CHAPTER - 3

Review of Literature
EPILEPSY

Epilepsy is a common chronic neurological disorder characterized by seizures (Commission on Epidemiology and Prognosis, 1993; Blume, et al., 2001) which are transient signs and symptoms of abnormal, excessive or hypersynchronous neuronal activity in the brain (Fisher, et al., 2005).

CARBAMAZEPINE

Carbamazepine (CBZ) is a derivative of iminostilbene with a carbamyl group at the 5th position, which is essential for potent anticonvulsant activity (McNamara, 2006). In 1953, Schindler discovered CBZ as an anticonvulsant drug. CBZ is also used in the treatment of trigeminal neuralgia (Schindler and Häfliger, 1954) and bipolar disorders (McNamara, 2006).

MECHANISM OF ACTION OF CARBAMAZEPINE

CBZ acts by blocking the voltage gated sodium channels and inhibits high-frequency repetitive firing in neurons. In addition the drug acts presynaptically and decreases the synaptic transmission. CBZ also inhibits the uptake and release of norepinephrine from brain synaptosomes (Katzung, 2001). 10,11, epoxy CBZ, an active metabolite of CBZ slows Na⁺ channel recovery, which is also considered to be responsible for its
therapeutic effects (Golan, et al., 2008). CBZ also potentiates the GABA receptors thereby brings down neuronal firing (Granger, et al., 1995). CBZ depresses synaptic transmission in the reticular activating system, thalamus and limbic structure thereby reduces the high frequency neuronal excitation. CBZ appears to act by reducing polysynaptic response by blocking the post tetanic potentiation. CBZ exerts anticonvulsant activity by inhibiting the action of excitatory neurotransmitters such as glutamate (Bennett and Brown, 2003).

PHARMACOKINETICS

Absorption
CBZ is absorbed well and the peak levels are usually achieved within 6-8 hours after administration. The drug is given to patients after food to help the patient tolerate larger total daily doses and to slow the absorption process (Katzung, 2001). Its plasma half-life is about 30 hours when it is given as a single dose. As CBZ is a strong enzyme inducing agent, the plasma half-life shortens to about 15 hours on repeated administration (Rang and Dale, 2003).

Distribution
Distribution of CBZ is slow and the volume of distribution is roughly 1L/Kg. 70% of the drug is bound to plasma proteins (Katzung, 2001).

Metabolism
CBZ is metabolized by hepatic oxidative enzymes into 10, 11-CBZ epoxide which is further metabolized to inactive compounds and gets excreted in the urine. CBZ is also inactivated by conjugation and hydroxylation reactions. CBZ induces CYP3A4, CYP2C and CYP3A, enhancing the metabolizing capacity of the above enzymes (McNamara, 2006).
CBZ is also an autoinducer i.e. it induces CYP3A4, the self metabolizing enzyme and thus it has a short half life (Leduc, et al., 2009). Steady state concentration of CBZ occurs usually within 2-3 weeks after initiation of therapy (Bauer, 2001). CBZ epoxide, the primary active metabolite, is 50% bound to plasma proteins (Rane, et al., 1976) and is subjected to enterohepatic circulation (Laffey and Guzzardi, 1983). CBZ is hydroxylated to 2-hydroxy CBZ. CYP3A4 catalyzes secondary metabolism of 2-hydroxy CBZ to hydroxyiminostilbene which is followed by non enzymatic reduction to CBZ iminoquinone (Leduc, et al., 2009).

**Metabolism of carbamazepine in pregnancy**

Bernus, et al., (1995) revealed that the CBZ is metabolized and excreted as CBZ-10, 11-epoxide, CBZ-10, 11-trans-diol, 9-hydroxyacidan and 2- and 3-hydroxy CBZ in urine. Mean plasma CBZ clearance was apparently increased in pregnancy. CBZ clearance increases urinary excretion of unmetabolised drug in pregnancy due to increased glomerular filtration rate and formation of oxidative metabolites of the drug, particularly in women co-medicated with enzyme inducing anticonvulsants and by inhibition of the epoxide-diol pathway in pregnancy.

**Elimination**

After oral administration, 72% of the CBZ is excreted in the urine and 28% is eliminated in the faeces.

**PHARMACODYNAMICS**

CBZ acts by reducing polysynaptic responses and by blocking post-tetanic potentiation. CBZ is more effective in reducing stimulus induced discharges in the amygdala of stimulated rats. CBZ increases the latency of trigeminal neuronal response and decreases the number of neuronal discharges thereby reduces the
excitatory synaptic transmission in the spinal trigeminal nucleus. Thus, it is effective against neuralgia (Satoskar, et al., 2007).

**DRUG INTERACTION**

Drugs that increase the metabolism of CBZ include phenobarbitone, phenytoin and valproate. Erythromycin, fluoxetine and isoniazid inhibit the metabolism of CBZ (Stafstrom, et al., 1995; Tripathi, 2008). Valproic acid and valnoctamide inhibits microsomal epoxide hydrolase.

Drugs that are more rapidly metabolized with CBZ include warfarin, phenytoin, theophylline and valproic acid. CBZ increases the metabolism of the hormones in birth control pills and reduces their effectiveness, potentially leading to unexpected pregnancies (Bagheri and Shahrokh, 2004).

**Therapeutic applications**

CBZ is typically used in the treatment of seizure disorders and neuropathic pain.

**Epilepsy:** Generalized tonic-clonic (grand mal) and partial (focal) seizures.

**Pain syndromes:** CBZ is used in trigeminal neuralgia and glossopharyngeal neuralgia.

**Psychosis:** CBZ is used in the treatment of manic depression and as adjuvant with antipsychotic agents in schizophrenia.

**Adverse effects**

The adverse effects that appear within the first week of treatment include nausea, vomiting, ataxia, mental confusion, anorexia, giddiness, diplopia, blurred vision, fluid retention, mental slowness and skin rash (Satoskar, et al., 2007). The rare but serious toxic effects reported include EEG slowing (Miyaoka, et al., 2000), cell apoptosis
(Gao, et al., 1995), changes in auditory perception (Yoshikawa and Abe, 2003; Konno, et al., 2003; Kashihara, et al., 1998), obstructive jaundice, peripheral neuritis, agranulocytosis, thrombocytopenia and aplastic anemia.

The therapeutic range of CBZ is found to be 4.0-12.0 µg/mL. The signs of toxicity appear at plasma concentrations above 12 µg/mL causing changes in the central nervous system (Salcman and Pippenger, 1975), gastrointestinal irritation (Lehrman and Bauman, 1981), arrhythmogenic properties (Beerman, et al., 1975) and fluid retention anti-diuretic action (Stephens, et al., 1977).

- **Toxicity in Pregnancy and Lactation**

  **a) Fetal toxicity**

  CBZ is reported to induce intrauterine growth retardation, poor neonatal performance, postnatal growth deficiency, developmental delay, microcephaly, upslanting palpebral fissures, hypoplastic nails and cardiac defect (Jones, et al., 1989a). In another study, it was reported that CBZ administration in pregnant mothers resulted in offsprings with spina bifida (Jones, et al., 1989b). The incidence of 0.6% of spina bifida represented a 9-fold relative risk for the neural tube defect.

  Little, et al., (1993) reported neural tube defect in a woman ingested CBZ after conception. Thoracolumbar spinal defect was observed on sonographic examination at 20th week of gestation. Fetal autopsy of the infant revealed hypoplastic left cerebral hemisphere and the latter defect is thought to be the result of focal necrosis.

  Ornoy and cohen, (1996) described typical dysmorphic facial features in children who had been exposed to CBZ monotherapy during pregnancy. The authors concluded that the facial features and mild mental retardation were consistent with CBZ syndrome.
Wide, et al., (2000) in a study found increased number of facial anomalies. Jick, et al., (1997) reported the congenital anomalies involving CBZ monotherapy and experienced ventricular septal defect, pulmonary stenosis, cleft palate, hare lip, sensorineural deafness, congenital megaureter, hydronephrosis syndrome and vesicoureteric reflux. Canger, et al., (1999) in a prospective study in pregnant epileptic mothers identified genetic and chromosomal defects, hydrocephalus, diaphragmatic hernia, pyloric stenosis, renal dysplasia, hydronephrosis and inguinal hernia. Toxic oxidative metabolite of CBZ, i.e CBZ 10, 11-epoxide binds covalently to macromolecules and produce mutagenic or teratogenic effects. This was proposed to be a possible mechanism of CBZ induced teratogenecity (Lindhout, et al., 1984).

