CHAPTER - 3

Review of Literature


3.1 EPILEPSY

Epilepsy is a disorder characterized by paroxysmal cerebral dysrhythmia, manifesting as brief episodes of loss or disturbance of consciousness, with or without characteristic body movements. Epilepsy is the second most common chronic neurological condition (Wells, et al., 2006).

Classification

Depending upon the involvement of the brain, the seizures are grouped into two types:

- Generalized seizures
- Partial seizures

Generalized seizures

Generalized seizures involve both the hemispheres of brain and the initial clinical event is loss of consciousness. Various types of generalized seizures are differentiated by absence or presence of specific motor activity.

- Generalized tonic-clonic seizures (Grandmal)
- Absence seizures (Petitmal)
- Tonic seizures
- Atonic seizures
- Myoclonic seizures
- Atypical absences.

Partial seizures

In this type, initial onset arises from a localized area of the brain. These are caused by localized injury to brain where diagnostic evaluation for the presence of a focal lesion (i.e., trauma, tumor, vascular malformation, stroke, neurodegenerative disease) is required. Partial seizures are further subdivided into:

- Simple partial seizures
- Complex partial seizures or psychomotor or temporal lobe epilepsy
- Partial seizures with secondary generalization
Etiology

In infants and children, the common causes of seizures are perinatal injuries, hypoxia, congenital malformations, metabolic disturbances, developmental disorders, febrile seizures and acute CNS infections. Among young adults, the predisposing factors for occurrence of seizures are brain tumors, head trauma, CNS infection, alcohol withdrawal and arteriovenous malformations. In case of geriatrics, metabolic disorders, cerebrovascular disorders and brain tumors are known to be the reasons for epileptic seizures. Seizures may also be idiopathic.

Pathophysiology

The onset of seizures occurs due to prolonged depolarization of a small group of abnormal neurons associated with the rapid firing of repeated action potentials. These neurons recruit adjacent neurons by which they are also connected in the process.

When the electrical discharges of a large number of cells become abnormally linked together, it creates a storm of electrical activity in the brain, resulting in a clinical seizure, which may spread to adjacent areas of the brain.

Seizures result from an imbalance between excitatory and inhibitory processes in the brain. Proposed mechanisms for the generation and spread of seizure activity within the brain are abnormalities in the membrane properties of neurons, changes in the ionic microenvironment surrounding the neuron, decreased inhibitory neurotransmission primarily by gamma amino butyric acid (GABA) or enhanced excitatory neurotransmission mediated by glutamate.

Diagnosis of epilepsy

For diagnosis of epilepsy, Electro Encephalo Gram (EEG), imaging tests and blood tests are performed.
**Electro Encephalo Gram**

An EEG measures the electrical activity in the brain and helps to uncover many abnormalities. 24-hour EEG is necessary in some cases to determine the precise frequency and nature of any unusual electrical activity in the brain. An abnormal EEG does not necessarily confirm the disease and a normal EEG does not rule out the possibility of epilepsy.

**Imaging tests**

Imaging techniques like Magnetic Resonance Imaging (MRI), Computed Tomography (CT) scan, Positron Emission Tomography (PET) scan, Single Positron Emission Computed Tomography (SPECT) or cerebral angiogram are useful in identifying structural abnormalities within the brain such as tumours and lesions that provoke seizures.

**Blood tests**

Routine blood tests are performed to estimate the liver and kidney functions, sodium, calcium and glucose levels in the blood. These tests help to identify some non-epilepsy related sources of seizures, such as hyperglycemia, hypoglycemia, or electrolyte imbalances. Specific blood tests help in the determination of etiological factors like infection, poisoning and alcohol or other drug abuse in seizures.

**Treatment of epilepsy**

Therapy for a patient with a seizure disorder always includes treatment of underlying conditions which cause seizures, avoidance of precipitating factors and suppression of recurrent seizures by prophylactic therapy with AEDs or surgery.

Phenytoin and carbamazepine act by modulation of voltage dependent ionic channels. Benzodiazepines, phenobarbitone and tiagabine act by enhancing the activity of
GABA, the major inhibitory neurotransmitter in the brain. Felbamate and Lamotrigine act by suppressing the excitatory neurotransmission.

### 3.2 PHENYTOIN

![Chemical Structure of Phenytoin](image)

IUPAC Name: 5,5-diphenylimidazolidine-2,4-dione

Phenytoin (diphenylhydantoin) is one of the most widely used antiepileptic drugs recommended against generalized or partial seizures. It is used as a first line drug for generalized tonic-clonic, simple and complex partial seizures, second line drug for atonic and atypical absences. It is not effective in typical generalized absences and myoclonic seizures (Levine and Chang, 1990; David, et al., 1994). Phenytoin was first synthesized by German chemist Heinrich Biltz in 1908. Phenytoin suppresses the abnormal brain activity seen in seizure by reducing the electrical conductance among brain cells via stabilizing the inactive state of voltage-gated sodium channels. The drug limits the spread of seizure activity without causing CNS depression. It is occasionally used in status epilepticus and trigeminal neuralgia and as a second drug of choice in cardiac arrhythmias induced by digitalis.

**Mechanism of action of phenytoin**

Phenytoin blocks voltage sensitive neuronal sodium channels in neuronal tissue causing prolongation of the rate of recovery and reduces the frequency of sustained repetitive firing of action potentials (Macdonald and Kelly, 1994). It blocks post tetanic potentiation, limits development of maximal seizure activity and reduces the spread of seizures.
Pharmacokinetics of phenytoin

Absorption of phenytoin by oral route is slow, mainly because of its poor aqueous solubility. The peak serum levels are attained at about 2 to 8 hr, inter individual differences were observed during long-term therapy. 80-90% of drug is bound to plasma proteins, its oral bioavailability is approximately 95% and the volume of distribution is 0.5 - 0.8 L/Kg. It is excreted through kidneys and its elimination half-life is 7-42 hrs. The unbound fraction of phenytoin corresponds to its concentration in cerebro spinal fluid (CSF) and saliva (Schmidt and Kupferberg, 1975).

Phenytoin is metabolized principally in the hepatic endoplasmic reticulum mainly by the enzyme Cytochrome P450 (CYP). Small increments in dose produce disproportionately high plasma concentrations. The $t_{1/2}$ (12-24 hrs) progressively increases (up to 60 hrs) when plasma concentration rises above 10 µg/ml as metabolising enzymes get saturated. Monitoring of plasma concentration is very helpful in tailoring dosage.

Drug interactions of phenytoin

Interactions between phenytoin, phenobarbitone and carbamazepine are well established. Chloramphenicol, isoniazid, cimetidine, dicoumarol and warfarin inhibit phenytoin metabolism and precipitate its toxicity. Phenytoin induces microsomal enzymes and increases the degradation of steroids, digoxin, doxycycline, theophylline etc. Sucralfate binds with phenytoin in the gastrointestinal tract and decreases its absorption.

Adverse effects of phenytoin

Serum levels of phenytoin within a range of 10 to 20 µg/ml offer satisfactory seizure control in most of the patients (Dawson and Jamieson, 1971; Borofsky, et al., 1972;
Houghton, et al., 1975; Richens, 1979; Tozer and Winter, 1980; Bergman, et al., 1981) and the toxic signs are rare below 15µg/ml.

- **Neurotoxicity**

Phenytoin at therapeutic concentration causes changes in cognitive function and psychomotor function (Reynolds and Travers, 1974). Phenytoin is known to induce cognitive dysfunction, in terms of long term memory loss (Pandhi and Balakrishnan, 1999). Acute or chronic phenytoin administration in epileptic patients causes cerebellar dysfunction and cerebellar degeneration (McClain, et al., 1980; Baier, et al., 1984; Luef, et al., 1994) and hence leads to disturbances in motor coordination, sensory-motor integration, motor learning and cognition. The dose related adverse effects of phenytoin include ataxia (lack of coordination of muscle movements), nystagmus (involuntary eye movement), lethargy and slurred speech (Reynolds, 1975).

Montenegro, et al., (1999) described the clinical characteristics of phenytoin induced dyskinesia by investigating the occurrence of involuntary movements in adult and paediatric epileptic patients. There were three patients with phenytoin induced dyskinesia: one adult with axial and orofacial dyskinesia and two children with choreoathetosis and had complete recovery after phenytoin withdrawal. Chronic treatment with phenytoin was found to induce dyskinesia. Ohmori, et al., (1999) investigated the neurotoxic effect of phenytoin in the developing cerebellum by oral administration of phenytoin to newborn mice once a day during 2-4 postnatal days. Many apoptotic cells were observed in the external granular layer on postnatal day 5 which was thicker than the control on postnatal day 14. These results showed phenytoin induced cell death of external granule cells in cerebellum. The motor
performance of phenytoin treated mice in a Rota Rod test was impaired indicating phenytoin induced motor in-coordination, as a result of phenytoin induced damage in granule as well as purkinje cells in the developing cerebellum (Ohmori, et al., 1999).

- **Haematotoxicity**

Phenytoin was observed to cause reduction in folic acid levels and induce megaloblastic anemia. Phenytoin also induced agranulocytosis, aplastic anemia, leukopenia, and thrombocytopenia. Phenytoin induced haematotoxicity was believed to be mediated via epoxide metabolites of phenytoin (Holtzer and Reisner-Keller, 1997).

In another study, Sorrell and Forbes, (1975) observed the depression of cellular and humoural immune responses in 60% of patients treated with phenytoin. Phenytoin treated patients failed to manifest delayed hypersensitivity (DHS) reactions to common antigens and to produce antibody to Salmonella typhi and tetanus toxoid. Serum levels of IgA, IgM and DNA content in circulating leucocytes were low. Depression of IgA, DHS reactivity and antibody responsiveness to S. typhi were shown to develop after the commencement of phenytoin therapy in a study of eleven patients. The presence of immunological defects was independent of dosage of the drug, its serum concentration, duration of therapy and sex of the subject. *In vitro* studies provided evidence for immunosuppression which was the result of a direct effect of phenytoin on the metabolism of lymphoid cells. High concentrations of the drug in addition caused depression of cell counts, lymphocyte blastogenesis, deoxyribonucleic acid and protein synthesis (Sorrell and Forbes, 1975).
**Teratogenicity**

Phenytoin is a known teratogen. The syndrome consists of craniofacial anomalies which include broad nasal bridge, cleft lip and palate, microcephaly and a mild form of mental retardation. This syndrome resembles foetal alcohol syndrome and is called the foetal hydantoin syndrome (FHS). Goes and Azoubel, (2003) studied the teratogenic effect of single daily dose of phenytoin (75 mg/Kg), on the epithelial layer of choroid plexus in rat foetus, during gestation. The histological sections were analysed for nuclear alterations in the cuboidal epithelium of choroid plexuses of lateral ventricles in the rat foetus. A distinctive pattern of nuclear abnormalities in choroid plexus epithelium of rat fetus was associated with low dose of phenytoin during pregnancy. Variations in nuclear size might reflect in fundamental nuclear alterations of significance during the process of embryogenesis and represent teratogenic influence of phenytoin in rats. Even at low dose and short period of use during gestation, phenytoin induced embryonic toxicity.

Strickler, et al., (1985) experimented to know whether arene oxide metabolites of phenytoin and a genetic defect in arene oxide detoxification contribute to susceptibility to phenytoin induced birth defects. Lymphocytes from 24 children exposed to phenytoin throughout gestation and from their families were challenged in a blind protocol with phenytoin metabolites generated by a murine hepatic microsomal drug metabolising system. 14 children had a "positive" assay result i.e., a significant increase in cell death associated with phenytoin metabolites. Each child with a positive result had one parent whose cells also were positive. A positive *in vitro* challenge was highly correlated with major birth defects, including congenital heart disease, cleft lip and palate, microcephaly and major genitourinary, eye and limb defects. It was concluded that a genetic defect in arene oxide detoxification seems to
increase the risk of the baby having major birth defects during pregnancies in epileptic women treated with phenytoin (Strickler, et al., 1985).

Adams, et al., (1990) made a review focused on the human and animal evidence for the teratogenicity of phenytoin, with emphasis on neurobehavioral end points. The FHS was characterised by craniofacial defects, pre or postnatal growth deficiency, limb defects, major malformations and mental deficiency. Available data suggested 10-30% prevalence of FHS in infants of women ingesting 100-800 mg/Kg of phenytoin during the first trimester or beyond. Animal models of FHS have been developed and those focusing on neuro-behavioural effects showed that phenytoin produced multiple behavioural dysfunctions in rat offspring at subteratogenic and non growth retarding doses comparable to those found in humans. The dysfunction in rats is dependent on dose and period of exposure, but independent of nutritional, maternal rearing or seizure disorder. Effects include vestibular dysfunction and deficits in learning and memory.

