress induced by the same. This investigation reports the beneficial effect of antioxidants against phenytoin induced metabolic disorders.
Results and Discussion

5.5 Pharmacokinetic and pharmacodynamic interactions of selected antioxidants with phenytoin in rats
RESULTS

Investigation of pharmacokinetic interactions of selected antioxidants with phenytoin

There was no significant difference in the serum concentration of phenytoin between phenytoin alone treated group and the groups subjected to combination of phenytoin and graded doses of antioxidants (Vit C, Vit E, ALA and NAC). The serum phenytoin level, 3 hrs after the administration of phenytoin (45th day) was 15.740 ± 1.8 µg/ml in the groups treated with phenytoin. The serum phenytoin levels of phenytoin supplemented with the studied antioxidants were observed to be between 14-17 µg/ml, which fits well within the normal therapeutic range (10–20 µg/ml) of phenytoin (Table 5), and were not statistically significant.

Investigation of pharmacodynamic interactions of selected antioxidants with phenytoin

Phenytoin as well as phenytoin supplemented with Vit C, Vit E, ALA and NAC (50, 100 and 200 mg/kg) offered same degree of protection (100%) against MES induced convulsions in rats (Graph50).
Graph 49
Standard graph of phenytoin

Table 5
Phenytoin Concentration Vs Peak Area

<table>
<thead>
<tr>
<th>S. No</th>
<th>Phenytoin Concentration (µg/ml)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>14572.4740</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>24711.2440</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>36369.0500</td>
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<tr>
<td>4</td>
<td>8</td>
<td>48110.4980</td>
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<tr>
<td>5</td>
<td>10</td>
<td>59769.2480</td>
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<tr>
<td>6</td>
<td>12</td>
<td>70404.1640</td>
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<td>14</td>
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<td>16</td>
<td>93305.5520</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>115901.2500</td>
</tr>
</tbody>
</table>
Results - Pharmacokinetic and Pharmacodynamic interactions

Figure 13
Blank Chromatogram

Type of Instrument: LC  Gradient: High Pressure  Detector: UV
Model No: Grace  Wavelength (nm): 230nm
Column Part No. 5144984
Mobile Phase: Methanol: Water: Glacial acetic acid (67:33:1)
Flow rate: 1ml/min

[Blank Chromatogram Image]
Figure 14

Chromatogram of Control

Type of Instrument: LC  
Gradient: High Pressure  
Detector: UV  
Model No: Grace  
Wavelength (nm): 230nm  
Column Part No. 5144984  
Mobile Phase: Methanol: Water: Glacial acetic acid (67:33:1)  
Flow rate: 1ml/min  
Internal Standard: Carbamazepine

Retention time for Carbamazepine: 5.157
Figure 15

Chromatogram of Phenytoin (standard)

Type of Instrument: LC
Gradient: High Pressure
Detector: UV
Model No: Grace
Wavelength (nm): 230nm
Column Part No. 5144984
Mobile Phase: Methanol: Water: Glacial acetic acid (67:33:1)
Flow rate: 1ml/min
Internal Standard: Carbamazepine

Retention time for Phenytoin: 4.490 min
Retention time for Carbamazepine: 5.157 min
Results - Pharmacokinetic and Pharmacodynamic interactions

Figure 16

Chromatogram of Phenytoin supplemented with Vitamin C

Type of Instrument: LC  Gradient: High Pressure  Detector: UV
Model No: Grace  Wavelength (nm): 230nm
Column Part No. 5144984
Mobile Phase: Methanol: Water: Glacial acetic acid (67:33:1)
Flow rate: 1ml/min
Internal Standard: Carbamazepine

Retention time for Phenytoin: 4.490 min
Retention time for Carbamazepine: 5.157 min
Results - Pharmacokinetic and Pharmacodynamic interactions

Figure 17

Chromatogram of Phenytoin supplemented with Vitamin E

Type of Instrument: LC
Gradient: High Pressure
Detector: UV
Model No: Grace
Wavelength (nm): 230nm
Column Part No. 5144984
Mobile Phase: Methanol: Water: Glacial acetic acid (67:33:1)
Flow rate: 1ml/min
Internal Standard: Carbamazepine

Retention time for Phenytoin: 4.490 min
Retention time for Carbamazepine: 5.157 min
Results - Pharmacokinetic and Pharmacodynamic interactions

Figure 18

Chromatogram of Phenytoin supplemented with Alpha Lipoic Acid

Type of Instrument: LC  Gradient: High Pressure  Detector: UV
Model No: Grace  Wavelength (nm): 230nm
Column Part No. 5144984
Mobile Phase: Methanol: Water: Glacial acetic acid (67:33:1)
Flow rate: 1ml/min
Internal Standard: Carbamazepine

Retention time for Phenytoin: 4.490 min
Retention time for Carbamazepine: 5.157 min
Figure 19

Chromatogram of Phenytoin supplemented with N Acetyl Cysteine

Type of Instrument: LC
Model No: Grace
Column Part No. 5144984
Mobile Phase: Methanol: Water: Glacial acetic acid (67:33:1)
Flow rate: 1ml/min
Internal Standard: Carbamazepine

Retention time for Phenytoin: 4.490 min
Retention time for Carbamazepine: 5.157 min
Table 6