**b) Toxicity during Lactation**

CBZ is found to be excreted in the breast milk. Wisner and Perel, (1998) measured the amount of CBZ excreted into breast milk. The amount of CBZ measured in infant serum is low, around 0.4 mg/mL, but levels may be as high as 0.5-1.8 mg/mL. Frey, et al., (2002) reported transient cholestatic hepatitis in an infant between the 3rd and 7th week of life, due to CBZ exposure during breast feeding. The baby born to an epileptic mother treated with CBZ monotherapy throughout breast feeding experienced asphyxia since birth with transient hepatic dysfunction in the first week of life. After full recovery from asphyxia, the baby experienced a second period of liver dysfunction, presenting as cholestatic hepatitis that lasted approximately for 5 weeks. CBZ is known to induce hepatic damage in children and adults.

- **Ophthalmic toxicity**

CBZ is reported to cause impairment of oculomotor control, gaze-evoked nystagmus and impaired eye movements (Umeda and Sakata, 1977), downbeat nystagmus (Wheeler, et al., 1982), slowing and prolongation of saccade velocity (Tedeschi, et al.,
1989; Hamilton, et al., 1993), prolonged saccade latencies (Tedeschi, et al., 1989) and saccade dysmetria (Remler, et al., 1990). Verrotti, et al., (2004) evaluated the deficits in color vision in epileptic adolescents who received CBZ. 45 epileptic patients were examined before the beginning of therapy and after 1 year of CBZ therapy. The study demonstrated that treatment with CBZ affects significantly both central and paracentral color vision.

- **Ototoxicity**


- **Neurotoxicity**

Treatment with CBZ produced cerebellar dysfunction (Diener and Dichgans, 1988). The rate of rise in plasma CBZ concentration is the most important determinant of psychomotor side effects (Wildin, et al., 1993). Animal studies indicates that CBZ and CBZ-10,11 epoxide are equipotent in producing neurotoxicity (Bourgeois and Wad, 1984).

- **Behavioural abnormalities**

Increasing doses of CBZ was reported to produce side effects such as dizziness, ataxia, drowsiness and reduced alertness. Pleak, et al., 1988 reported CBZ induced adverse behavioural and neurological reactions in subjects, who were diagnosed with attention deficit hyperactivity disorder and conduct disorder. The untoward effects included a severe manic episode, hypomania, increased irritability, impulsivity, aggressiveness and worsening of behaviour. Myers and carrera (1989) reported
irritability, insomnia, agitation, talkativeness and prepubescent hypersexuality in patients on CBZ therapy.

- **Nephrotoxicity**

CBZ is reported to induce granulomatous interstitial nephritis (Hegarty, et al., 2002). Eijgenraam, et al., (1997) reported tubulointerstitial nephritis, a rare side-effect of CBZ and discontinuation of CBZ rapidly improved the renal function.

- **Pulmonary toxicity**


- **Genotoxicity**

Sarikaya and Yüksel, (2008) evaluated the genotoxic effect of different concentrations of CBZ in the wing spot test of Drosophila melanogaster. The wing spot test detects different kinds of somatic mutations and allows detection of mitotic recombinations. Survival rates of flies used in the experiments were significantly lower than that of the control group revealing the toxic effects of CBZ on Drosophila melanogaster larvae. Celik, (2006) investigated the genotoxic effect of CBZ particularly in *in vitro* micronucleus test using cytogenesis-block technique. *In vitro* analysis was performed in human blood lymphocytes from four healthy persons at five different concentrations of CBZ (6, 8, 10, 12, 14 μg/mL).

The results of the study indicated that CBZ caused genotoxicity under *in vitro* conditions, except at the lowest dose of 6 μg/mL and cytotoxic effects of CBZ were revealed at all the selected concentrations.
• **Cardiotoxicity**

CBZ caused lethal cardiovascular complications, sinus bradycardia, atrioventricular block and sinus tachycardia (Fisher and Cysyk, 1988). Todorović, et al., (1993) reported the manifestations of cardiotoxicity in 9 patients with acute CBZ poisoning. The most common clinical signs of cardiotoxicity are tachycardia, hypotension, electrocardiographic extrasystoles, ventricular extrasystoles and repolarization disorders. Salzman, et al., (1997) described a 13-year-old boy with attention deficit hyperactivity disorder in whom fever, rash, conjunctivitis, hepatitis, myocarditis and eosinophilia are developed and died about two months after the initiation of CBZ therapy. When CBZ dose was gradually increased from 100 mg twice a day to 800 mg per day, electrocardiography showed ventricular tachycardia with frequent multiform ventricular ectopy. Echocardiography revealed decreased ventricular function, with a left ventricular shortening fraction of 25 percent and the following day the patient died from uncontrollable dysrhythmias.

**Intervention of antioxidants and other supplements in carbamazepine induced toxicity**

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

• **Influence of melatonin on carbamazepine induced genotoxicity**

Pretreatment of human lymphocytes with melatonin (0.5 mM) exhibited a significant decrease in the occurrence of CBZ induced chromosomal aberrations and sister chromatid exchanges as compared with non-treated cultures and improved the depressed mitotic and proliferation indices. The study suggests that CBZ
monotherapy resulted in chromosomal damages (genotoxic) and melatonin was observed to possess anti-mutagenic effect (Awara, et al., 1998).

- **Effect of curcumin on carbamazepine induced memory impairment**

  Reeta, et al., (2010) reported cognitive impairment with chronic CBZ administration. The increase in free radical generation has been implicated as one of the important mechanisms of cognitive impairment by AEDs. The administration of CBZ for 21 days caused a significant impairment of learning and memory as well as an increased oxidative stress. Concomitant curcumin administration prevented the cognitive impairment and decreased the AED induced oxidative stress. Curcumin coadministration did not cause any significant alteration in the serum concentrations of CBZ. The study reported the beneficial effects of curcumin in mitigating the deterioration of cognitive functions and oxidative damage in rats treated with CBZ without significantly altering the bioavailability of CBZ. These findings suggest that curcumin is considered as a safe and effective adjuvant to CBZ therapy in preventing cognitive impairment associated with CBZ.

- **Effect of *Cassia auriculata* and *Cardiospermum halicacabum* teas on pharmacokinetics of carbamazepine**

  CBZ toxicity was assessed by changes in general behaviour, haematological parameters and by liver and kidney function. Thabrew, et al., (2004) demonstrated that in rats receiving the *Cassia auriculata* tea and CBZ, the blood levels of the CBZ was significantly enhanced by 47.1%, when compared to normal. In animals receiving the *Cardiospermum halicacabum* tea, there were no significant changes in the blood levels of CBZ or drug-related toxicity. *Cassia auriculata* tea has therefore the potential to increase the bioavailability of CBZ, which may further increase the
toxicity profile. Concurrent ingestion of CBZ with herbal teas containing *Cassia auriculata* should be avoided by epileptic patients.

**VITAMIN C**

![Chemical structure of Vitamin C](image)

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

(or)

(R)-3,4-dihydroxy-5-((S)-1,2-dihydroxyethyl)furan-2(5H)-one

Vitamin C (Vit C) or L-ascorbic acid or L-ascorbate is an essential nutrient for human beings. Vit C is an important water soluble antioxidant in biological fluids (Frei, et al., 1989; Frei, et al., 1990). An antioxidant is defined as a substance which even at low concentration significantly delays or prevents oxidation of substrates such as proteins, lipids, carbohydrates and nucleic acids (Halliwell, 1996).

Vit C is an essential micronutrient required for normal metabolic functioning of the body (Jaffe, 1984). Ascorbate was reported to scavenge peroxyl radical and offer protection against oxidative stress induced cytotoxicity (Padayatty, 2003; Yen, 2002).