- **Gingival toxicity**

Phenytoin was associated with drug induced gingival enlargement, gum hypertrophy, (hyperplasia) in the oral cavity probably due to folate deficiency. Phenytoin was observed to induce bleeding upon probing, increased gingival exudates and pronounced gingival inflammatory response to plaque levels. A 17-year-old boy with generalized tonic clonic seizures was treated with 300 mg of phenytoin per day for a period of two years. Examination revealed coarsening of facial features and severe gingival hyperplasia, brisk deep tendon reflexes and cerebellar ataxia. Withdrawal of phenytoin was followed by marked regression of the gingival hyperplasia within three months, however, ataxia persisted (Sharma and Dasroy, 2000).
• **Hepatotoxicity**

Many studies have reported on phenytoin induced hepatotoxicity. Brindha and Geetha, (2009) proved that 250 mg/Kg of phenytoin treatment for 21 days caused hepatocellular damage in albino rats as evidenced by increased serum enzymes, bilirubin, TG and cholesterol. Santos, et al., (2008a) evaluated the induction of oxidative stress by three classical AEDs such as carbamazepine, phenytoin and phenobarbital as well as by their metabolites. AED induced hepatotoxicity has been attributed to a defective detoxification by the epoxide hydrolase and accumulation of arene oxides leading to oxidative stress. The toxic effects of the metabolites were evaluated by incubating the drug with rat liver microsomes. Results suggested that the hepatotoxicity associated with AED was mediated by the oxidative stress induced by the drug metabolites. Ahmed and Siddiqi, (2006) made a review on AED and found that 95% of phenytoin is bio transformed by the liver and less than 5% is eliminated unchanged in the urine. Phenytoin at clinically accepted doses saturated the hepatic enzymatic system that metabolizes the drug which is particularly significant in the presence of liver disease. GGT is elevated in 50-90% of patients on phenytoin therapy. Phenytoin induced hepatic injury in 10-38% of cases progressed to fatal outcome. The interval between the initiation of phenytoin therapy and the onset of clinical abnormalities ranged from 1 to 6 weeks in majority of patients. The most common presenting symptoms were fever, rashes, lymphadenopathy, jaundice and hepatosplenomegaly, whereas a substantial proportion of patients experience haemorrhagic complications. Spielberg, et al., (1981) reported that a heritable defect in response to arene oxides may predispose some patients to phenytoin hepatotoxicity. Thus, arene oxide metabolites of phenytoin are observed to be involved in the pathogenesis of drug induced hepatotoxicity. Dreifuss and Langer, (1987) made a
review on the effects of different antiepileptic drugs on liver and found that phenytoin 
and carbamazepine therapy appears to develop serious and fatal hepatotoxicity in 
older adolescents and adults than in children. After the development of hepatotoxicity, 
mortality rates were found to be 10–38% with phenytoin. Phenytoin induces 
microsomal P_{450} enzymes which enhance the production of toxic metabolites and 
consequently the greater risk of hepatotoxicity.

- **Osteomalacia**

Khaira, et al., (2008) presented a case of severe osteomalacia with serious disabilities, 
multiple pseudofractures and contractures secondary to use of phenytoin. Long term 
use of anticonvulsants is associated with an increased risk of fractures. Phenytoin 
induced osteomalacia occurs in approximately one third of the patients receiving this 
drug and changes in the bone mineral density were noticed in nearly 47% of the 
patients. Osteomalacia with hypocalcemia and elevated ALP levels occurs frequently 
in patients on long term phenytoin therapy. This has been attributed to both altered 
metabolism of vitamin D and the inhibition of intestinal absorption of calcium. It also 
increases the metabolism of vitamin K and reduces the concentration of vitamin K 
dependent proteins that are important for normal calcium metabolism. AEDs also 
directly stimulate the osteoblastic activity resulting in osteomalacia. The progress of 
osteomalacia was associated with continued phenytoin use and the greater fracture 
risk from recurrent seizures combine to result in multiple fractures, severe disability 
and complete immobilization. Varkey, et al., (1973) reported that phenytoin therapy 
(200-300 mg/day for 8 years) induced osteomalacia in addition to folic acid 
deficiency and concluded that defect in calcium metabolism was responsible for 
osteomalacia.
• **Infertility**

Falokun Olutunde, et al., (2010) observed that oral administration of phenytoin at the dose of 50 and 100 mg/Kg twice daily for four weeks significantly reduced the fertility in male rats which was however reversible upon withdrawal. Phenytoin significantly reduced the testicular weight, serum testosterone concentration, sperm count and high doses of phenytoin considerably reduced the sperm motility. The percentage of fertility success was also appreciably reduced by phenytoin treatment.

• **Hypersensitivity syndrome**

Vittorio and Muglia, (1995) reported that anticonvulsant hypersensitivity syndrome is a potentially fatal drug reaction with cutaneous and systemic reactions to the arene oxide producing anticonvulsants such as phenytoin, carbamazepine and phenobarbital sodium. In most cases, the hallmark features like fever, rashes and lymphadenopathy are accompanied by multi organ system abnormalities. Fatal outcomes are most often associated with liver failure. As this reaction is genetically inherited there is increased risk for the siblings of patients to develop this hypersensitivity syndrome. Thus, timely recognition of anticonvulsant hypersensitivity syndrome is important as accurate diagnosis avoids potentially fatal exposure. Paul, (1997) reported a life-threatening case of phenytoin hypersensitivity syndrome, which was characterized by a skin eruption and multisystem organ failure. A 64 year old male with alcohol withdrawal seizure was treated with phenytoin for six weeks. Approximately, four weeks after starting phenytoin he developed diffuse erythematous skin rash and later he developed fever and chills along with increased severity of rashes. The patient was found to be confused, hypotensive and in severe distress. He was noted to have periorbital edema with a diffuse morbilliform maculo-papular rash covering his entire body including his palms and soles, with small follicular pustules on his face.
Enlarged discreet lymph nodes were palpable in the cervical, axillary and inguinal regions. The symptoms are characteristic features of the phenytoin hypersensitivity syndrome (PHS), and the patient developed multisystem organ failure after treatment with phenytoin. Bhargava, (2001) examined sixty patients of anticonvulsant hypersensitivity syndrome where all the patients exhibited fever and skin rash. Phenytoin was the commonest offending drug (39 patients) followed by carbamazepine (19 patients) that accounted for the development of anticonvulsant hypersensitivity syndrome which appeared after 2-12 weeks of initiation of the therapy. It was reported that most of the patients recovered well upon withdrawal of the offending drugs and treatment with topical corticosteroids and antihistamines.

- **Visual toxicity**

Thakral, et al., (2003) reported the development of blurred vision and xanthopsia in a 19 year old male after phenytoin treatment for status epilepticus. Phenytoin toxicity resulted in acute visual dysfunction and colour blindness. Discontinuation of phenytoin therapy resulted in partial recovery and after ten months of withdrawal, electro retinogram confirmed diffuse bilateral cone and rod dysfunction. Phenytoin toxicity resulted in acute visual dysfunction a rarely reported phenomenon.

- **Dermatological disturbances**

Danno, et al., (1989) reported the incidence of generalized erythematous rash, fever and systemic lymphadenopathy after two months of phenytoin treatment. Laboratory examinations revealed increased WBC counts with eosinophilia, abnormal liver function and increased gamma globulin levels. Skin biopsy findings showed nonspecific cellular infiltrates in the upper dermis whereas, in lymph nodes the normal architecture was replaced by massive cellular infiltrates consisting largely of
lymphocytes, immunoblasts and eosinophils suggesting a pattern of phenytoin induced lymphadenopathy. No malignant changes were noted. All the manifestations subsided after discontinuation of phenytoin followed by treatment with oral corticosteroids for a period of two months (Danno, et al., 1989). Dwivedi, et al., (2004) correlated the in vitro lymphocyte toxicity of arene oxide metabolites with phenytoin induced skin eruptions. The intermediate metabolites of arene oxides accumulate due to deficiency of the enzyme epoxide hydrolase and are postulated to be associated with PHS.

**Mechanism of phenytoin induced toxicity**

The toxicity associated with phenytoin, may be due to its bioactivation to reactive intermediates (Winn and Wells, 1995). Phenytoin is bioactivated by peroxidases such as prostaglandin H synthase to free radical intermediates that initiate the formation of ROS such as hydroxyl radicals (Kim and Wells, 1996), which in turn oxidize the lipids, proteins, carbohydrates and DNA (Liu and Wells, 1995; Winn and Wells, 1995; 1999). This damage ultimately leads to cellular disruption (Winn and Wells, 1995; 1999), implicating a role for oxidative stress in phenytoin initiated toxicity. Although ROS is generated in many physiological processes, oxidative stress occurs with bioactivation of drugs leading to an imbalance between ROS formation and detoxification (Gutteridge and Halliwell, 2000). Excessive production of ROS including superoxide radical anions, hydroperoxyl radicals, hydrogen peroxide and the highly reactive hydroxyl radical were implicated in many disease processes. DNA damage is a critical cellular lesion involved in cell death, carcinogenesis and teratogenesis (Nicol, et al., 1995).
Intervention of antioxidants and other supplements in phenytoin induced toxicity

Phenytoin induced toxicity was proved to have an etiologic background of oxidative stress and many researchers investigated on the effect of antioxidants and other supplementation against phenytoin induced oxidative stress and toxicity.

- **Influence of spirulina on phenytoin induced behavioural abnormalities**
  Thaakur and Pushpa, (2008a) studied the influence of spirulina (1000 mg/Kg) on regional brain lipid peroxidation and selected behavioural abnormalities induced by phenytoin (10 mg/Kg) administration (42 days) in rats. Phenytoin was found to significantly decrease the motor co-ordination, alertness, spontaneous motor activity and memory. Phenytoin also considerably increased lipid peroxidation in hippocampus, striatum, hypothalamus followed by medulla, pons, midbrain, cerebellum and cortex. Spirulina appreciably reversed the lipid peroxidation induced by phenytoin in hypothalamus, hippocampus, medulla, pons and striatum to varying degrees along with reversal of phenytoin induced behavioural abnormalities. Phenytoin is metabolised to produce free radicals which cause lipid peroxidation in different brain regions and this may attribute to memory impairment, ataxia, decreased alertness and locomotion. Spirulina being a cocktail of nutrients and antioxidants was able to significantly combat the lipid peroxidation in brain regions and thereby reverse the behavioural abnormalities induced by phenytoin.

- **Effect of curcumin on phenytoin induced memory impairment**
  Reeta, et al., (2009) investigated the effect of chronic curcumin administration on phenytoin induced cognitive impairment and oxidative stress in rats. Phenytoin (75 mg/Kg, i.p.) for 21 days produced significant deficits in learning and memory as indicated by the significant increase in retention transfer latency in elevated plus maze test and a significant decrease in retention latency in the passive avoidance paradigm.
Chronic administration of phenytoin also produced significant elevations in brain malondialdehyde (MDA) and reduction of brain GSH levels. Co-administration of curcumin (100, 200 and 300 mg/Kg, orally) with phenytoin, significantly prevented phenytoin induced cognitive impairment and oxidative stress in a dose dependent manner in rats without altering the serum phenytoin levels, suggesting the potential of adjuvant curcumin therapy in ameliorating cognitive impairment caused by phenytoin.

- **Influence of piracetam on phenytoin induced memory impairment**

  Shahid, et al., (2004) studied the effect of the combined treatment of phenytoin and piracetam on seizure control, cognition and motor functions in mice. The study showed that piracetam when co administered with phenytoin, significantly reversed phenytoin induced reduction in spontaneous motor and cognitive functions without altering the efficacy of phenytoin against increasing current electroshock seizure in both acute and chronic studies. Further, it also reversed phenytoin induced increase in acetyl cholinesterase (Ach E) activity. Thus, piracetam was found to alleviate the phenytoin induced cognitive impairment without compromising its antiepileptic efficacy.