Effect of antioxidants on serum phenytoin levels

<table>
<thead>
<tr>
<th>S. No</th>
<th>Groups</th>
<th>Serum phenytoin concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Phenytoin</td>
<td>15.740±1.8</td>
</tr>
<tr>
<td>3</td>
<td>Phenytoin + Vit C 50 mg/Kg</td>
<td>14.820±1.2</td>
</tr>
<tr>
<td>4</td>
<td>Phenytoin + Vit C 100 mg/Kg</td>
<td>16.380±1.4</td>
</tr>
<tr>
<td>5</td>
<td>Phenytoin + Vit C 200 mg/Kg</td>
<td>15.400±1.6</td>
</tr>
<tr>
<td>6</td>
<td>Phenytoin + Vit E 50 mg/Kg</td>
<td>16.120±1.1</td>
</tr>
<tr>
<td>7</td>
<td>Phenytoin + Vit E 100 mg/Kg</td>
<td>14.410±1.5</td>
</tr>
<tr>
<td>8</td>
<td>Phenytoin + Vit E 200 mg/Kg</td>
<td>14.220±1.3</td>
</tr>
<tr>
<td>9</td>
<td>Phenytoin + ALA 50 mg/Kg</td>
<td>14.480±1.6</td>
</tr>
<tr>
<td>10</td>
<td>Phenytoin + ALA 100 mg/Kg</td>
<td>15.340±2.2</td>
</tr>
<tr>
<td>11</td>
<td>Phenytoin + ALA 200 mg/Kg</td>
<td>15.650±1.2</td>
</tr>
<tr>
<td>12</td>
<td>Phenytoin + NAC 50 mg/Kg</td>
<td>16.230±1.6</td>
</tr>
<tr>
<td>13</td>
<td>Phenytoin + NAC 100 mg/Kg</td>
<td>14.230±1.4</td>
</tr>
<tr>
<td>14</td>
<td>Phenytoin + NAC 200 mg/Kg</td>
<td>15.720±1.5</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SEM of 6 animals.
Graph 50

Pharmacodynamic interactions of antioxidants with phenytoin

Values are expressed as mean± SEM of 6 animals.

*** p< 0.001 Vs Control group
DISCUSSION

The present study investigates the pharmacokinetic and pharmacodynamic interaction of the selected antioxidants (Vit C, Vit E, ALA and NAC) with phenytoin in rats.

Phenytoin was reported to have a narrow therapeutic index. It was also observed that bioavailability of phenytoin gets altered with co-administration other drugs or supplements leading to toxic effects or therapeutic failure (Arnold, et al., 1970). Enzyme inducing characteristics along with other special physicochemical and solubility properties of phenytoin provide a great scope for researchers and clinicians to investigate the interactions of phenytoin with other drugs and food supplements (Potsalos, et al., 2002; Lalonde and Botez, 1985). Phenytoin also has a complex kinetics and saturable biodisposition (Porter and Meldrum, 2004) and it is necessary for clinicians to be aware of the possibilities in phenytoin toxicity or therapeutic failure during its co-administration with antioxidants like Vit C, Vit E, ALA and NAC.

Phenytoin (20 mg/Kg) was administered orally for 45 days, and on 45th day, the drug was administered 3hr prior to MES induced seizures. The antioxidants were also co-administered with phenytoin for 45 days, and on 45th day, the antioxidants and phenytoin were administered 3hr prior to MES induced seizures.

Pharmacodynamic study was carried out to evaluate whether antioxidant supplementation hinders the therapeutic efficacy of phenytoin. In the present study, administration of phenytoin (20 mg/Kg for 45 days) produced 100% protection against MES induced seizures. Co-administration of antioxidants with phenytoin also offered the same degree of protection against MES induced convulsions. This finding
suggests that antioxidant supplementation with phenytoin did not reduce the therapeutic effect of phenytoin, revealing that there was no pharmacodynamic interaction between phenytoin and the selected antioxidants.

Immediately after observation of MES induced convulsions, the blood samples were collected from retro orbital plexus and the plasma concentration of phenytoin was estimated by HPLC UV-detector. In view of the fact, that phenytoin is reported to reach its peak plasma concentration at 3 hrs after oral dosing; the blood samples were withdrawn 3hrs after oral administration (Osborn, et al., 1987; Rantanakorn, et al., 1997; Wilder, et al., 1973).

The serum drug concentration was estimated because it is related to the therapeutic or toxic effects of phenytoin. The findings of the present study revealed that the serum phenytoin concentration was not different in phenytoin group and antioxidant supplemented groups. In both phenytoin treated and supplemented groups the serum phenytoin levels were maintained in therapeutic range (10-20 µg/ml). This suggests that co-administration of antioxidants do not alter the serum phenytoin concentration. This is an advantage since pharmacokinetic interactions often make drug combinations ineffective and drug levels are to be monitored frequently to optimize drug dosage in case of interactions. This finding has proved that all the above selected antioxidants reversed the toxicities of phenytoin without interfering with its bioavailability.

Despite the availability of a wide range of conventional as well as newer AEDs and a remarkable progress in the understanding of pathophysiological processes underlying seizures, there are still approximately 30% of epileptic patients who remain refractory to the current therapies. Another concern associated with epilepsy is the side effects
associated with AEDs. Therefore, a need was felt for some novel combinations of AEDs with natural products or antioxidants to get rid of serious adverse effects. An improved therapy to counteract the side effects and drawbacks of the existing AEDs is the need of the hour.

The use of traditional, complementary and alternative medicine along with allopathic drugs are very common (Barnes, et al., 2004; Eisenberg, et al., 1998; 2001; World Health Organization, 2002). The relatively fewer side effects of traditional medicines and antioxidant supplementation in comparison with modern drugs have led to exploration of the concomitant use of alternative medicines. Moreover, there is a renewed public interest in naturopathy and dietary supplementations related to health and disease. Herbal medicines and antioxidant supplementations are the most common forms of traditional, complementary and alternative medicine, since they are considered to be safe and effective.

Therefore, the above selected antioxidants are recommended as potential supplements along with phenytoin since the antioxidants help in decreasing the toxicity of phenytoin with no interference on the bioavailability of the drug and its therapeutic efficacy.