**Importance of Vitamin C supplementation**

Human beings and other primates do not have the ability to synthesize Vit C as a result of 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
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Ames, 1997). Thus, Vit C must be obtained through the diet; however, rodents have the capacity to synthesize Vit C.

Food sources of Vitamin C

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

Dietary recommendations of Vitamin C

The average daily intake level that is sufficient to meet the nutritional requirement of ascorbic acid is 90 mg/day for men and 75 mg/day for women (Frei and Traber, 2001). Consumption of 100 mg/day of ascorbic acid is found to be sufficient to saturate the body pools (neutrophils, leukocytes and other tissues) in healthy individuals.

Based on clinical and epidemiological studies it has been suggested that a dietary intake of 100 mg/day of ascorbic acid is associated with reduced incidence of mortality from heart diseases, stroke and cancer (Carr and Frei, 1999).

Biological role of Vitamin C

- **In catecholamine biosynthesis**

Vit C is a cofactor for catecholamine biosynthesis, in particular the conversion of dopamine to norepinephrine, which is catalyzed by dopamine β-monooxygenase (Burri and Jacob, 1997).

Vit C maintains 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 (Tsao, 1997).

- **In Cholesterol metabolism**

Vit C is implicated in the metabolism of cholesterol to bile acids via the enzyme cholesterol 7α-monoxygenase and in steroid metabolism in the adrenals (Tsao, 1997).

- **In Detoxification process**

Hydroxylation of aromatic drugs and carcinogens by hepatic cytochrome P450 is enhanced by Vit C. The role of Vit C in the above metabolic pathways is to reduce the active center metal ion of the various mono and dioxygenases (Tsao, 1997).

**Antioxidant potential**

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). Vit C readily scavenges reactive oxygen 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 acids 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. Vit C also acts as a co-antioxidant by regenerating Vit E (Packer, 1997b; Bowry, et al., 1995). Vit C also regenerates urate, GSH and β-carotene in vitro from their respective one-electron oxidation products, i.e, urate, glutathiyl and β-carotene radicals (Edge and Truscott, 1997).
Two major properties of Vit C makes it as an ideal antioxidant. First, the low one-electron reduction potential of ascorbate (Halliwell, 1996) enables 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 an NADH-dependent semidehydroascorbate reductase (Wells and Jung, 1997). The 2-electron oxidation product of ascorbate, dehydroascorbic acid, itself be reduced back to ascorbate by glutathione dehydroascorbate oxidoreductase or the NADPH-dependent selenoenzyme thioredoxin reductase (Wells and Jung, 1997). Alternatively, dehydroascorbic acid is rapidly and irreversibly hydrolyzed to 2,3-diketogulonic acid (DKG) (Halliwell, 1996).

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

where equation 1 shows the conversion of 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-enedioli form of DKG (3,4-DKGL) and 2,3-enedioli 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 (Faya, 1991) whereas oxalate complexes with divalent metals and excrete them in urine and thus prevents metal induced oxidation (Betsche and Fretzdorff, 2005).

Ascorbic acid present in food is readily available and easily absorbed by active transport in the intestine (Sauberlich, 1985). Most of it (80–90%) will be absorbed when the intake is up to 100 mg/day, whereas at higher levels of intake (500 mg/day) the efficiency of absorption of ascorbic acid rapidly declines. Ascorbic acid is sensitive to air, light, heat and easily destroyed by prolonged storage and over processing of food. Hence, ascorbic acid has to be regularly supplemented through diet or tablets to maintain ascorbic acid pool in the body. The major metabolites of ascorbic acid in human are dehydroascorbic acid, 2, 3-diketogulonic acid and oxalic acid. The main route of elimination of ascorbic acid and its metabolites is through urine. It is excreted unchanged when high doses of ascorbic acid are consumed.

**Physiological functions of Vitamin C**

Ascorbic acid, α-tocopherol and GSH are important chain-breaking antioxidants responsible for scavenging free radicals and suppression of peroxidation in the cytosol and membrane of the cell (Niki, 1987; Mann, 1975). The central nervous system is vulnerable to free radical damage because of the brain’s high oxygen consumption, its abundant lipid content and the relative paucity of antioxidant enzymes as compared to other tissues (Skaper, 1999). The brain is deficient in oxidative defense mechanisms and hence is at greater risk of damage mediated by ROS, resulting in molecular and cellular dysfunction (Gupta, 2003).

The physiological functions of ascorbic acid are largely dependent on the oxido-reduction properties of this vitamin. L-ascorbic acid is a co-factor for hydroxylases and monooxygenase enzymes involved in the synthesis of collagen, carnitine and
neurotransmitters (Levine, 1986). Ascorbic acid accelerates hydroxylation reactions by maintaining the active center of metal ions in a reduced state for optimal activity of enzymes hydroxylase and oxygenase. Ascorbic acid plays an important role in the maintenance of collagen which represents about one third of the total body protein. It constitutes the principal protein of skin, bone, teeth, cartilage, tendons, blood vessels, heart valves, inter vertebral discs, cornea and eye lens. Ascorbic acid is essential to maintain the enzyme prolyl and lysyl hydroxylase in active form. The hydroxylation of proline and lysine is carried out by the enzyme prolyl hydroxylase using ascorbic acid as co-factor required for collagen synthesis.

Ascorbic acid is essential for the synthesis of muscle carnitine (β-hydroxy butyric acid) (Hulse, 1978). Carnitine is required for transport and transfer of fatty acids into mitochondria where it can be used for energy production. Ascorbic acid acts as a co-factor for hydroxylations involved in carnitine synthesis. Further, ascorbic acid acts as co-factor for the enzyme dopamine-β-hydroxylase, which catalyzes the conversion of neurotransmitter dopamine to norepinephrine. Thus, ascorbic acid is essential for the synthesis of catecholamines. In addition, ascorbic acid catalyzes other enzymatic reactions involving amidation necessary for maximal activity of hormones oxytocin, vasopressin, cholecystokinin and alpha-melanotropin (Cameron and Pauling, 1973). Ascorbic acid is also necessary for the transformation of cholesterol to bile acids as it modulates the microsomal 7 α-hydroxylation, the rate limiting reaction of cholesterol catabolism in liver. In ascorbic acid deficiency, this reaction becomes slowed down thus, resulting in an accumulation of cholesterol in liver, hypercholesterolemia, formation of cholesterol gall stones etc., (Ginter, 1982a).

Ascorbic acid is a well-known antioxidant, has been suggested to act synergistically with tocopherol to regenerate the tocopheryl radicals. Ascorbic acid scavenges
peroxyl radical and inhibit cytotoxicity induced by oxidants. In addition, ascorbic acid reduces or prevents $\text{H}_2\text{O}_2$ induced lipid peroxidation and the formation of hydroxy deoxyguanosine (Retsky and Frei, 1995; Tsou, et al., 1996) and acts as a free radical scavenger (Deutsch, 1998).

Ascorbic acid is an antioxidant which efficiently scavenges toxic free radicals and other ROS formed in cellular metabolism. Actually, ROS are associated with several forms of tissue damage, disease and also with the process of ageing (Ames, 1993). Enzymatic mechanisms include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-px) and in plants, ascorbate peroxidase (AA-px).

![Figure 1. The role of ascorbic acid in the detoxification of ROS.](image)

(Blue dotted lines indicate non-enzymatic reactions)

SOD catalyses the conversion of superoxide anion to $\text{H}_2\text{O}_2$ and oxygen; in turn $\text{H}_2\text{O}_2$ is converted by CAT into water and molecular oxygen. CAT turnover number is very high, but its affinity for $\text{H}_2\text{O}_2$ is relatively low, and consequently a certain amount of $\text{H}_2\text{O}_2$ remains in the cell. This is potentially troublesome, since $\text{H}_2\text{O}_2$ reacts with superoxide anion formed in oxidative metabolism and generate highly reactive hydroxyl radical. GSH-px and AA-px are capable of removing low amounts of $\text{H}_2\text{O}_2$ due to their high affinity for $\text{H}_2\text{O}_2$. Thus, the combined effect of SOD, CAT and peroxidases ensures low levels of superoxide anion and $\text{H}_2\text{O}_2$ and therefore limits the
risk of hydroxyl radical formation (Fig.1). In addition to enzymatic mechanisms, endogenous non enzymatic antioxidants are involved in redox reactions and neutralise free radicals to non-reactive species.