- **Influence of Bacopa monniera on phenytoin induced cognitive deficit**

  Vohora, et al., (2000) evaluated the effect of *Bacopa monniera* (BM) alone and in combination with phenytoin on passive-avoidance (PA) task, maximal electroshock seizures and locomotor activity in mice. Phenytoin (25 mg/Kg p.o. for 14 days) adversely affected cognitive function in the PA task. Supplementation of BM extract (40 mg/Kg for 7 days), along with phenytoin in the second week of the two week regimen, significantly reversed phenytoin induced memory impairment. Both acquisition and retention of memory showed improvement without affecting its anticonvulsant activity. It was evidenced that BM is effective in the treatment of cognitive deficit associated with phenytoin therapy.
Influence of spirulina on phenytoin induced haematotoxicity

Thaakur and Puspha, (2007) studied the influence of spirulina on phenytoin induced haematotoxicity in rats. Administration of phenytoin at a dose of 20 mg/Kg/day significantly decreased the haemoglobin content, total leukocyte and erythrocyte count. Supplementation of spirulina (200 mg/Kg) along with phenytoin for 30 days significantly elevated the haemoglobin content, and blood cell count decreased by phenytoin. Phenytoin by depleting vital nutrients such as calcium, folic acid, vitamin D, vitamin K, biotin, carnitine, copper, selenium and zinc caused haematotoxicity whereas spirulina being a cocktail of antioxidants, reversed the phenytoin induced toxicity.

Influence of melatonin on phenytoin induced foetal toxicity

Navarova, et al., (2004) stated that phenytoin (150 mg/Kg p.o) administration from day 7 to 18 of gestation induced chronic hypoxia during pregnancy in rats leading to toxic damage associated with an increase in lysosomal enzyme N-acetyl-β-D-glucosaminidase activity and decrease of GSH level in placenta, maternal serum and heart. Pre and postnatal study was carried out to investigate the effect of melatonin on phenytoin induced toxicity. Melatonin (40 µg/ml of drinking water) was administered from day 0 to 19 of gestation. Melatonin partially inhibited the changes of glucosaminidase activity and increased the level of GSH in maternal heart, liver and lungs, but not in foetal brain. In the postnatal study a significant increase in liver GSH level was found, while the activity of glucosaminidase remained unchanged. Melatonin was found to partially inhibit the biochemical changes induced by phenytoin.
• **Influence of folic acid and Vitamin B_{12} on phenytoin induced hepatotoxicity**

Ekaidem, et al., (2007) observed that phenytoin (70 µg/Kg) significantly reduced serum protein and increased the levels of SGOT, SGPT, ALP and lipids in rats. The effect of folic acid and Vitamin B_{12} was explored on liver integrity of growing rats following phenytoin administration. Supplementation of phenytoin with oral administration of folic acid resulted in a significant increase in serum total protein and decrease in SGOT and SGPT activities. Vitamin B_{12} supplementation offered no significant protection against the toxic effect of phenytoin but rather phenytoin toxicity was exacerbated. However, the combined effects of Vitamin B_{12} and folic acid ameliorated the effects of phenytoin on hepatic enzymes.

### 3.3 VITAMIN C

![](image)

2-oxo-L-threo-hexono-1,4-lactone-2,3-enediol

Vitamin C (Vit C) is an important water soluble antioxidant in biological fluids (Frei, et al., 1989; 1990). An antioxidant is defined as a substance which even at low concentrations significantly delays or prevents oxidation of substrates such as proteins, lipids, carbohydrates and nucleic acids (Halliwell, 1996). Vit C (ascorbic acid) is an essential micronutrient required for normal metabolic functioning of the body (Jaffe, 1984).

**Significance of Vitamin C supplementation**

Human beings and other primates do not have the ability to synthesize Vit C as a result of a mutation in the gene coding for L-gulonolactone oxidase, an enzyme
required for the biosynthesis of Vit C via the glucuronic acid pathway (Woodall and Ames, 1997). Thus, Vit C must be obtained through the diet; however, rodents have the capacity to synthesize Vit C.

**Source**

Vit C is especially plentiful in Broccoli, Cauliflower, Strawberries, Lemon, Lettuce, Mustard greens, Papaya, Turnip greens, Grape fruit, Kiwi fruit, Oranges, Cabbage, Tomato, Asparagus, Spinach, Cucumber, Fennel, Pineapple, Watermelon, Green beans, Cloves, Blueberries, Carrots, Garlic, Apricots, Sweet potato, Plum, Green peas, Onion, Potato, Yam, Banana, Apples, Mushroom, Pear and Corn (Bendich, 1997).

**Role of Vitamin C in biochemical pathways**

Vit C acts as a cofactor for catecholamine biosynthesis, in particular the conversion of dopamine to norepinephrine catalyzed by dopamine β hydroxylase (Burri and Jacob, 1997). It also maintains the reduced state of tetrahydrofolate, a cofactor required for catecholamine biosynthesis. Depression and mood changes frequently occur during Vit C deficiency which is related to deficient dopamine hydroxylation and reduced catecholamine synthesis (Burri and Jacob, 1997; Tsao, 1997). Vit C has also been implicated in the metabolism of cholesterol to bile acids via the enzyme cholesterol 7 α-monooxygenase and in steroid metabolism in the adrenals (Burri and Jacob, 1997; Tsao, 1997). Detoxification of aromatic drugs and carcinogens by hepatic CYP_{450} is also enhanced by Vit C (Tsao, 1997). The role of Vit C in the above metabolic pathways is to reduce the active central metal ion of the various mono and dioxygenases (Burri and Jacob, 1997; Tsao, 1997). Vit C maintains the enzyme thiols in a reduced state and helps to retain the reduced state of GSH, an important intracellular antioxidant and enzyme cofactor (Meister, 1994). Several biochemical, clinical and epidemiologic studies discussed about the beneficial effect of Vit C in
chronic diseases such as cardiovascular diseases, cancer, and cataract (Weber, et al., 1996; Enstrom, 1997; Gey, 1998).

Vit C readily scavenges ROS and nitrogen species, such as superoxide and hydroperoxyl radicals, aqueous peroxyl radicals, singlet oxygen, ozone, peroxynitrite, nitrogen dioxide, nitric oxide radicals and hypochlorous acid (Halliwell, 1996), thereby effectively protects other substrates such as lipids, proteins and nucleic acid from oxidative damage. Although Vit C reacts rapidly with hydroxyl radicals, it is unable to preferentially scavenge this radical over other substrates (Niki and Noguchi, 1997) as hydroxyl radicals are extremely reactive and will combine indiscriminately with any substrate in their immediate environment at a diffusion limited rate. Vit C also acts as a co antioxidant by regenerating \( \alpha \)-tocopherol (Vit E) from the tocopheroxy radical, produced via scavenging of lipid soluble radicals (Packer, 1997; Bowry, et al., 1995). This is a potentially important function because \textit{in vitro} experiments have shown that \( \alpha \)-tocopherol acts as a pro-oxidant in the absence of co antioxidants such as Vit C (Bowry, et al., 1995; Neuzil, et al., 1997). Vit C also regenerates urate, GSH and \( \beta \)-carotene \textit{in vitro} from their respective one electron oxidation products like urate, glutathiol and \( \beta \)-carotene radicals (Halliwell, 1996; Edge and Truscott, 1997). Two major properties of Vit C make it an ideal antioxidant. First is the low one-electron reduction potentials of both ascorbate (282 mV) and its one electron oxidation product, the ascorbyl radical (2174 mV), which is derived from the ene-diol functional group in the molecule (Halliwell, 1996). These low reduction potentials enable ascorbate and the ascorbyl radical to react with and reduce basically all physiologically relevant radicals and oxidants. The second major property that makes Vit C an effective antioxidant is the stability and low reactivity of the ascorbyl radical formed when ascorbate scavenges a reactive oxygen or nitrogen species. The
ascorbyl radical readily dismutates to form ascorbate and dehydroascorbic acid, or is reduced back to ascorbate by semidehydroascorbate reductase (Tsao, 1997; Packer, 1997; Wells and Jung, 1997). The 2-electron oxidation product of ascorbate is dehydroascorbic acid which can itself be reduced back to ascorbate by glutathione dehydroascorbate oxidoreductase or selenoenzyme thioredoxin reductase (Tsao, 1997; Packer, 1997; Wells and Jung, 1997). Dehydroascorbic acid is rapidly and irreversibly hydrolyzed to 2,3- diketogulonic acid (DKG) (Halliwell, 1996).

\[ \text{AH}^- \leftrightarrow \text{A}^-^- \leftrightarrow \text{A} \quad (1) \]
\[ \text{A}^-^- + \text{A}^-^- \leftrightarrow \text{AH}^- + \text{A} \quad (2) \]
\[ \text{A} \leftrightarrow \text{DKG} \leftrightarrow \text{oxalate, threonate} \quad (3) \]

where equation 1 shows the reversible 1- and 2-electron oxidation of ascorbate (AH\(^-\)) to the ascorbyl radical (A\(^-^-\)) and dehydroascorbic acid (A) respectively; equation 2 shows the dismutation of the ascorbyl radical to form ascorbate and dehydroascorbic acid; and equation 3 shows the hydrolysis of dehydroascorbic acid to DKG, which then decomposes to oxalate, threonate, and many other products. DKG is known to be very unstable and easily converts into two delta-lactones of DKG, the 3,4-enediol form of DKG (3,4-DKGL) and 2,3-enediol form of DKG (2,3-DKGL) depending on both pH and temperature. 3,4-DKGL plays an antioxidant role which prevents oxidation of lipoproteins induced by copper ion or peroxyl radicals (Li, et al., 2001). Threonate increases the absorption of ascorbic acid and oxalate forms complex with divalent metals thereby, excretes them in urine and thus prevents metal induced oxidation (Betsche and Fretzdorff, 2005).

Vit C has been recognized and accepted by the US Food and Drug Administration (FDA) as one of the 4 dietary antioxidants, other 3 being Vit E, Vit A (the precursor of β-carotene) and selenium, an essential component of the antioxidant enzymes GPx
and thioredoxin reductase. FDA stated that Vit C serves as an effective free radical scavenger to protect cells from damage by reactive oxygen molecules.

**Applications of Vitamin C**

- **Role of Vitamin C in pregnancy and lactation**
  Pregnant and lactating women also require a higher intake of Vit C to maintain their plasma Vit C concentrations (Burri and Jacob, 1997). This increased requirement is probably due to active placental Vit C transport, whereby Vit C concentrations are significantly higher in cord blood as well as in newborn infants than in the mothers and additional loss of Vit C through milk during lactation (Burri and Jacob, 1997; Berger, et al., 1996). The recommended dietary allowance (RDA) of Vit C for women during pregnancy and lactation are 80 and 100 mg/day respectively.

- **Role of Vitamin C on memory**
  A cohort study showed that consumption of Vit C supplements reduced the occurrence of severe cognitive impairment (Paleologos, et al., 1998).

  Siamak, et al., (2008) investigated the effects of acute, short and long-term pre-training administration of ascorbic acid (60, 120 mg/Kg) on passive avoidance learning (PAL) and memory in rats. The study concluded that short and long term supplementation with ascorbic acid had beneficial effects on acquisition and retrieval processes of PAL and memory in rats.

  Protective effect of Vit E and Vit C supplements against development of dementia and cognitive impairment was evaluated. The study observed that the aged males using both Vit E and Vit C regularly had a reduced risk of vascular dementia and had higher levels of cognitive functioning in late life. The study suggested
supplementation of Vit E and Vit C daily to reduce the risk of certain types of dementia and to maintain cognitive functioning (Masaki, et al., 2000).

- **Hepatoprotective effect of Vitamin C**
  Ahn, et al., (2004) studied the protective effect of Vit C on the hepatotoxicity induced by radiation. Spraque Dawley rats were exposed to radiation and radiation along with Vit C treatment. SOD activity, catalase, MDA and liver enzymes were analyzed to assess the antioxidant effects of Vit C. Exposure to radiation increased the MDA levels in liver and decreased catalase activity whereas Vit C treatment improved the activity of catalase and SOD and decreased the lipid peroxidation, also reversed the elevated levels of marker liver enzymes such as SGOT, SGPT, ALP and LDH which were remarkably elevated by radiation. Electromicrographic findings revealed decreased destruction of hepatic cells in Vit C treated group. Thus, Vit C is thought to be an effective antioxidant against the hepatotoxicity induced by radiation. Ademuyiwa, et al., (1994) studied the protective effect of Vit C against CC1₄ (8 ml/Kg body weight) induced hepatotoxicity in rats. Administration of CC1₄ caused liver damage whereas Vit C (2 g/Kg body weight) prevented liver damage induced by CC1₄. Vit C and paracetamol are known to compete for the sulphate pool in the body. Raghuram, et al., (1978), studied the effect of Vit C on paracetamol induced hepatotoxicity. In therapeutic doses, simultaneous administration of Vit C and paracetamol did not result in liver dysfunction in undernourished subjects as judged by SGOT and GGT levels. On the other hand, even after toxic doses of paracetamol, Vit C had a protective role in mice possibly through its antioxidant property.