Many chemicals serve this purpose because the high reactivity of free radicals results in extracting an electron from almost any available molecule. An efficient biological antioxidant is expected to be present in an adequate amount in the cell to react with a variety of free radicals, and be suitable for regeneration (Rose, 1993).

Applications of Vitamin C

- **Against genotoxicity**

Turkez, (2011) investigated the protective effect of ascorbic acid against titanium dioxide induced genotoxicity. The protective effects of ascorbic acid and titanium dioxide induced genotoxicity were assessed by sister chromatid exchange, micronucleus and the comet assays. There were significant increases in both sister chromatid exchange and micronucleus frequencies of cultures treated with titanium dioxide as compared to controls. Co-application of ascorbic acid (4.87 and 9.73 μM) and titanium dioxide resulted in reduction of sister chromatid exchange, micronucleus rates and DNA damage as compared to the group treated with titanium alone. Thus, ascorbic acid has a preventive role in alleviating titanium dioxide-induced DNA damage and genotoxicity.

Rao, et al., (2001) investigated the effect of L-ascorbic acid (9.734 μM) against mercuric chloride (1.052, 5.262 and 10.524 μM) induced genotoxicity in human leucocyte cultures. The proliferative rate index, sister chromatid exchange and chromosomal aberrations in control and mercuric chloride treated cultures with and without Vit C supplementation were assessed. Vit C prevented the mutagenic activity of mercuric chloride due to its strong antioxidant and nucleophilic nature.
• **Hepatoprotective effect**

Ahn, et al., (2004) studied the protective effect of Vit C on radiation induced hepatotoxicity. Spraque Dawley rats were exposed to radiation and radiation along with Vit C treatment. Exposure to radiation increased malondialdehyde levels of liver and decreased catalase activity whereas Vit C treatment improved the catalase and SOD activity of the liver and decreased the lipid peroxidation, also reversed the elevated levels of SGOT, SGPT, LDH and ALP. On the electromicrographic findings, the hepatic cell destruction was considerably decreased by Vit C. Thus, Vit C is thought to be an effective antioxidant against radiation induced hepatotoxicity.

Ademuyiwa, et al., (1994) studied the protective effect of Vit C against CCl₄ (8 ml/Kg body weight) induced hepatotoxicity in rats. Vit C (2 g/Kg body weight) prevented liver damage induced by CCl₄.

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 (Gamma glutamyl transferase) levels. On the other hand, even after toxic doses of paracetamol, Vit C had a protective role in mice possibly through its antioxidant property.

• **Against 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 (Simon, 1992). Many epidemiologic studies have shown inverse associations between antioxidant intake
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I particularly Vit C) and cardiovascular as well as cerebrovascular disease risk (Jha, et al., 1995; Enstrom, et al., 1992; Enstrom, 1993; Manson, et al., 1992; Manson, et al., 1993; Sahyoun, et al., 1996). Several studies showed a reduced risk with moderate intake of Vit C between 45 and 113 mg/day (Gale, et al., 1995; Fehily, et al., 1993). Knekt, et al., (1994) reported a 51% lower risk of coronary artery disease in women consuming > 91 mg Vit C/day than in those consuming < 61 mg/day. In a population of elderly men and women, Gale, et al., (1995) found that daily intakes of > 45 mg Vit C 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 >113 mg Vit C/day. Sahyoun, et al., (1996) reported a significant 62% lower risk of cardiovascular disease in a population of elderly men and women consuming > 388 mg Vit C/day. Finally, Kritchevsky, et al., (1995) measured carotid artery wall thickness as an index of atherosclerosis and found significantly decreased intima thickness in men and women aged more than 55 years consuming > 982 and > 728 mg Vit C/day respectively.

Hypercholesterolemia is a significant risk factor for cardiovascular disease (Lynch, et al., 1996; Simon, 1992). Consumption of 1000 mg Vit C/day for 4 weeks resulted in a reduction in total serum cholesterol (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 hydroxylation of cholesterol to form bile acids (Burri and Jacob, 1997). Vit C also modulates the activity of hydroxymethylglutaryl-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, in which decreased concentrations of HDL and increased concentrations of LDL are potential risk factors.
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; Hallfrisch, et al., 1994; Jacques, 1992). Ness, et al., (1996) also found an inverse correlation between Vit C status and triacylglycerol concentrations as it 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). It was reported 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 physiologic concentrations of Vit C increases PGE1 and PGI1 (prostacyclin) production, resulting in a reduction in platelet aggregation and thrombus formation (Frei, 1997). Low concentration of Vit C is 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 vitamin E along with 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 of Vit C on intima-media thickness of common carotid artery for 6 year. 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 benefit from the supplementation in terms of cholesterol levels and slowing the
progression of heart disease. Vitamin E had no effect on HDL cholesterol whereas the supplementation with combination of vitamin E and slow release Vit C slowed down atherosclerotic progression in hypercholesterolemic persons (Salonen, et al., 2003).

Deficiency in endothelium derived nitric oxide (EDNO) levels, contributes 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 improves 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 by high resolution vascular ultrasound at baseline, 2 hours after the single dose, and 30 days after long term treatment in 46 patients with CAD. Flow mediated dilation and plasma ascorbic acid concentration was 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.

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 contracted coronary heart disease suggested that a dietary supplement including Vit C reduced the risk of CHD (Osganian, et al., 2003).
Mullan, et al., (2002) studied the haemodynamic effects of chronic oral supplementation of Vit C on type II diabetes. Patients of type II diabetes (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 diabetes, the use of Vit C supplementation is an economic way to help control blood pressure.

- **Against cancer**

High intake of Vit C has been associated with decreased risk of certain cancers, particularly cancers of the pharynx, oral cavity, oesophagus, lung and stomach (Jacob and Sotoudeh, 2002). Although the anticancer actions of Vit C are not well defined, it is believed that the antioxidant properties of Vit C protect against molecular damage that is associated with carcinogenesis and/or that Vit C may modulate signal transduction and gene expression (Li and Schellhorn, 2007). Meta-analysis indicated that individuals with high intakes of Vit C are at reduced risk for oesophageal cancer (Kubo and Corley, 2007), lung cancer (Cho, et al., 2006) and breast cancer (Howe, 1990). 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 (Tannenbaum, 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 formed from NO generated by inflammatory cells expressing inducible NO synthase (Parsonnet, 1995; Satarug, et al., 1996). Epidemiological studies have shown an inverse association between Vit C intake and cancers (Block, 1991; Fontham, 1994). Vit C reduces in-
vivo nitrosation by scavenging nitrite and hence preventing its reaction with amines to form nitrosamines (Hecht, 1997; Tannenbaum, 1987).

Concentration of fecapentaenes, fecal mutagens that have been implicated in colon cancer (Parsonnet, 1995) are 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), activity of natural killer cells and 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 antibodies as well as complement components (Haskell and Johnston, 1991; Tanaka, et al., 1994).

- **Against alcoholic liver disease**

Alcoholic liver disease (ALD) is commonly accompanied with hepatic iron overload and liver injuries. Oxidative stress plays an important role in pathogenesis of alcoholic liver disease and also leads to iron-metabolic disorders.

Xiaoqiang, et al., (2010) investigated the effect of Vit C on iron metabolism related gene expression and liver protection in mice. Vit C ameliorated the increased serum
alanine aminotransferase activity and hepatic iron overload in mice. Vit C increased the expression of iron-regulated hormone hepcidin and decreased the transferrin receptor 1 expression in liver and decreased the iron release to blood. Vit C ameliorated the alcoholic liver injuries through regulation of iron metabolism-related gene expression.