- **Role of Vitamin C on cardiovascular disease**
  Coronary artery disease and stroke are the leading causes of morbidity and mortality in general population. Many cohort studies investigated the association between Vit C
intake and the risk of cardiovascular disease. Major risk factors associated with cardiovascular disease are age, male sex, smoking, hypercholesterolemia, hypertension, family history, obesity, and physical inactivity (Lynch, et al., 1996; Simon, 1992). Many epidemiologic studies have shown inverse associations between antioxidant intake, particularly Vit C and cardiovascular as well as cerebrovascular disease risk (Jha, et al., 1995; Enstrom, et al., 1992; Enstrom, 1993; Knekt, et al., 1994; Gale, et al., 1995; Kritchevsky, et al., 1995; Pandey, et al., 1995; Sahyoun, et al., 1996). Several studies observed a reduced risk with moderate intake of Vit C between 45 and 113 mg/day (Knekt, et al., 1994; Gale, et al., 1995; Pandey, et al., 1995; Fehily, et al., 1993). Knekt, et al., (1994) reported a 51% lower risk of coronary artery disease in women consuming Vit C (more than 91 mg/day) than in those consuming less than 61 mg/day. In a population of elderly men and women, Gale, et al., (1995) found that daily intakes of Vit C (> 45 mg) were associated with a 50% lower risk of stroke. Pandey, et al., (1995) observed a moderate but significant 25% lower risk of coronary artery disease in men consuming more than 113 mg of Vit C/day. Sahyoun, et al., (1996) reported a 62% lower risk of cardiovascular disease in a population of elderly men and women consuming more than 388 mg of Vit C/day. Finally, Kritchevsky, et al., (1995) measured carotid artery wall thickness as a measure of atherosclerosis and found significantly decreased intima thickness in men and women aged above 55 years consuming 982 and 728 mg Vit C/day respectively.

Hypercholesterolemia is a significant risk factor for cardiovascular disease (Lynch, et al., 1996). A supplementation study showed that consumption of 1000 mg Vit C/day for 4 weeks resulted in a reduction in serum TC (Gatto, et al., 1996; Toohey, et al., 1996; Simon and Hudes, 1998). One putative pathway is through Vit C’s role as a cofactor for cholesterol 7α-monooxygenase, an enzyme involved in the in vivo
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hydroxylation of cholesterol to form bile acids (Burri and Jacob, 1997). Vit C also modulates the activity of hydroxy methyl glutaryl-CoA reductase, the rate-limiting enzyme in the biosynthesis of cholesterol (Lynch, et al., 1996). The plasma lipoprotein profile is also an important consideration for cardiovascular disease, with decreased concentrations of HDL and increased concentrations of LDL being significant risk factors (Lynch, et al., 1996). Numerous observational studies have found a significant association between elevated plasma Vit C concentrations and increased concentrations of HDL cholesterol and reduced concentrations of LDL cholesterol (Toohey, et al., 1996; Simon and Hudes, 1998; Ness, et al., 1996; Hallfrisch, et al., 1994; Jacques, 1992). Similar modulatory effects were reported after supplementation with 1000 mg Vit C/day for 4 weeks (Gatto, et al., 1996). Ness, et al., (1996) also found an inverse correlation between Vit C status and triacylglycerol concentrations (Ness, et al., 1996). Vit C modulates the activity of lipoprotein lipase (Lynch, et al., 1996). The thrombotic risk of cardiovascular disease is associated with increased concentrations of the coagulation factor fibrinogen (Woodward, et al., 1997). Two earlier studies indicated that supplementation of heart disease patients with 2000–3000 mg Vit C/day for 1 to 6 weeks increased fibrinolytic activity and reduced platelet adhesiveness (Bordia, 1980; Bordia and Verma, 1985).

*In vitro* studies showed that physiological concentrations of Vit C increases PGE₁ and PGI₁ (prostacyclin) production, resulting in a reduction of platelet aggregation and thrombus formation (Frei, 1997). Low concentrations of Vit C are also associated with increased concentrations of plasminogen activator inhibitor 1, a protein that inhibits fibrinolysis (Woodhouse, et al., 1997).

A three years study of antioxidant supplementation in atherosclerosis prevention showed that supplementation with 136 IU of Vit E plus 250 mg of slow-release Vit C
twice daily slowed down the progression of carotid atherosclerosis in men. Salonen, et al., (2003) examined the effect of supplementation (6-year) on common carotid artery intima-media thickness. 520 smoking and nonsmoking men and postmenopausal women aged 45 to 69 years with serum cholesterol ≥193 mg/dl were selected for the study. Atherosclerotic progression was assessed ultrasonographically. Those participants who were deficient in Vit C had more benefits from the supplementation in terms of cholesterol levels and the rate of progression of heart disease. Vit E had no effect on HDL cholesterol where as the supplementation with combination of Vit E and slow release Vit C slowed down atherosclerotic progression in hypercholesterolemic persons (Salonen, et al., 2003).

Loss of endothelium derived nitric oxide (EDNO) contributed to the clinical expression of coronary artery disease (CAD). Increased oxidative stress was considered to be linked to impaired endothelial vasomotor function in atherosclerosis and recent studies demonstrated that short term ascorbic acid treatment improved endothelial function. In a randomized, double blind, placebo controlled study, Gokce, et al., (1999) examined the effects of single dose (2 g) and long term (500 mg/day) ascorbic acid treatment on EDNO dependent flow mediated dilation of the brachial artery in patients with angiographically established CAD. Flow mediated dilation was examined 2 hours after the single dose and 30 days after long term treatment in 46 patients with CAD. Both flow mediated dilation and plasma ascorbic acid concentration were improved after single dose treatment and the effect was sustained after long-term treatment whereas no improvement was observed in placebo group. In patients with CAD, long-term ascorbic acid treatment has a sustained beneficial effect on EDNO action. As endothelial dysfunction contributes to the pathogenesis of
cardiovascular events, the study recommended the supplementation of ascorbic acid in patients with CAD (Gokce, et al., 1999).

Osganian, et al., (2003) studied the effect of Vit C on contracting coronary heart disease in females. A total of over 85,000 women were interviewed about their dietary intake, and then a follow up was done years later to track the rate of the disease among these women. Analysis of the women who had contracting coronary heart disease suggested that a dietary supplement including Vit C reduced the risk of coronary heart disease (Osganian, et al., 2003).

Mullan, et al., (2002) studied the hemodynamic effects of chronic oral supplementation of Vit C on type II diabetes mellitus (DM). Patients of type II DM (n=30) were supplemented with Vit C daily and cardiac functioning was measured in all participants before treatment and 4 weeks after treatment. The Vit C group had lowered diastolic and systolic blood pressure along with reduction in stiffness of arteries. Since high blood pressure is a risk for people with type II DM, the use of Vit C supplementation is an economical way of controlling blood pressure (Mullan, et al., 2002).

- **Role of Vitamin C in cancer**

Vit C protects against cancer through several mechanisms in addition to inhibition of DNA oxidation. One potential mechanism is chemoprotection against mutagenic compounds such as nitrosamines (Hecht, 1997; Tannenbaum and Wishnok, 1987). N-Nitroso compounds are formed by reaction of nitrite or nitrate (common in cigarette smoke) with amines and amides (Hecht, 1997). Nitrosating compounds are also formed from NO generated by inflammatory cells expressing inducible NO synthase (Hecht, 1997; Parsonnet, 1995; Satarug, et al., 1996). N-Nitroso compounds undergo
activation by CYP_{450} dependent enzymes and have been implicated in gastric and lung cancer (Hecht, 1997). Epidemiologic studies have shown an inverse association between Vit C intake (from fruit, vegetables) and cancers (Block, 1991; Fontham, 1994). Additionally, Vit C reduced *in vivo* nitrosation by scavenging nitrite and thereby, prevented its reaction with amines to form nitrosamines (Hecht, 1997; Tannenbaum and Wishnok, 1987). Concentrations of fecal mutagens that have been implicated in colon cancer (Parsonnet, 1995) were also reduced by Vit C (Jacob, et al., 1991). In addition, Vit C reduces carcinogenesis through stimulation of the immune system. Two of the major functions of the immune system are to fight off infections and to prevent cancer (Bendich, 1997). It is hypothesized that the immune system recognizes tumor forming cells as nonself. Cytotoxic T lymphocytes, macrophages and natural killer cells degrade tumor cells (Bendich, 1997). Vit C protects host cells against harmful oxidants released into the extracellular medium. Therefore, an optimal immune response requires a balance between free radical generation and antioxidant protection (Bendich, 1997). Many studies have investigated the effects of Vit C on leukocyte function (Hemila, 1997). Vit C modulates the functions of phagocytes, such as chemotaxis (Vohra, et al., 1990; Johnston, et al., 1992; Levy, et al., 1996; Maderazo, et al., 1991), also alters the activity of natural killer cells along with the functions and proliferation of lymphocytes (Hemila, 1997; Heuser and Vojdani, 1997; Smit and Anderson, 1990). Vit C also affects the production of immune proteins such as cytokines and complement components (Hemila, 1997; Haskell and Johnston, 1991; Tanaka, et al., 1994).

Vit C is required for the optimal activity of several important biosynthetic enzymes and is therefore essential for various metabolic pathways in the body. A deficiency of
this vitamin results in the symptoms like scurvy. Vit C acts as an important co-substrate for several mono- and dioxygenases and oxidases and maintains the active site metal ions of these enzymes in the reduced state. Vit C also acts as an efficient scavenger of aqueous radicals and oxidants, thus protecting other biomolecules from oxidative damage. In addition, Vit C spares or recycles GSH and Vit E to other important physiological antioxidants. Oxidative biomarker studies indicate that Vit C protects against *in vivo* oxidation of lipids and DNA in humans, particularly in persons exposed to enhanced oxidative stress. Numerous epidemiological studies strongly suggest that Vit C lowers the incidence of mortality from most prevalent human diseases: cardiovascular disease and cancer. This role of Vit C in lowering the incidence of disease is most likely derived from its antioxidant activity, although other mechanisms may also contribute. In addition, Vit C seems to have a substantial effect against cataract formation, most likely through antioxidant mechanism. The antioxidant potential of Vit C was believed to benefit public health and reduce the medical costs associated with many chronic diseases. The totality of evidence from the human studies strongly suggest that a dietary intake of 90-100 mg Vit C/day is associated with reduced risk of cardiovascular disease and cancer; furthermore, chronic 500 mg/day doses or acute 1-3 g doses of Vit C significantly improved vasoreactivity, an important consideration for the clinical expression of cardiovascular and cerebrovascular disease (eg., angina pectoris, myocardial infarction, and stroke).
3.4 VITAMIN E

![Alpha Tocopherol](image)

Alpha Tocopherol

(2R)-2,5,7,8-Tetramethyl-2-[(4R,8R)-(4,8,12-trimethyltridecyl)]-6-chromanol

The term Vitamin E (Vit E) describes a family of eight antioxidants: four tocopherols (alpha, beta, gamma and delta) and four tocotrienols (alpha, beta, gamma and delta). Alpha-tocopherol is the only form of Vit E that is actively maintained in blood and tissues (Traber, 1999). The isomeric form of alpha-tocopherol found in food is RRR-alpha-tocopherol (also referred to as "natural" or d-alpha-tocopherol).

Alpha-tocopherol functions as an antioxidant, against free radicals which are formed primarily in the body during normal metabolism and upon exposure to environmental factors such as cigarette smoke, pollutants and drugs etc. Fats which are considered to be integral part of all cell membranes are vulnerable to destruction through oxidation by free radicals. The fat soluble vitamin, alpha-tocopherol is uniquely suited to interrupt free radicals and thus prevent a chain reaction of lipid destruction. Aside from maintaining the integrity of cell membranes throughout the body, alpha-tocopherol also prevents oxidation of LDL. Lipoproteins are particles composed of lipids and proteins that transport fat through the bloodstream. LDL specifically transports cholesterol from the liver to the tissues of the body. Oxidized LDL has been implicated in the development of cardiovascular diseases. As Vit E prevents LDL oxidation, it reduces the risk of cardiovascular diseases.
When a molecule of alpha-tocopherol neutralizes a free radical, it is altered in such a way that its antioxidant capacity is lost. However, other antioxidants such as Vit C are capable of regenerating the antioxidant capacity of alpha-tocopherol (Traber, 2006; Bruno, et al., 2006). Apart from its antioxidant property, alpha-tocopherol also inhibits the activity of protein kinase C (an important cell-signaling molecule), inhibits enzymes involved in inflammation and platelet aggregation (Traber, 2001).

Vit E is an example of phenolic antioxidant, which readily donates the hydrogen from the hydroxyl (OH) group on the ring structure to free radicals, which in turn become unreactive. On donating the hydrogen, the phenolic compound itself becomes a relatively non reactive free radical because the unpaired electron on the oxygen atom is usually delocalized into the aromatic ring structure thereby increasing its stability. The major biological role of Vit E is to protect PUFAs, other components of cell membranes and LDL from oxidation by free radicals. Vit E is located primarily within the phospholipid bilayer of cell membranes. It is particularly effective in preventing lipid peroxidation, a series of chemical reactions involving the oxidative deterioration of PUFAs. Elevated levels of lipid peroxidation products are associated with numerous diseases and clinical conditions (Duthie, 1993). Although Vit E is primarily located in cell and organelle membranes where it can exert its maximum protective effect, its concentration may only be one molecule for every 2000 phospholipid molecules. This suggests that after its reaction with free radicals it is rapidly regenerated, possibly by other antioxidants (Kagan and Tyurina, 1998). Absorption of Vit E from the intestine depends on adequate pancreatic function, biliary secretion and micelle formation. Conditions for absorption are like those for dietary lipid, that is, efficient emulsification, solubilisation within mixed bile salt micelles, uptake by enterocytes and secretion into the circulation via the lymphatic system (Gallo-Torres,
Emulsification takes place initially in the stomach and then in the small intestine in the presence of pancreatic and biliary secretions. The resulting mixed micelle aggregates, solubilises and transports the Vit E molecules into brush border membrane of the enterocyte probably by passive diffusion. Within the enterocyte, tocopherol is incorporated into chylomicrons and secreted into the intracellular space and lymphatic system and subsequently into the blood stream. Tocopherol esters, present in processed foods and vitamin supplements, must be hydrolysed in the small intestine before absorption. Vit E is transported in the blood by the plasma lipoproteins and erythrocytes. Chylomicrons carry tocopherol from the enterocyte to the liver, where they are incorporated into parenchymal cells as chylomicron remnants. The catabolism of chylomicrons takes place in the systemic circulation through the action of cellular lipoprotein lipase. During this process tocopherol is transferred to HDL. The tocopherol in HDL can be transferred to other circulating lipoproteins, such as LDLs and very low-density lipoproteins (VLDLs) (Traber, et al., 1990). During the conversion of VLDL to LDL in the circulation, some α-tocopherol remains within the core lipids and thus is incorporated in LDL. Most α-tocopherol then enters the cells of peripheral tissues within the intact lipoprotein through the LDL receptor pathway, although some may be taken up by membrane binding sites recognising apolipoprotein A-I and A-II present on HDL (Traber, 1996). Although the process of absorption of all the tocopherol homologues in our diet is similar, the alpha form predominates in blood and tissue. This is due to the action of binding proteins that preferentially select the alpha form. In the first instance, a 30-KDa binding protein unique to the liver cytoplasm preferentially incorporates α-tocopherol in the nascent VLDL (Traber and Kayden, 1989). This form also accumulates in non-hepatic tissues, particularly at sites where free radical production is greatest, such as in the
membranes of mitochondria and endoplasmic reticulum in the heart and lungs (Kornbrust and Mavis, 1980). Hepatic intracellular transport may be expedited by a 14.2-KDa binding protein that binds α-tocopherol in preference to the other homologues (Dutta-Roy, et al., 1993). Other proteinaceous sites with apparent tocopherol-binding abilities have been found on erythrocytes, adrenal membranes, and smooth muscle cells (Dutta-Roy, et al., 1994). These serve as Vit E receptors which orient the molecule within the membrane for optimum antioxidant function. The primary oxidation product of α-tocopherol is a tocopheryl quinone that can be conjugated to yield the glucuronate after prior reduction to hydroquinone. This is excreted in bile or further degraded in kidneys to α-tocopheronic acid. Vit E homologues not preferentially selected by the hepatic binding proteins are eliminated during the process of nascent VLDL secretion in the liver and probably excreted via the bile (Drevon, 1991). Some Vit E may also be excreted via skin sebaceous glands (Shiratori, 1974).

Vit E deficiency has been observed in individuals with severe malnutrition, genetic defects affecting the alpha-tocopherol transfer protein and fat malabsorption syndromes. For example, children with cystic fibrosis or cholestatic liver disease along with impaired capacity to absorb dietary fat and fat soluble vitamins may develop symptomatic Vit E deficiency. Severe Vit E deficiency results in neurological symptoms, including impaired balance and coordination (ataxia), injury to the sensory nerves (peripheral neuropathy), muscle weakness (myopathy) and damage to the retina of the eye (pigmented retinopathy). Patients with peripheral neuropathy, ataxia or retinitis pigmentosa are to be screened for Vit E deficiency (Traber, 2006). Developing nervous system is especially vulnerable to Vit E deficiency. Children with
severe Vit E deficiency from birth should be treated with Vit E immediately as they may develop neurological symptoms.

**Dietary sources of Vitamin E**

Major sources of alpha-tocopherol include vegetable oils such as Olive oil, Soybean oil, Corn oil, Canola oil, Safflower oil, Almonds, Peanuts, Spinach, etc.

**Therapeutic applications of Vitamin E**

- **In Cardiovascular disease**
  
  A protective effect of Vit E was reported in a number of European study populations, in which a strong inverse correlation was observed between Vit E levels and risk of mortality associated with cardiovascular disease (Gey, et al., 1991). The cardio protective effects of Vit E are attributed to its antioxidant properties. Vit E quenches single oxygen species as well as terminates free radical chain reactions (Giugliano, 2000). Alpha-tocopherol acts as an antioxidant by donating hydrogen radical to remove the free lipid radical, reacting with it to form non radical products or simply trapping the lipid radical (Upston, et al., 1999). It was assumed to exert its primary protective effects through the protection of LDL from oxidation. This effect has been demonstrated in laboratory animals *in vivo* (Keaney and Frei, 1994), in isolated tissues *in vitro* and in human populations (Pryor, 2000).

Vit E is the most potent inhibitor of lipid peroxidation because it is fat soluble and constitutes part of the LDL molecule. Oxidation of LDL particles initiates a plaque forming cascade, which involves the ingestion of oxidized LDL by macrophages, thereby creating foam cells. These foam cells secrete chemotactic molecules that
attract more white cells, which damage local endothelium, increase inflammatory cytokines and promote procoagulant activity (Pryor, 2000). Heavy metal like copper induced oxidation of LDL was decreased by Vit E supplementation (Suzukawa, et al., 1998). The protective activity of Vit E in LDL molecule depends on Vit C to recycle oxidized Vit E (Gey, 1998). Vit E also was found to affect the pathogenesis of atherosclerotic vascular disease beyond its direct effects on lipids. The majority of morbidity and mortality from cardiovascular disease occurs as a result of thrombosis at the site of an unstable atheromatous plaque in an atherosclerotic artery. Vit E affects the complications of cardiovascular disease by reducing platelet adhesion, inhibiting Vit K dependent clotting factors or stimulating NO formation by the endothelial cell (Pryor, 2000). Effects on platelet aggregation and adhesion, in turn affects intravascular clot formation (Steiner, 1993). Furthermore, the oxidized LDL interferes with the normal production of EDNO. NO is an essential vasodilator which plays an important role in the inhibition of platelet aggregation and smooth muscle cell proliferation (Vogel, 1999). Analysis of data from the Women's Health Study also showed that women receiving Vit E experienced a 21% reduction in risk of venous thromboembolism (Glynn, et al., 2007).

Hyperlipidemia is a risk factor for many chronic illnesses, such as atherosclerosis and coronary heart disease. Engler, et al., (2003) carried out a randomized, double blind, placebo controlled trial to determine the effects of antioxidant Vit C (500 mg/day) and Vit E (400 IU/day) for 6 weeks on endothelium dependent flow mediated dilation of the brachial artery in 15 children with familial hypercholesterolemia or familial combined hyperlipidemia. Antioxidant vitamin therapy improved flow mediated dilation of the brachial artery compared with baseline. Antioxidant therapy with Vit C and Vit E restored endothelial function in hyperlipidemic children. Early detection
and treatment of endothelial dysfunction in high risk children may retard the progression of atherosclerosis. Daily supplement of both Vit C and Vit E helped to improve heart function and reduced the risk of atherosclerosis as well as coronary heart disease.

Boshtam, et al., (2002) investigated the effect of Vit E supplements on blood pressure and heart rate in people with hypertension. Seventy participants who have been diagnosed with mild hypertension were included in the study. After 27 weeks of Vit E supplementation (daily), reduction in both systolic and diastolic blood pressure was observed and the study recommended a daily supplementation of Vit E in patients with mild hypertension.

- **In immune function**
  
  Alpha-tocopherol enhances specific aspects of the immune response that appear to decline with aging. For example, elderly adults administered with 200 mg/day of synthetic alpha-tocopherol for several months displayed increased formation of antibodies in response to hepatitis B and tetanus vaccine (Meydani, et al., 1997). A randomized, placebo controlled trial in elderly nursing home residents reported that daily supplementation with 200 IU of synthetic alpha-tocopherol for one year significantly lowered the risk of upper respiratory tract infections, especially the common cold, but had no effect on lower respiratory tract (lung) infections (Meydani, et al., 2004).

- **In Cancer**

  Cancer is caused by free radical induced oxidative damage to DNA, alpha-tocopherol neutralizes free radicals and prevents cancer. Cancer cells proliferate rapidly and are resistant to death by apoptosis (programmed cell death). Cell culture studies indicate
that the Vit E ester, alpha-tocopheryl succinate, inhibits proliferation and induce apoptosis in a number of cancer cell lines (Yu, et al., 2003; You, et al., 2002; Neuzil, et al., 2001). Vit E supplementation was found to reduce the risk of prostate cancer (Alkhenizan and Hafez, 2007; Heinonen, et al., 1998).

- **In chemotherapy induced mucositis**

  Wadleigh, et al., (1992) determined the efficacy of Vit E in the treatment of chemotherapy induced mucositis in patients with malignancy. A randomized, double blind, placebo controlled study was performed to evaluate the efficacy of topical Vit E in the treatment of oral mucositis in patients receiving chemotherapy for various types of malignancy. A total of 18 patients, 17 of whom had solid tumors and one with acute leukemia, were included and the lesions were observed daily prior to and 5 days after topical application of Vit E. The patients receiving Vit E had complete resolution of their oral lesions. The study suggested the supplementation of Vit E to be an effective therapy in patients with chemotherapy induced mucositis.

- **In Diabetes Mellitus**

  Alpha-tocopherol supplementation was recommended in patients with DM because DM increases the oxidative stress and eventually leads to cardiovascular complications (heart attack and stroke), the leading causes of death in DM. In a study it was found that urinary excretion of F2-isoprostanes, a biochemical marker of oxidative stress was elevated in type 2 DM individuals and supplementation with 600 mg of synthetic alpha-tocopherol for 14 days reduced the levels of the above biomarker (Davi, et al., 1999). Some studies showed that Vit E improved insulin action and glucose disposal in type 2 diabetic (Paolisso, et al., 1993) and non-diabetic (Paolisso, et al., 1993; 1994 c) individuals. Increased oxidative stress has also been
documented in insulin dependent diabetes mellitus (IDDM) (Davi, et al., 1999) and it was reported that supplementing IDDM with 100 IU/day of synthetic alpha-tocopherol for one month significantly decreased both glycosylated hemoglobin and TG levels (Jain, et al., 1996).

Siman and Eriksson, (1997) studied the protective capacity of Vit E in early and late pregnancy of streptozotocin induced diabetic rats. Treatment with 2% of Vit E enriched diet, yielding an approximate daily dosage of 2 g/kg of Vit E, was found to significantly restore both embryonic and foetal morphology. It was observed that maternal diabetes decreased embryonic content of Vit E and a fivefold increase of TBARS in foetal liver. When pregnant diabetic animals were supplemented with Vit E, increased concentrations of the vitamin was found in maternal, embryonic, and foetal tissues followed by a significant reduction in TBARS. Congenital malformations caused by experimental diabetes were prevented by Vit E in vivo and suggest a direction for antioxidant prophylactic treatment against diabetic embryopathy (Siman and Eriksson, 1997).