- **Against atherosclerosis**

Lipid peroxidation and oxidative modification of low density lipoproteins (LDL) are implicated in the development of atherosclerosis (Steinbrecher, 1990). Vit C protects against oxidation of LDL by different types of oxidative stress, including metal ion dependent and independent processes (Frei, 1997). Addition of iron to plasma devoid of ascorbic acid resulted in lipid peroxidation, whereas endogenous and exogenous ascorbic acid was found to inhibit the lipid oxidation in iron-over loaded human plasma (Berger, 1997). Similarly, when ascorbic acid was added to human serum supplemented with Cu\(^{2+}\), antioxidant activity rather than pro-oxidant effects were observed (Dasgupta and Zdunek, 1992).

Ascorbic acid is known to prevent the oxidation of LDL primarily by scavenging the free radicals and other ROS in the aqueous medium (Frei, 1989). In addition, *in vitro* studies have shown that physiological concentrations of ascorbic acid strongly inhibited LDL oxidation by vascular endothelial cells (Martin and Frei, 1997). Adhesion of leukocytes to the endothelium is an important step in initiating atherosclerosis.

*In vivo* studies have demonstrated that ascorbic acid inhibits leukocyte-endothelial cell interactions induced by cigarette smoke (Lehr, 1994; Lehr, 1997) or oxidized LDL (Lehr, 1995). Further, lipophilic derivatives of ascorbic acid showed protective effect on lipid-peroxide induced endothelial injury (Kaneko, 1993).
Against asthma

Asthma is characterized by airway obstruction and hyperreactivity to nonspecific stimulants like allergens, chemical agents, cold air, or exercise (Doelman and Bast, 1990). When inflammatory reaction is initiated, chemotactic factors are released and neutrophils and eosinophils infiltrates into lung tissue. Eosinophil, in particular, plays a key role in the pathogenesis of the late phase reaction and the development of the bronchial hyperresponsiveness (Frigas and Gleich, 1986).

Ascorbic acid has been reported to have a beneficial role in asthma and in prevention of airway hyperreactivity in asthmatics with an upper airway infection (Heffner and Repine, 1989). Increased responsiveness to a wide range of inhaled bronchoconstrictors is generally considered to be an essential component of asthma (Boushey, et al., 1980). Although it has been suggested that oxidative stress may contribute to bronchial hyperreactivity, the mechanisms for this process are unclear. Bronchial hyperreactivity is induced in the guinea pig by exposing to various periods of ozone and the degree of hyperreactivity is related to the duration of ozone exposure. A single intraperitoneal bolus of ascorbic acid given 30 min before ozone exposure prevented the bronchial hyperreactivity (Yeadon, et al., 1992).

In mild asthmatics, ascorbic acid supplements for 3 days did not alter pulmonary function (Ting, et al., 1983). In asthmatic subjects challenged with histamine inhalation, ascorbic acid supplements did not prevent the expected decrease in pulmonary function (Malo, et al., 1986). In contrast to the animal study discussed above, these studies suggest that short-term ascorbic acid supplements do not alter bronchial hyperreactivity.

In other studies, ascorbic acid pretreatment led to a mild but significant attenuation of the bronchospasm seen postexercise in asthmatics (Schacter and Schlesinger, 1982).
In studies where the bronchial hyperresponsive reaction was initiated with a methacholine challenge, ascorbic acid significantly reduced the response of the airways (Mohsenin and Dubois, 1987). This protective effect of ascorbic acid was abolished by indomethacin, which suggests ascorbic acid produced its protection through the modulation of leukotriene production. In human embryo lung fibroblast, ascorbic acid was demonstrated to modulate prostanoid production (Taylor and Polgar, 1981). These effects of ascorbic acid may not be solely due to its antioxidant property.

In a study of the cellular and humoral immunity of asthmatic children, supplementation of ascorbic acid improved the neutrophil chemotaxis, phagocytosis, secretory immunoglobulin A concentrations and immunoglobulins (Anderson, et al., 1983). These improvements were maintained as long as the children remained on the ascorbic acid supplementation for 6 months. Schwartz and Weiss (1994) demonstrated a relationship between pulmonary function and ascorbic acid intake and found a fivefold increase in protection in bronchitis and asthma (Schwartz and Weiss, 1994).

These studies in the lung demonstrate that ROS play a role in a number of different pathologies of the lung. These ROS may be from the primary injurious agent or they may be secondary to activation of the inflammatory cascade. Ascorbic acid appears to function as an antioxidant and is an important mediator in reducing injury from these reactive radicals.

- Enhancement of immune system

Phagocytosis and the destruction of microorganisms include multiple steps such as production and mobilization of phagocytes, opsonization, ingestion, metabolic activation, killing, and extrusion. Ascorbic acid supplements have positive antiviral
and antibacterial effects by enhancing the production of interferon by T cells (Siegel, 1974).

B-cell functions in the humoral immune system may be similarly affected by ascorbic acid. In cultured murine spleenocytes, ascorbic acid stimulated antibody production possibly through improved lymphocyte viability (Yamamoto, 1993).

Ascorbic acid has been shown to inactivate a broad spectrum of viruses including poliovirus, herpesvirus, hepatitis virus and bacteriophages. Inhibition of replication and infectivity of Rous sarcoma virus by ascorbic acid has been demonstrated in avian tendon cells and chicken embryo fibroblasts (Bissell, et al., 1980). In latently infected cells exposed to activating agents, ascorbic acid inhibited the induction of human T-cell leukemia virus (Blakeslee, et al., 1985). In cells chronically infected with human immunodeficiency virus (H9/HTLV-IIIB cells), ascorbic acid significantly suppressed the activity and growth of the same (Harakeh and Jariwalla, 1991). HIV suppression was maintained as long as the ascorbic acid supplements were present. This improved immunity may be through an effect of ascorbic acid on the cell or through direct effects on the virus.

*In vitro* studies with influenza A virus demonstrated that trypsin and HOCl treated α1-antiprotease increased virus infectivity 10,000-fold (Hennet, et al., 1992). The ability of ascorbic acid to protect α1-antiprotease from inactivation (Pryor and Stone, 1993) suggests that ascorbic acid may modulate the infectivity of influenza A.

- **Against eye disorders**

Ascorbic acid protected against light induced oxidation and cataract development. In organ culture, ascorbic acid protected the rat lens against light induced damage to the cation pump and ROS induced damage to the membranes (Varma, 1987). Similar studies in guinea pigs demonstrated that high dietary ascorbic acid protected the lens
against ultraviolet light-induced or heat-induced damage. In the lens of diabetic rats, ascorbic acid supplementation decreased membrane damage. Ascorbic acid has also been suggested to play a role in lens development and maintenance of transparency during development (Garland, 1991).

- **Against kidney disorders**
  The compound 4-aminophenol is a metabolite of acetaminophen (paracetamol), aniline (an industrial chemical), the pesticide isopropyl carbanilate, hair dyes, and photographic processing chemicals. This compound is acutely nephrotoxic, producing necrosis to the pars recta of the proximal tubule with a single dose (Gartland, et al., 1989). Dose related alterations in renal function and blood urea nitrogen (BUN) were associated with the morphological changes. However, co-administration of ascorbic acid prevented the nephrotoxicity due to 4-aminophenol (Fowler, et al., 1993). This protection included prevention of diuresis, glucosuria and proteinuria as well as the elevation of blood urea nitrogen. Morphologically, it decreased the extent and severity of necrosis of the proximal tubules.

In male Syrian hamsters, chronic administration of estrogens induced kidney tumors. Ascorbic acid administration inhibited the estrogen-induced carcinomas as assessed by a decrease in the number of foci or the number of tumors/tumor-bearing animal (Liehr and Wheeler, 1983).

**VITAMIN E**

\[
(2R)-2,5,7,8\text{-Tetramethyl-2-[(4R,8R)-(4,8,12\text{-trimethyltridecyl}]}-6\text{-chromanol}
\]
α-tocopherol is one of the eight forms of Vitamin E (Vit E) which have a 6-chromanol ring structure and a side chain at the 2nd position. The tocopherols have a 4', 8', 12'-trimethyl tridecyl side chain, called the phytol or phytetyl side chain. The term Vit E describes a family of eight antioxidants with 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 tocopherols have three asymmetrical carbons at the 2nd position in the ring and at the 4' and 8' positions of the side chain. Thus, eight optical isomers exist. Of all of the isomers and analogs, RRR-α-tocopherol, formerly known as d-α-tocopherol, has the highest biological activity (Burton, et al., 1988; Cheeseman, et al., 1984; Bjorneboe, et al., 1990) and is found abundant in plasma and tissues. It is generally accepted that individuals with plasma Vit E levels of less than 0.5 mg/dl are Vit E deficient (Machlin, 1984). Daily dietary Vit E intakes of 10-30 mg in healthy individuals will maintain serum Vit E concentrations in the normal range. In a study of well nourished adults, mean plasma Vit E concentrations were 1.06 mg/L at baseline and doubled to 2.03 mg/dL after supplementation with 800 IU Vit E for 8 weeks (Willett, et al., 1983).

Sources of Vitamin E

Major sources of alpha-tocopherol include Olive oil, Soybean oil, Corn oil, Canola oil, Safflower oil, Almonds, Peanuts, Spinach, etc., (Bendich, 1997).

Biological role of Vitamin E

Vit E protects polyunsaturated fatty acids found in the cell membranes which are important for membrane function and structure (Burton, et al., 1982). Increased Vit E intake enhanced the immune response. Vit E regulates platelet aggregation by inhibiting platelet cyclooxygenase activity which in turn decreased prostaglandin
Inhibition of Lipoprotein oxidation by α-tocopherol

Vit E is abundantly present in LDL, which prevents LDL oxidation (Steinbrecher, et al., 1984; Jialal and Grundy, 1992). In case of Vit E depletion, the oxidation proceeds at faster rate. It was observed that oral supplementation with α-tocopherol increased both the Vit E content of LDL and its resistance to oxidation (Dieber-rotheneder, et al., 1991).

Interaction of α-tocopherol with other Antioxidants

α-tocopherol functions as an antioxidant not only individually but also synergistically with other antioxidants (Packer, 1992a). The radical scavenging antioxidants act as the second-line defense to suppress the attack of radicals on the substrate and also to break the chain propagation. Hydrophilic antioxidants, such as ascorbate scavenge aqueous radicals, whereas lipophilic antioxidants such as Vit E are responsible for scavenging radicals in the lipophilic compartment (Niki, et al., 1985). The lipophilic antioxidants play a vital role in breaking the chain propagation. The concentration of α-tocopherol, the most abundant lipophilic antioxidant in vivo, is in general less than those of antioxidants such as Vit C (Stocker and Frei, 1991). This drawback is offset partly by the recycling of α-tocopherol by reducing antioxidants such as Vit C, GSH and ubiquinol.

Vit E is reported to show synergism with Vit C in terms of antioxidant activity (Golumbic and Mattill, 1941). Tappel (1968) suggested that Vit C might reduce the tocopheroxyl radical to regenerate tocopherol.
Therapeutic applications of Vitamin E

- **Cardiovascular disease**

An U.S. based study evaluated the association between serum antioxidant levels and risk of myocardial infarction. The study correlated myocardial infarction with high serum cholesterol concentration and low Vit E levels. The Cambridge Heart Antioxidant Study evaluated the effect of supplementation of Vit E (400 or 800 IU/day) in case of myocardial infarction in 2002 patients with angiographic evidence of atherosclerosis. The risk of nonfatal myocardial infarction was reduced by 77% in the Vit E supplemented group (Stephens, et al., 1996). Thus, Vit E was reported to exhibit protection against myocardial infarction.

There was increased resistance of LDL oxidation in non-smoking subjects supplemented with Vit E and smokers supplemented with Vit E (Princen, et al., 1992; Reaven, et al., 1993). Resistance of LDL to oxidation also increased significantly in animals, which showed the possible protective effects of Vit E on the development and progression of atherosclerosis. In studies of specific types of hens and rabbits that are susceptible to development of atherosclerosis, early aortic lesion development was significantly inhibited by Vit E supplementation (Smith and Kummerow, 1989; Willingham, et al., 1993).

Prevention and regression of atherosclerosis by Vit E were studied in male monkeys on an atherosclerosis promoting diet. Stenosis progressed more rapidly and to a greater extent in unsupplemented monkeys compared to Vit E treated monkeys. Stenosis in the group of animals with established atherosclerosis significantly decreased from 33 to 8% after 8 months of Vit E therapy. The result shows that Vit E may be effective in both prevention and treatment of atherosclerosis (Verlangieri and Bush, 1992). In a population case-control study in the United Kingdom, there was a
significant inverse association between plasma Vit E concentrations and angina risk. It was reported that some populations with a high coronary heart disease incidence may benefit from a diet rich in antioxidants, particularly Vit E (Riemersma, et al., 1991).

In the Harvard-based study of 39,910 male health professionals in the United States, a 36% lower relative risk of coronary heart disease was demonstrated in men who consumed more than 60 IU Vit E per day compared to men consuming less than 7.5 IU daily. Men who consumed 100 IU Vit E per day for at least 2 years had a 37% lower relative risk of coronary heart disease than men who did not take Vit E supplements (Rimm, et al., 1993). The relative risk of coronary heart disease was 48% lower in women taking Vit E supplements of more than 100 mg per day for at least 2 years. The study confirms that Vit E supplements may reduce the risk of heart disease (Stampfer, et al., 1993).

- **Aging and Immunity**

It has been suggested that free radical generation associated with aging depressed the immunity in aged rodents and supplementation of antioxidants may improve the immunity (Meydani, et al., 1989). The effects of antioxidants on free radical levels were evaluated in a study in Poland of 100 subjects between 60 to 100 years of age. Average blood malondialdehyde (MDA) levels decreased 26% in subjects receiving 200 IU Vit E (Meydani, et al., 1989).

Administration of Vit E supplementation (800 mg/day for 30 days) or placebo on cell mediated immune response in 32 healthy adults of 60 years showed improvement in delayed type hypersensitivity skin test response in the Vit E supplemented group. The immune response was enhanced in most, but not all, of the Vit E supplemented subjects (Meydani, et al., 1990).
Vit E supplements (200 or 800 mg/day for 235 days) improved certain clinical relevant indexes of cell-mediated immunity in a study of 88 healthy elderly subjects in the United States. The age associated decline in immune response is associated with increased incidence of morbidity and mortality and recommendations to increase Vit E intake should be considered for the elderly to improve immunity (Meydani, et al., 1997).

- **Cataracts**

The lens of the eye is very susceptible to light-induced lipid peroxidation and oxidation is believed to be an early and significant event in development of the majority of cases of senile cataract (Bunce and Hess, 1988; Robertson, et al., 1989). Animal studies showed that Vit E can arrest and reverse cataract development against light induced lipid peroxidation.

Vit E delayed or minimized cataract development induced by experimental oxidative stress in isolated animal lenses (Trevithick, et al., 1981; Ross, et al., 1983; Bhuyan, et al., 1982; Varma, et al., 1984). Cataract risk was 56% lower in the Vit E supplemented group than who did not take Vit E.

- **Air pollution**

Smokers inhale high levels of free radicals in the gaseous and tar phase of tobacco (Duthie, et al., 1989). A study on young adult smokers showed decreased Vit E in the lower respiratory tract fluid compared to nonsmokers.

Vit E supplementation (2400 IU/day for 3 weeks) increased the Vit E concentrations in lower respiratory tract fluid, but levels remained much lower than baseline levels of nonsmokers, providing clear evidence that Vit E utilization may be increased in lung cells of smokers. Vit E supplementation increased the lung's defense against cigarette smoke induced free radical damage (Pacht, et al., 1986).
In another study, red blood cells of smokers showed increased peroxidation when incubated with hydrogen peroxide compared to nonsmokers, which suggest that smoking lead to changes in antioxidant status (Duthie, et al., 1989). Supplementation of Vit E (600 mg) exhibited a protective effect against free radical induced cell damage due to air pollutants like photochemical smog and hydrogen peroxide (Duthie, et al., 1989).