- **In Dementia**

Brain is particularly vulnerable to oxidative stress, which is involved in the pathology of neurodegenerative diseases like AD (Meydani, 2001). Additionally, some studies have documented low levels of Vit E in cerebrospinal fluid of patients with AD (Kontush and Schekatolina, 2004). A large placebo-controlled intervention trial in individuals with moderate neurological impairment found that supplementation with 2,000 IU of synthetic alpha-tocopherol daily for two years significantly slowed progression of dementia in AD (Sano, et al., 1997). A case-control study examining the risk factors for vascular dementia in elderly Japanese-American men found that
Vit E and Vit C intake was associated with a significantly decreased risk of vascular and other types of dementia (Masaki, et al., 2000).

Morris, et al., (2002) examined the effect of intake of antioxidant nutrients such as Vit E, Vit C, and carotene in cognitive decline associated with increased age. Almost 3000 elderly people were interviewed to determine their vitamin intake and their cognitive functioning was then measured and monitored. Those adults who had higher levels of Vit E consumption had fewer declines in cognitive functioning over time. This suggested that the use of a Vit E supplement daily prevented cognitive decline associated with aging.

- **In menstrual migraine**

Women with migraine experience a change in migraine frequency associated with the menstrual cycle. Ziaei, et al., (2009) studied the effect of Vit E as a prophylactic agent on women with menstrual migraine. During a placebo controlled double blinded trial, 72 women with menstrual migraine received placebo daily for five days, two days before and three days after menstruation for two cycles followed by one month wash out and one Vit E softgel (400 IU) daily for five days in the next two cycles. Each woman was evaluated monthly throughout the study and the daily headache severity, concomitant symptoms and functional disability derived from questionnaires were compared between the Vit E and the placebo treatment periods using four point anchored scales. Vit E exhibited statistically significant differences in the pain severity and functional disability scales when compared with placebo treatment along with reduction in photophobia, phonophobia and nausea. Thus it was evident that Vit E was effective in relieving symptoms due to menstrual migraine.
• *In hot menopausal flashes*

Hot flashes affect as many as 75% of menopausal women. Oestrogen reliably reduces the severity of hot flashes and remains the single most effective treatment. Ziaei, et al., (2007) conducted a placebo double blind controlled trial to study the effect of Vit E on hot menopausal flushes. After 1 week baseline period, the enrolled patients (n = 51) received placebo daily for 4 weeks, followed by 1 week wash out and 400 IU Vit E (soft gel cap) daily for the next 4 weeks. Vit E therapy significantly reduced severity score and daily frequency of hot flashes. The study recommended Vit E in the treatment against hot flashes.

• *In dysmenorrhea*

Ziaei, et al., (2001) evaluated the use of Vit E in treating dysmenorrhea. A total of 100 adolescent girls were enrolled and were subjected to daily supplements of Vit E prior to their menstrual period and during the first 3 days of menstrual cycle. The girls under the supplementation of Vit E reported a larger improvement in symptoms suggesting the beneficial application of Vit E against dysmenorrhea (Ziaei, et al., 2001).

• *Against atopic dermatitis*

Tsourel-Nikita, et al., (2002) studied the effect of Vit E supplementation against of atopic dermatitis. A total of 96 participants with atopic dermatitis were enrolled and were subjected to daily supplementation of Vit E. Vit E supplementation showed a significant improvement in symptoms of atopic dermatitis suggesting the beneficial role of Vit E in the treatment of atopic dermatitis.
3.5 ALPHA LIPOIC ACID

IUPAC Name: (R)-5-(1,2-dithiolan-3-yl) pentanoic acid

Synonyms: α-lipoic acid (alpha lipoic acid), thioctic acid, 6,8-dithiooctanoic acid

Alpha lipoic acid (ALA) is a powerful antioxidant synthesized in human beings and animals (Carreau, 1979). ALA is often termed as "universal antioxidant" as it neutralizes free radicals in both aqueous and lipid media of cells, in contrast to Vit C (water soluble) and Vit E (fat soluble). R-Lipoic acid is unique in that it functions as both fat and water soluble antioxidant that easily crosses cell membranes. Thus, it confers free radical protection to both interior and exterior cellular structures. The antioxidant capacity of ALA is retained in both its reduced and oxidised forms (Packer, et al.,1995). Free radicals cause damage to physiological structures such as DNA, lipids and proteins. ALA by donating electrons to certain key enzyme systems plays a role against oxidative stress induced damages (Spector, et al., 1988).

Biological significance of ALA

ALA was observed to have an important role in free radical scavenging, antioxidant generation, metal chelation, mitochondrial function etc.

- Free radical scavenging

ALA is one of the most versatile antioxidants which is effective in both aqueous and lipid media. It is capable of neutralizing a wide variety of free radicals: singlet oxygen, superoxides, peroxyl and hydroxyl radicals, hypochlorite and peroxynitrite
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(Biewanga, et al., 1997). These types of radicals are believed to play a significant role in disease processes such as hardening of the arteries, atherosclerosis, cancer, cataract formation and diabetes.

- **Metal chelation**

  *In vitro* tests showed that ALA, like other antioxidants, forms insoluble complexes with toxic metals such as arsenic, cadmium, lead, mercury (Keith, et al., 1997; Ou, et al., 1995; Ou, et al., 1996), copper, manganese and zinc, thereby reduces their systemic availability (Sigel, et al., 1978) and thus prevents tissue damage induced by metal poisoning. *In vivo* tests reported that pre treatment with ALA significantly decreased the liver damage and overall toxicity in cadmium (Sumathi, et al., 1996; Muller and Menzel, 1990) and arsenic poisoning (Grunert, 1960).

- **Antioxidant regeneration**

  Once an antioxidant neutralizes a free radical it usually loses its antioxidant ability. In living systems, however, antioxidants are regenerated, often with the help of other antioxidants. GSH regenerates Vit C. Vit C in turn regenerates Vit E (Scholich, et al., 1989). True to its versatile nature, ALA regenerates a variety of antioxidants including GSH (Busse, et al., 1992), Vit C, Vit E and the mitochondrial antioxidant coenzyme Q10 (Kagan, et al., 1990; Packer, et al., 1995).

  Vit E is a potent biological antioxidant that stabilises highly reactive free radicals in lipid tissues and cell membranes. In the process of quenching fatty free radicals, Vit E becomes a free radical itself. The Vit E radical is then regenerated by Vit C (ascorbic acid). This process although restores the lost antioxidant potential of Vit E, it results in the formation of new free radical in the form of unstable Vit C. Vit C is next
recycled by GSH. Vit E, Vit C and GSH work interdependently and control free radicals thereby, prevent cellular damage. But, the antioxidant regeneration cycle runs into a limiting factor determined by the availability of GSH. The concentration of these key antioxidants, Vit E, Vit C and GSH diminishes with age and the individual becomes more susceptible to oxidative damage and inflammation. Cell membrane as well as DNA integrity and immune system go downhill as antioxidants diminish. GSH also plays a vital role in protecting against cataract formation, enhances immune function, prevents liver damage, slows the initiation of cancers and eliminates heavy metals. GSH is quickly depleted when the body experiences high levels of oxidative stress from infection, trauma, medication and environmental toxins. GSH deficiency is observed in patients with low protein intake, DM, liver disease, cataracts, HIV infection, respiratory distress syndrome, cancer and idiopathic pulmonary fibrosis.

ALA enhances GSH levels. GSH is the most important water soluble antioxidant and is linked to detoxification of xenobiotics, modulation of signal transduction, prostaglandin metabolism, regulation of immune response, control of enzyme activity and peptide hormones, etc. The availability of the amino acid cysteine is the rate limiting factor in GSH synthesis. ALA is taken up rapidly by the cells and reduced to dihydro lipoic acid (DHLA), which in turn reduces cystine to cysteine and accelerates the biosynthesis of GSH. ALA acts as a potent antioxidant on its own and also regenerates other antioxidants like Vit E, Vit C, and GSH. Figure 1 depicts the role of ALA in recycling other antioxidant systems (Packer, et al., 1995).
- **Mitochondrial function**

Mitochondria act as the power house of cell. As the cells age, the activity of the mitochondria decreases resulting in lowered energy production, decreased metabolic rate and increased oxidative stress and damage. Clinical studies have demonstrated that supplementation with ALA improved mitochondrial function and metabolic rate along with a decrease in oxidative damage.

- **Delayed aging**

Glycation is the formation of chemical bonds between protein molecules and glucose which impairs the physiological function of proteins and contributes to the effects of aging and many disease processes, especially those associated with DM. These sugar damaged proteins are referred to as advanced glycosylation end products (AGEs). AGEs increase with hyperglycemia and are thought to be responsible for the kidney damage and advanced atherosclerosis associated with DM. Researchers have found that non-covalent binding of ALA to albumin protected proteins against glycation.
thus, ALA acts as an anti-aging nutrient by both its antioxidant properties and its antiglycation properties.

Clinical applications of ALA

- **Protection against atherosclerosis**
  
  In animal models of atherosclerosis, ALA had a protective effect. Shih, (1983) induced atherosclerotic lesions in two groups of Japanese quail fed with atherogenic diet. One of the groups received ALA along with atherogenic diet and exhibited 75 percent lesser atherogenic lesions than the control group. The researchers concluded that ALA protected against atherosclerosis by preventing oxidation of LDL cholesterol and by recycling Vit E.

- **Against cataract**
  
  The enzyme aldose reductase plays an important role in the development of cataracts in DM. ALA inhibited aldose reductase activity in the rat lens (Ou, et al., 1996). Cataracts are associated with reduced antioxidant activity in the lens of the eye and ALA is known to regenerate several important lens antioxidants like GSH. ALA administration maintained the levels of GSH, ascorbic acid and alpha-tocopherol in the lens. It was observed that ALA at a dose of 25 mg/Kg protected 60% of the animals from cataract formation (Maitra, et al., 1995).

- **In HIV treatment**
  
  ALA is shown to improve the antioxidant status in HIV infected patients. In a study conducted in Germany, researchers administered 450 mg ALA per day for 14 days and found significant increase in plasma Vit C and GSH in a majority of the patients.
These observations coincided with a decrease in blood lipid peroxidation products and increase in WBC counts (Fuchs, et al., 1993).

- **In Diabetes and its complications**

ALA, a naturally occurring dithiol compound has long been known as an essential cofactor for mitochondrial bioenergetic enzymes. It is a very important micronutrient with diverse pharmacological and antioxidant properties. Pharmacologically, ALA improves glycemic control and polyneuropathies associated with DM. DM is a common metabolic disorder, usually accompanied by increased production of ROS or by impaired antioxidant defences. Singh and Jialal, (2008) reviewed the efficacy of ALA with reference to IDDM and NIDDM. It appeared that the patients with diabetic neuropathy experienced major benefit from ALA supplementation along with improved glycemic control and insulin sensitivity.

a) **Diabetic neuropathy**

ALA has a therapeutic potential against DM and its complications. It favourably influences the vascular abnormalities of DM such as impaired microcirculation, increased indices of oxidative stress and vascular dysfunction (Ziegler, 2004). ALA is involved in the regulation of carbohydrate and lipid metabolism (Malinska and Winiarska, 2005). Diabetics frequently suffer from peripheral neuropathy, a degenerative nerve condition that involves numbness, tingling and sometimes burning pain in the extremities. Studies indicated that ALA reduced the nerve dysfunction symptoms. The exact mechanism behind this pain relief was not clear, however, it was believed that ALA prevented free radical damage within nerve cells. In one randomized, double-blind, placebo-controlled, multicenter study conducted in
Germany, 82.5% of a group of 63 NIDDM with peripheral neuropathy experienced a significant reduction in their symptoms after three weeks of daily intravenous infusions of 600 mg of ALA (Ziegler, et al., 1995).


b) Insulin resistance

ALA offered the additional benefit of reducing insulin resistance in diabetics, where the ability of insulin receptors to effectively uptake glucose at the cellular level was increased. Insulin resistance appears to be the underlying problem for many people with NIDDM. To compensate, the pancreas secretes large amounts of insulin, resulting in hyperinsulinemia. Individuals who compensate in this way may be at increased risk for developing heart disease and hypertension as a result of their hyperinsulinemic condition. Studies indicate that ALA reduces hyperglycemia and is a safer alternative to oral hypoglycemic agents (Jacob, et al., 1996; Konrad, et al., 1999). In one multicentered, placebo-controlled trial performed in Germany, 74 NIDDM patients received 600, 1200 or 1800 mg ALA daily or a placebo. After four weeks, all treatment groups showed an improvement in glucose disposal compared to placebo, with no significant differences between the ALA groups. The combined results of the treated groups (vs) the placebo group showed a 27 percent increase in
insulin stimulated glucose uptake, with no serious side effects in any of the treatment groups (Jacob, et al., 1999)

c) **Diabetic retinopathy**

Treatment with ALA improved significantly the diabetes induced deterioration of Vit C and GSH in blood and possessed a significant protective role against diabetic retinopathy (Ametov, et al., 2003). Stoyanovsky, et al., (1995) had documented that Vit E (alpha tocopherol) is considered the major lipid soluble antioxidant in retinal cell membranes. It scavenges peroxyl radicals forming peroxyl-α tocopherol radical. Vit C in retinal cells reduces the oxidized Vit E radical to its reduced state and protects it from oxidation induced by ultraviolet radiation. ALA enhanced the protective effect of Vit C by regenerating it from dehydroascorbate. Thus, by increasing the levels of Vit C and GSH, ALA protected the retina from oxidative stress caused by different stressful conditions (El-Hossary, et al., 2010; Akpinar, et al., 2007; Berkowitz, et al., 2007; Derin, et al., 2009 and Komeima, et al., 2007).

d) **Diabetic nephropathy**

ALA is effective in the prevention of early diabetic glomerular injury and provides more protection than high doses of Vit C or Vit E (Melhem, et al., 2001). The study observed that ALA (30 mg/Kg daily for two months) either prevented or significantly decreased the urinary albumin excretion, fractional albumin clearance, glomerular volume and glomerular content of immune reactive transforming growth factor β as well as collagen α1 in diabetic rats. In addition, it was found that ALA, but not Vit C or Vit E, significantly increased renal-cortical GSH content.
e) **Obesity**

Obesity is a predisposing factor for NIDDM and is associated with oxidative stress due to increased mitochondrial uncoupling and β oxidation of free fatty acids. Increased oxidative stress and impaired antioxidant defence mechanisms are important factors in the pathogenesis and progression of diabetes mellitus. ALA and DHLA reduced the oxidative stress by scavenging a number of free radicals in both lipid and aqueous domains. Obese diabetic rats had higher oxidative stress as compared to diabetic rat. ALA significantly lowered the elevated serum glucose, TG, TC, LDL-C, MDA levels and increased the HDL-C along with enzymatic antioxidant levels in obese diabetic rats. ALA decreased the oxidative stress produced by DM and obesity by increasing the sensitivity of insulin thereby, maintained glycemic control and thus decreased the ROS generated by hyperglycemia and dyslipidemia. Further it increased the expression of antioxidants by preventing ROS mediated DNA damage, restored the enzymatic antioxidants and quenched ROS generated by obesity and DM. ALA possessed considerable benefit in the management of obesity and DM in conjunction (Thaakur and Sangeetha, 2008).

- **Hepatoprotection**

The liver stores nutrients and converts them to hormones, proteins and ready sources of energy. It detoxifies drugs and toxins. Many free radicals are produced in the liver. If the number of free radicals overwhelms the body’s natural protection system, the liver gets damaged. 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 encouraged its use in liver damage.
Pari and Murugavel, (2004) investigated the hepatoprotective effect of ALA against chloroquine induced toxicity. Rats were treated orally with ALA (10, 30 and 100 mg/Kg/day) for 7 days before a single oral administration of chloroquine (970 mg/Kg/day) and ALA treatment was continued for three more days. Chloroquine induced increase in the levels of SGOT, SGPT, ALP, bilirubin, lipids and plasma TBARS were significantly reversed in rats treated with ALA. A significant decrease in plasma antioxidants such as GSH, Vit C and Vit E were observed in chloroquine treated rats while ALA significantly improved the levels of plasma antioxidants GSH, Vit C and Vit E in chloroquine treated rats. ALA at a higher dose (100 mg/Kg/day) exhibited a highly significant effect than at its lower doses (10 and 30 mg/Kg/day). It was observed that ALA had a better protective effect than silymarin, a reference hepatoprotective drug, against chloroquine induced hepatotoxicity.

Tamoxifen citrate (TAM), 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. Liver injury was induced in female rats by intraperitoneal (i.p.) administration of TAM at a dose of 45 mg/Kg/day for 7 successive days. ALA at a dose of 20 mg/Kg/day was administered 4 days before and for 14 days concurrently during TAM administration as a prophylactic therapy. On the other hand, another group was subjected to concurrent administration of ALA alone with TAM as a curative therapy. TAM intoxication elicited a significant decline in antioxidant enzymes such as GPx, SOD and catalase along with reduction in levels of GSH. TAM was also observed to cause significant elevations in TBARS, levels of liver enzymes such as SGPT, SGOT and LDH. The
prophylactic administration of ALA to TAM intoxicated rats produced significant increase in all the antioxidant enzymes and GSH whereas significantly decreased the levels of liver TBARS, transaminases and LDH. In addition, it was noted that TAM intoxicated rats exhibited a degree of DNA fragmentation which was partially inhibited by ALA treatment. ALA scavenged the free radicals, prevented DNA fragmentation, reduced liver injury and protected 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 (post TAM administration) agent.

**In improvement of cognitive function**

ALA has a positive effect on patients with AD and other types of memory dysfunction secondary to trauma or cerebro vascular accident. By decreasing oxidative damage in the CNS, ALA decreased the severity of CNS disorders (Packer, et al., 1995). An animal study observed that supplementation with ALA improved long term memory in aged mice; however, no effect in young mice was seen (Stoll, et al., 1993). This lack of therapeutic effect in young mice suggested that ALA did not improve memory from a general standpoint whereas it compensated age related memory deficits.

ALA protected the brain cells from damaging effects of hazardous chemicals. Researchers at the University of Rochester reported that ALA effectively prevented N-methyl-D-aspartate (NMDA) induced neuronal damage (Greenamyre, et al., 1994). Another study found buthionine sulfoximine stimulated neurotoxicity in rat brain was partially prevented by ALA.
In the treatment of Alzheimer's disease

AD is a progressive neurodegenerative disorder that destroys patient memory, cognition and communication ability with the social environment and the ability to carry out daily activities. Holmquist, et al., (2007) suggested the intervention of ALA in AD. ALA was observed to activate choline acetyl transferase and increase the synthesis of ACh. Chelating property of ALA with transition metals inhibited the formation of hydroxyl radicals and scavenged ROS, thereby increased the levels of GSH and down regulated redox sensitive inflammatory processes. Furthermore, ALA scavenges lipid peroxidation products such as hydroxynonenal and acrolein. DHLA, the reduced form of ALA is the active compound responsible for most of the above beneficial effects. This review suggested the application of ALA to be effective in the treatment of AD and related dementias.

In human plasma, ALA exists in an equilibrium of free and plasma protein bound form. ALA binds to high affinity fatty acid sites on human serum albumin, suggesting that one large dose rather than continuous low doses (as provided by "slow release" ALA) will be beneficial for delivery of ALA to the brain. In an open-label study, ALA at a dose of 600 mg was administered daily to 43 patients with AD (receiving a standard treatment with choline-esterase inhibitors) for a period of 48 months showed no significant improvement in patients with moderate dementia but the progress of the disease was extremely slow. Data from cell culture and animal models suggested that ALA combined with nutraceuticals such as curcumin, epigallocatechin gallate (from green tea) and docosahexaenoic acid (from fish oil) synergistically decreased the oxidative stress along with inflammation and thus provided a combined benefit in the treatment of AD (Mazurek, et al., 2008).
Oxidative stress and neuronal energy depletion are characteristic biochemical hallmarks of AD. Pro-energetic and antioxidant drugs such as ALA appear to delay the onset or slow down the progression of the disease. ALA at a dose of 600 mg was administered daily to nine patients with AD (receiving a standard treatment with choline-esterase inhibitors) in an open label study over an observation period of 12 months resulted in stabilization of cognitive functions, demonstrated by constant scores in two neuropsychological tests (the mini mental state exam (MMSE) and the Alzheimer's Disease Assessment Score cognitive subscale-(ADAScog)). Hager, et al., (2007) extended the analysis to 43 patients over an observation period of up to 48 months. The study suggested ALA treatment to be a successful ‘neuroprotective’ therapy option for AD (Hager, et al., 2007).

- **In the treatment of Tardive dyskinesia**

Haloperidol (HAL) is a widely used neuroleptic drug used in the treatment of acute and chronic psychosis. HAL was observed to induce tardive dyskinesia, a complex hyperkinetic syndrome consisting of choreiform and athetoid movements, which persists for months or years even after drug withdrawal. Increased levels of TBARS are found in the CSF and plasma of patients treated with neuroleptics, especially those with movement disorders. ALA is effective in both prevention and treatment of numerous types of neurological disorders. HAL (1 mg/Kg/i.p.) induced vacuous chewing movements in rats. ALA supplementation significantly decreased HAL induced tardive dyskinesia and catalepsy dose dependently, by decreasing lipid peroxidation and by scavenging reactive oxygen and reactive nitrogen species induced by HAL (Thaakur and Himabindu, 2009).
• **In regulation of brain acetylcholinesterase activity**

Arivazhagan, et al., (2006) investigated the activity of acetylcholinesterase (Ach E) in discrete regions of young and aged rat brain before and after ALA supplementation in male albino rats (4 and 24 months of age). ALA was administered intraperitoneally with a regimen of 100 mg/Kg/day for 7 and 14 days. The activity was measured in the cerebral cortex, cerebellum, striatum, hippocampus and hypothalamus, and found to be significantly decreased in brain regions of control rats. Administration of ALA into aged rats reversed the above. The results suggested that ALA is effective in restoration of the activity of Ach E in aged rats.

• **In the treatment of glaucoma**

ALA was administered to 75 subjects with open-angle glaucoma at dosages of either 75 mg daily for two months or 150 mg daily for one month. Thirty one others served as controls and were given only local hypotensive therapy. The greatest improvements in the biochemical parameters of glaucoma and visual function were observed in the group receiving 150 mg ALA (Filina, et al., 1995).

• **Protection against ischemic reperfusion injury**

After an area of tissue has been deprived of blood for a period of time, such as occurs in the brain after a stroke or in the heart after clot dissolution, reperfusion of the tissues causes a burst of free radical formation. Several animal studies have demonstrated the effectiveness of DHLA in the prevention of reperfusion injury (Scheer and Zimmer, 1993; Assadnazari, et al., 1993; Prehn, et al., 1992; Panigrahi, et al., 1996; Cao and Phillis, 1995). Animal studies supported the efficacy of ALA as a neuroprotectant after ischemia (Mitsui, et al., 1999; Haramaki, et al., 1995). One week
after ischemic injury and reperfusion in rats, the amplitude of sensory action potential and sensory conduction velocity in heart was significantly improved with ALA (Mitsui, et al., 1999).

- **In Heavy metal toxicity**

*In vitro* and animal studies suggested ALA supplementation to be beneficial in the treatment of heavy metal toxicity, particularly toxicity involving lead, cadmium, mercury or copper (Packer, et al., 1995; Gurer, et al., 1999; Muller and Menzel, 1990; Muller, 1989; Sumathi, et al., 1996; Anuradha and Varalakshmi, 1999; Yamamoto, et al., 2001). In one study an intraperitoneal injection of 25 mg/Kg ALA administered to rats for seven days was able to significantly alter the oxidative stress induced by lead toxicity (Gurer, et al., 1999). Another study demonstrated ALA, at concentrations of 5 mM, was able to protect rat hepatocytes from cadmium toxicity (200 µM) by increasing the total GSH and decreasing lipid peroxidation (Muller and Menzel, 1990). Furthermore, a study on mercury intoxication revealed an injection of 10 mg/Kg/day ALA in rats inoculated with 1 mg/Kg/day mercuric chloride prevented damage to nerve tissue caused by lipid peroxidation (Yamamoto, et al., 2001). Long Evans Cinnamon rats have a genetic defect that causes them to accumulate copper in the liver in a manner similar to patients with Wilson’s disease and spontaneously develop acute hepatitis. ALA has been shown to protect these rats from developing hepatitis. ALA appeared to improve tissue redox status in metal toxicity (Pande and Flora, 2002).
3.6 N-ACETYL CYSTEINE

Acetylcysteine, also known as N-acetylcysteine or N-acetyl-L-cysteine (NAC), is an acetylated derivative of the amino acid L-cysteine. NAC was used primarily as a mucolytic agent in chronic respiratory illnesses as well as in the management of paracetamol toxicity (Geier and Geier, 2006). Animal and human studies showed NAC to be a powerful antioxidant and potential therapeutic agent in the treatment of cancer, heart disease, HIV infection, heavy metal toxicity and other diseases characterized by oxidative damage. NAC has also been shown to be of value in the treatment of influenza, hepatitis C and myoclonic epilepsy.