- **Lipid peroxidation**

Lemoyne, et al., (1987) showed that daily supplementation of 1000 IU Vit E for 10 days significantly decreased the breath pentane excretion in healthy adults. Based on these results, it is found that there are undesirably high levels of lipid peroxidation in the body, which can be reduced by Vit E supplementation. These results showed the evidence of involvement of free radical damage in normal body processes and certain diseases and the effectiveness of Vit E in controlling or preventing lipid peroxidation (Van Gossum, et al., 1988a).

- **Hepatotoxicity**

Zaki, (2009) investigated the effect of co-administration of Vit E along with amiodarone in rats. The rats received amiodarone (5.4 mg/Kg) and Vit E (5 mg/Kg) for 2 weeks. After two weeks the animals were sacrificed and the isolated liver was subjected to microscopic examination. On examination, liver tissue showed disrupted hepatocytes with increased vacuolations in amiodarone alone received rats. The amiodarone plus Vit E administered rats showed minor damage in comparison to the amiodarone administered group. The result suggests that Vit E ameliorated the effects of amiodarone induced liver damage.
• **Restoration of mitochondrial dysfunction in asthma**

Mabalirajan, et al., (2009) found IL-4 to induce mitochondrial dysfunction in allergic asthma. Administration of Vit E reduced the level of IL-4 and restored the mitochondrial dysfunctions, thus alleviating asthma in an experimental allergic murine model. These findings suggest that Vit E reduced the key mitochondrial dysfunctions and alleviates asthmatic features.

• **Against chronic obstructive pulmonary disease**

Chronic oxidant burden and depletion of endogenous antioxidants have been proposed to play a key role in the pathogenesis of chronic obstructive pulmonary disease (COPD). Nadeem, et al., (2008) investigated the effect of supplementation of standard therapy of inhaled long-acting β₂ agonists, anticholinergics and corticosteroids with Vit E on oxidant and antioxidant balance in patients with COPD. Group A received only standard therapy, and group B received 400 IU of Vit E capsules twice daily in addition to standard therapy.

Spirometry and clinical assessment were carried out at the start and completion of 8 week treatment along with measurements of several biochemical parameters of oxidant-antioxidant status in plasma, leukocytes and red cells separated from venous blood. There was a similar degree of lung function and clinical improvement in both the groups and the findings showed that an 8 week supplementation of standard treatment with 400 IU twice daily of Vit E did not provide any additional clinical benefit although it augmented certain endogenous antioxidants in patients with COPD.

• **Osteonecrosis**

Kuribayashi, et al., (2010) examined the potential of Vit E to reduce the incidence of corticosteroid-induced osteonecrosis in an animal model. The Japanese white rabbits
in the control group were fed with a normal diet and the experimental group was fed with Vit E supplemented diet in which Vit E (600 mg/kg diet) was added to the normal diet. High-dose methylprednisolone acetate (20 mg/kg body weight) was injected once into the right gluteus medius muscle of all rabbits to induce osteonecrosis. 4 weeks after the injection of methylprednisolone acetate, the presence or absence of osteonecrosis of bilateral femurs was examined histopathologically. The finding suggests that the Vit E supplemented diet reduced the incidence of osteonecrosis.

• **In the treatment of Vitamin E deficiency**

Clinical Vit E deficiency states have been observed in individuals with a chronic malabsorption syndrome, severe malnutrition, genetic defects affecting the alpha-tocopherol transfer protein, fat malabsorption syndromes, premature infants and patients on total parenteral nutrition (Horwitt, 1986). Severe Vit E deficiency results mainly 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). The developing nervous system is especially vulnerable to Vit E deficiency and children with severe Vit E deficiency from birth should be treated with Vit E immediately, as they may develop neurological symptoms. Conditions interfering with normal digestion, absorption or transport of dietary fat have been associated with low serum Vit E concentrations (Carpenter, 1985). In patients with malabsorption syndromes such as celiac disease, biliary atresia and cystic fibrosis, serum Vit E concentrations can be less than 20 % of normal. Serum Vit E levels are often too low to measure in patients with abetalipoproteinemia
(Fritsma, 1983). Hemolysis and reduced life span of red blood cells have been reported with low Vit E plasma concentrations (0.5 mg/dL) (Machlin, 1984).

- **Against brain injury**

Wu, et al., (2010) examined the possibility of Vit E supplementation in the diet to counteract the effects of traumatic brain injury. Rats were fed with 500 IU/kg of Vit E for 4 weeks before performing a mild fluid percussion injury (FPI). FPI increased protein oxidation as evidenced by elevated levels of protein carbonyls and reduced levels of SOD. In addition, FPI resulted in poor performance in the Morris water maze test and supplementation of Vit E in the diet counteracted all the observed effects of FPI. The results suggest that Vit E dietary supplementation can protect the brain against traumatic brain injury.

**ALPHA LIPOIC ACID**

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

ALA or Lipoic acid is an antioxidant synthesized in the body in extremely small amounts. ALA is supplied from food and other supplement sources. ALA is sulphur containing vitamin like substance that plays a vital role in mitochondrial electron transport reactions, which inturn is intrinsically related to the metabolism of glucose into energy.

ALA has been characterized as an efficient antioxidant and is a potential therapeutic agent in the treatment or prevention of different pathologies that may be related to an imbalance of the oxidoreductive cellular status. This occurs in the case of neurodegeneration, ischemia-reperfusion, polyneuropathy, diabetes, AIDS and hepatic
disorder status (Packer, et al., 1995). After supplementation with ALA for 5 weeks, free ALA was found in various tissues, the highest being the heart (Podda, et al., 1994a).

ALA is amazing that, being a single nutrient it has so many actions. The ALA is described as the metabolic antioxidant. ALA is a coenzyme that is involved in metabolism that directly and indirectly helps protect every tissue component from the damage of oxidative stress (Passwater, 1998).

**Food sources**

**Mechanism**
ALA is a disulfhydryl coenzyme that gets converted into Dihydrolipoic acid (DHLA) once it enters inside the cellular structures. DHLA is considered as more powerful free radical deactivator than the original acid. ALA is readily absorbed from diet and is rapidly converted to DHLA by Nicotinamide adenine dinucleotide (NADH) or Nicotinamide adenine dinucleotide phosphate (NADPH) in most tissues. Both the oxidized and reduced forms of ALA are anti-oxidants. ALA is active against hydroxyl radicals, hypochlorous acid and singlet oxygen, but not against hydrogen peroxide or superoxide. DHLA is active against hydroxyl radicals, peroxyl and peroxynitrite free radicals and hypochlorous acid, but not against hydrogen peroxide or singlet oxygen. DHLA regenerates Vit C and Vit E from their oxidized forms (Biewenga, et al., 1997). Although GSH has twice the chemical reactivity in its thiol group, DHLA is superior to GSH in regenerating Vit C and E (Bast and Haenen, 2003). DHLA donates two hydrogens and neutralize free radicals without itself becoming a free radical.
Enantiomers of ALA

Lipoic acid exists as two enantiomers: the R-enantiomer and the S-enantiomer. Naturally occurring lipoic acid is the R-form, but synthetic lipoic acid is a racemic mixture of R-form and S-form. Although the R-enantiomer is more biologically active than the S-enantiomer, administration of ALA actually results in greater formation of DHLA due to a synergistic effect which each enantiomer exerts on the reduction of the other (Bast and Haenen, 2003).

Role of ALA as metal chelator

ALA and DHLA chelates a number of metal ions including Cu$^{2+}$, Fe$^{3+}$ (Scott, et al., 1994; Marangon, et al., 1999) Mn$^{2+}$, Zn$^{2+}$ and Cd$^{2+}$ (Sigel, et al., 1978). Chelation of Cu$^{2+}$ by ALA was reported to inhibit the Cu$^{2+}$ catalyzed oxidations in vitro (Ou, et al., 1995).