**Mechanism of action**

NAC acts as a source of sulfhydryl groups and its effectiveness is primarily attributed to its ability to reduce extracellular cystine to cysteine. NAC enhances glutathione-S-transferase activity thereby increases the synthesis of GSH, promotes liver detoxification by inhibiting xenobiotic biotransformation and acts as a powerful nucleophile capable of scavenging free radicals (De Vries and De Flora, 1993; De Flora, et al., 1985). GSH is a predominant antioxidant in the cytoplasm of cells which is synthesized from three amino acids in a two-step process which initiates with the combination of glutamic acid and cysteine and ends with the addition of glycine. The liver and lungs are the primary sites of GSH synthesis. Glycine and glutamic acid are plentiful in cells and thus it is the availability of cysteine that controls the reaction.
rate, which in turn depends on NAC. NAC acts as a mucolytic agent by interacting with disulfide bonds in mucoproteins, forms less viscous units by breaking into small units. NAC also acts as an expectorant by stimulating ciliary action and the gastropulmonary vagal reflex, thereby clearing mucus from the airways (Zimet, 1988). Studies have also shown NAC to be beneficial in heart disease as it lowers homocysteine and lipoprotein levels via dissociation of disulfide bonds (Gavish and Breslow, 1991; Wiklund, et al., 1996), protects against ischemia and reperfusion damage via replenishment of the GSH redox system (Ceconi, et al., 1988) as well as potentiates the activity of nitroglycerin, a vasodilator (Horowitz, et al., 1988).

Applications of NAC

- **In heavy metal poisoning**
  Heavy metals like lead, mercury and arsenic are detoxified and removed from the body by NAC. NAC lengthened the survival time of arsenite treated mice and prevented the risk of mortality in animals subjected to copper poisoning (Henderson, et al., 1985). NAC is also effective against other heavy metal poisoning like gold, silver, mercury, lead, etc., and increased the excretion of zinc and other essential minerals when taken over an extended period. It is therefore necessary to supplement zinc, copper and other trace minerals during administration of NAC (Zimet, 1988).

- **In enhancement of immune system**
  GSH assists the transport of nutrients to lymphocytes and phagocytes, thus protects cell membranes. Purified GSH is available as a dietary supplement but has a low absorption. So, NAC is thought to be a better supplement for boosting cellular GSH levels.
• **Against HIV infection**

HIV positive individuals generally exhibit low GSH and cysteine levels, which has prompted studies on NAC’s effectiveness as a therapeutic tool for AIDS patients. NAC enhances T cell immunity by stimulating T cell colony formation (Wu, et al., 1989) and blocks NF kappa B (Nuclear factor kappa) expression (Breithaupt, et al., 1996; Droge, et al., 1992). In a double-blind, placebo controlled trial, NAC positively impacted plasma cysteine levels and lymphocyte cell counts (Akerlund, et al., 1996). Thus, NAC was suggested to prevent the progression of AIDS.

• **Against respiratory illness**

NAC’s is effective against various types of respiratory disorders. It is a good expectorant which reduces the severity of cough (Jackson, et al., 1984) and treats diaphragm fatigue (Hida, et al., 1996). NAC (600 mg) thrice daily for 12 weeks resulted in improvement of pulmonary function and GSH levels in patients with fibrosing alveolitis which is characterized by severe oxidative stress and depleted GSH levels (Behr, et al., 1997). In chronic bronchitis conditions NAC treatment was found to decrease the exacerbations of severe airway obstruction (British Thoracic Society Research Committee, 1985, Gotz, et al., 1980).

• **In mucolytic therapy**

NAC is indicated for mucolytic therapy in respiratory conditions like emphysema, bronchitis, tuberculosis, bronchiectasis, amyloidosis, pneumonia, cystic fibrosis and Chronic Obstructive Pulmonary Disease (COPD) associated with excessive and thick mucus production. NAC reduces mucus viscosity by splitting disulfide bond linking proteins present in the mucus (mucoproteins), resulting in reduction in chain length and disintegration of mucoproteins in lung mucus which in turn reduces the viscosity of mucus. It was reported that high dose NAC modulated inflammation in cystic
fibrosis and counters the redox and inflammatory imbalances in cystic fibrosis (Tirouvanziam, et al., 2006).

- **In treatment of Cancer**

NAC reduced the proliferation of cells lining the colon and reduced the risk of colon cancer in people with recurrent polyps in the colon. Its action as an antioxidant and a GSH precursor contributes a protective effect against cancer. NAC possess a chemo preventive potential against the treatment of certain types of cancer, including lung, skin, head, neck, breast and liver cancer. *In vitro* and *in vivo* studies have demonstrated NAC to be directly anti-mutagenic and anti carcinogenic (De Flora, et al., 1986). NAC administration in cell cultures and animal studies selectively protected normal cells, but not malignant ones from chemotherapy and radiation toxicity (De Flora, et al., 1996). It was also observed that NAC inhibited cell growth and proliferation of human melanoma, prostate and astrocytoma cell lines (Chiao, et al., 2000; Redondo, et al., 2000; Arora-Kuruganti, et al., 1999).

- **In acetaminophen and other poisonings**

Historically the most prevalent and well accepted use of NAC was as an antidote for acetaminophen or paracetamol poisoning. The metabolite of acetaminophen depletes hepatocytic GSH, causes hepatocellular damage and even death. Administration of NAC intravenously or orally within 10 hours of overdose was effective to prevent hepatotoxicity; however, if the treatment is initiated within 8-10 hours of acetaminophen overdose it was observed that improvement was most appreciable. NAC’s effectiveness declines when treatment is delayed beyond 10 hours, where the risk of mortality is significantly increased (Smilkstein, et al., 1988, Wang, et al., 1997; Perry and Shannon, 1998). NAC is also effective against poisoning by CCl₄.
Review of Literature

Acrylonitriles, halothane, paraquat, acetaldehyde, coumarins, and interferons (Pajoumand, et al., 2003).

- **Against viral hepatitis**
  The standard therapy for chronic hepatitis C involves the usage of interferon alpha, however, many patients are either resistant to interferon alpha therapy or they develop resistance after a period of time. Beloqui, et al., (1993) found supplementation with NAC for six months (600 mg thrice daily) enhanced the response to interferon alpha therapy in chronic hepatitis C patients resistant to interferon alpha, with normalization of SGPT in 41 percent of patients (Beloqui, et al., 1993).

- **In cardiac disorders**
  NAC is an effective therapeutic agent in the management of heart disease. Wiklund, et al., (1996) found NAC reduced plasma homocysteine levels by 45%, while Gavish and Breslow, (1991) demonstrated the ability of NAC to decrease lipoprotein by 70%. As NAC significantly increase the tissue GSH it is useful in treating ischemia and reperfusion associated with acute myocardial infarction and also treats the resultant depletion in cellular sulfhydryl groups (Ceconi, et al., 1988). In addition, NAC potentiated nitroglycerine’s coronary dilating and anti-platelet properties and therefore, is useful in combination therapy in patients with unstable angina pectoris and myocardial infarction (Winniford, et al., 1986; Chirkov and Horowitz, 1996).

- **Against hepatotoxicity**
  Ozaras, et al., (2003) investigated the effect of NAC (1g/Kg) in intragastrically ethanol fed rats. Ethanol elevated the levels of liver enzymes such as SGOT and SGPT, increased serum and tissue levels of MDA indicating augmented lipid peroxidation, on the other hand, decreased the GPx, SOD in serum and liver
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. Methanol administration increased lipid peroxidation products which resulted in the hepatic cell membrane damage and a leak of SGOT and SGPT into the blood. Ingestion of NAC with methanol prevented these methanol induced changes. The study suggested that NAC being an antioxidant protected free radical induced hepatocellular damage following methanol intoxication (Dobrzynska, et al., 2000).

The efficacy of NAC was compared with the antioxidant potential of aminoguanidine against azathioprine induced hepatotoxicity. Aminoguanidine (100 mg/Kg/i.p.) and NAC (100 mg/Kg/i.p.) were administered to rats for 7 days after which single dose of Azathioprine (15 mg/Kg/i.p.) was administered. Azathioprine caused an increase in SGOT, SGPT along with liver lipid peroxides and decreased the levels of GSH contents in rats which were not pre-treated with aminoguanidine and NAC. Pre-treatment with NAC reversed the aminotransferases, lipid peroxides and GSH levels after azathioprine treatment. Pre-treatment with aminoguanidine did not significantly reverse azathioprine induced changes. These observations indicated that NAC exhibited a protective mechanism by improving the levels of GSH. The study reported a significant protection offered by NAC against the toxic effects of azathioprine (Raza, et al., 2003).

Narasimhanaidu, et al., (2005) studied the efficacy of NAC on marker enzymes, lipid peroxidation and antioxidants in \( \text{CCl}_4 \) induced hepatotoxicity. Subcutaneous administration of \( \text{CCl}_4 \) (3 ml/Kg/week) to albino Wistar rats for a period of three
months significantly increased the activities of SGOT, GGT and ALP, also increased the levels of TBARS and hydroperoxides in plasma and tissues (liver and kidney). A significant decrease in the levels of plasma enzymatic and non enzymatic antioxidants was observed. 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 hepatoprotective effect of NAC against CCl₄ induced toxicity which was considered to be due to its antioxidant potential.

- **Against recurrent pregnancy loss**

Amin, et al., (2008) evaluated the effect of NAC therapy in patients diagnosed with unexplained recurrent pregnancy loss (RPL). Pregnancy is associated with a state of oxidative stress that could initiate and propagate a cascade of changes that may lead to pregnancy wastage. This process of oxidative stress may be suppressed by the antioxidant effect of NAC. The study was a prospective controlled study performed in the Women's Health Centre, Assiut University, Egypt. A group of 80 patients with history of unexplained RPL were treated with a combination of NAC (600 mg) and folic acid (500 µg) daily and compared with an age matched group of 86 patients treated with only folic acid (500 µg/day). Combination of NAC and folic acid caused a significantly increased rate of maintaining pregnancy up to and beyond 20 weeks, also significantly increased the take home baby rate as compared with folic acid alone treated cases. NAC is a well tolerated drug that could be a potentially effective treatment in patients with unexplained RPL (Amin, et al., 2008).
Against memory impairment

Jayalakshmi, et al., (2007) explored the effect of supplementation of NAC on hypobaric hypoxia induced deficits on spatial working, reference memory functions and oxidative stress in rats. The rats were exposed to hypobaric hypoxia for 3 days and received oral NAC supplementation (750 mg/Kg) daily. Rats from all the groups were trained in Morris water maze task for 8 consecutive days. Spatial working and reference memory was tested immediately after the termination of hypobaric hypoxia and oxidative stress markers were estimated in hippocampus. Rats displayed significant deficits in spatial working memory and had increased oxidative stress. Supplementation with NAC in hypoxia exposed group improved spatial memory performance and decreased oxidative stress. These findings indicated that NAC supplementation reversed the memory deficit and oxidative stress caused by hypoxic exposure in rats (Jayalakshmi, et al., 2007).

Aluminium is a potent neurotoxin involved in the initiation and progression of various cognitive disorders like AD via oxidative stress. Prakash and Kumar, (2009) designed a study to explore the possible role of NAC against aluminium mediated cognitive dysfunction and oxidative stress in rats. Administration of aluminium chloride (100 mg/Kg, p.o.) daily for 6 weeks resulted in poor retention of memory in Morris water maze, elevated plus maze task paradigms and caused marked oxidative damage. Chronic administration of NAC (50 and 100 mg/Kg/i.p.) significantly improved memory retention, attenuated oxidative damage in aluminium treated rats. NAC possessed a significant neuroprotective effect against aluminium induced cognitive dysfunction and oxidative damage (Prakash and Kumar, 2009).
• **In wound healing**

NAC was evaluated for its wound healing activity in albino rats by using incision and excision wound models. Oral administration of NAC was observed to increase the levels of SOD, catalase, GSH, Vit C and decreased lipid peroxidation with concomitant accelerated wound healing effect. NAC possessed an appreciable wound healing activity by enhancing the level of antioxidant enzymes, by scavenging free radical and by improving the antioxidant status resulting in better collagenation (Thaakur, et al., 2009).