Although DHLA chelates Fe$^{3+}$, it can also reduce Fe$^{3+}$ to Fe$^{2+}$ a pro-oxidant effect it shares with ascorbic acid. DHLA have the capacity to remove bound iron from ferritin. By chelating iron and copper in the brain, ALA reduces free-radical damage (Bush, 2002). ALA fed to old rats reduced the increased iron in the cerebral cortex associated with aging, without affecting normal protein-bound iron by an unknown mechanism other than chelation (Suh, et al., 2005). Even small amounts of cadmium (Cd$^{2+}$) can cause significant lipid peroxidation in the brain, which is prevented by ALA (Packer, 1997c).

Role of ALA in mitochondria

In mitochondria, ALA compensates for the low concentrations of GSH and chelates free radical generating heavy metal ions. R-lipoic acid supplementation in old rats significantly restored mitochondrial membrane potentials and oxygen consumption along with reduction in lipid peroxidation when compared to unsupplemented
rats (Hagen, et al., 1999). Age-related damage to myocardial mitochondria has been considerably reduced by ALA supplementation (Suh, et al., 2001). GSH synthesis declines considerably with age in mitochondria, but ALA has been shown to restore high levels of GSH in aging rat liver (Suh, et al., 2004). ALA supplementation improves mitochondrial metabolic function without increasing oxidative stress (Hagen, et al., 2002). ALA induces cystine/cysteine uptake, thereby increasing the synthesis of GSH.

**Role of ALA as a free radical scavenger**

The dithiol nature of lipoate makes it highly reactive against a number of ROS, and it also has the ability to regenerate oxidized antioxidants. ALA prevented the symptoms of both Vit C and Vit E deficiency in guinea pigs and vitamin E deficiency in rats (Rosenberg and Culik, 1959).

Free radicals are formed during various reactions. The respiratory burst of neutrophils in response to inflammatory stimuli produces highly ROS. Superoxide in the presence of superoxide dismutase, form H$_2$O$_2$. This can then be converted to hypochlorous acid (HOCI) by the action of myeloperoxidase. H$_2$O$_2$ may react with transition metals producing highly reactive hydroxyl radicals. ALA and DHLA scavenge both H$_2$O$_2$ and HOCl (Haenen and bast, 1991; Yan, et al., 1996). In addition DHLA scavenges superoxide (Suzuki, et al., 1991; Suzuki, et al., 1993). ALA is also reported to quench reactive oxygen and nitrogen species, hydroxyl radicals, peroxyl radicals and peroxynitrite (Marangon, 1999).

During the course of lipid peroxidation, peroxyl radicals are formed that propagate the reaction. DHLA can scavenge the radicals, formed from both lipophilic and hydrophilic peroxyl radical generators (Kagan, et al., 1992a). Therefore, the lipoate couple represents a potent radical scavenging unit.
In antioxidant regeneration

When antioxidants react with ROS, the antioxidant is converted to a form that is no longer able to function and is said to be consumed. Therefore, this oxidized product needs to be recycled to its native form to function again. Vit E, being a potent peroxyl radical scavenger, is the major chain-breaking antioxidant protecting biological membranes from lipid peroxidation (Burton, et al., 1981). A number of antioxidants can recycle Vit E including Vit C, ubiquinol and GSH (Sies, 1993). DHLA has only a weak interaction with the tocopheroxyl radical, so the major recycling of Vit E by DHLA occurs via the intermediary recycling of other antioxidants. Electronic spin resonance studies have demonstrated the recycling of the ascorbyl radical by DHLA, which in turn recycles the chromanoxyl radical produced by oxidation. This has been shown in dioleylphosphatidyl liposomes (Kagan, et al., 1992a), erythrocyte membranes (Constantinescu, et al., 1993) and LDL (Kagan, et al., 1992a). DHLA also recycles Vit E by reducing oxidized GSH, (Bast and Haenen, et al., 1990) which then reduces the Vit E radicals. There is now evidence that ALA supplementation increases tissue ubiquinol content, (Gotz, et al., 1994) and ubiquinol in turn recycles vitamin E (Kagan, et al., 1990). Therefore, there exists a network of antioxidants in which DHLA can interact and replenish to maintain both lipid and aqueous phase antioxidant status.

Modulation of cellular GSH status has long been discussed as a potential therapeutic strategy, taking into account the role of reduced GSH in a variety of detoxification reactions against oxidizing species, produced during the metabolism of xenobiotics, as well as its involvement in the formation of conjugates with electrophilic metabolites (Meister, 1989).
The influence of ALA on the cellular status of GSH was investigated in various in vitro and in vivo systems. In accordance with the lower redox potential of the ALA/DHLA couple, with respect to the GSH/GSSG couple, DHLA is a powerful reductant of GSSG (Jocelyn, et al., 1967). Moreover, in various cellular systems (human T-lymphocytes cell lines, C6 glial, NB41A3 neuroblastoma, Jurkat cells, Wurzburg cells, human erythrocytes and peripheral blood lymphocytes) ALA treatment (10-100 mM) induced an increase in the cellular level of GSH by 30-70% (Busse, et al., 1992).

In the Jurkat cell line, the ALA dependent increase in cellular GSH levels was proposed to be due to improved cysteine utilization. The authors suggest that DHLA (formed via cellular ALA reduction) reduced extracellular cystine to cysteine, which is transported to the cells more efficiently than cystine, and is promptly used as sources of GSH synthesis (Han, et al., 1997). They are also capable of interacting with other antioxidants such as ascorbate, GSH, and ubiquinol and are thought to participate in ascorbate recycling and indirectly in the regeneration of α-tocopherol (AT) (Kagan, et al., 1992b).

Clinical applications

- **In the treatment of Hepatic disorders**

Abdel-Zaher, et al., 2008 investigated the protective role of ALA in acetaminophen induced hepatotoxicity in rats. The rats were administered with ALA (100 mg/kg) orally and assessed for biochemical parameters. The acetaminophen induced profound elevation of NO production, oxidative stress, lipid peroxidation along with reduction of GPx and depletion of intracellular reduced GSH level in liver. These results provide evidence that ALA by inhibiting NO overproduction and maintaining
intracellular antioxidant status play a pivotal role in the treatment of acetaminophen (100 mg/Kg) reversed acetaminophen induced alterations in the above parameters.

Anandakumar, et al., 2007 evaluated the protective effect of ALA (75 mg/kg body wt/day i.p) on adriamycin induced hepatotoxicity in rats. Injection of adriamycin (15 mg/kg body wt i.p.) induced hepatotoxicity which was expressed by an elevation in ALP, LDH, SGOT and SGPT in serum. Pretreatment with ALA 24 h prior to administration of adriamycin significantly restored various cellular activities by reduction of elevated liver enzymes revealing the hepatoprotective effect of ALA.

Shanmugarajan, et al., 2008 assessed the effect of ALA supplementation on acute D-Galactosamine induced oxidative liver injury. Intra peritoneal injection of D-Galactosamine (500 mg/Kg body wt) increased lipid peroxidation and serum enzymatic levels such as aspartate transaminase, alanine transaminase, alkaline phosphatase and lactate dehydrogenase. D-Galactosamine decreased the activities of enzymatic antioxidants (SOD, catalase, GSH px and glutathione reductase (GR) as well as GSH levels causing hepatotoxicity. Pretreatment with ALA (50 mg/Kg body weight for 7 days) significantly precluded these changes and prevented the hepatic injury. Hence, this study clearly exemplified that ALA prevents D-Galactosamine-induced hepatocellular abnormalities. Saad, et al., 2010 investigated the protective effect of ALA against isoniazid (INH) and rifampicin (RIF) combination induced hepatotoxicity. Administration of INH-RIF combination (50 mg/kg each for 14 days) resulted in an elevation of serum hepatic enzymes. Co-administration of ALA significantly ameliorated INH-RIF combination induced hepatic damage.

- **Against diabetes**

In Type I diabetes, destruction of pancreatic β cells results in loss of insulin secretion, whereas Type II dependent diabetes is associated with insulin resistance of peripheral
tissues. ALA has potential preventive or ameliorative effects in both Type I and Type II diabetes.

Ansar, et al., (2011) examined the effect of ALA on fasting blood glucose, insulin resistance and GPx activity in type 2 diabetes patients. Type 2 diabetes mellitus patients (n=57) were divided into 2 groups to receive either ALA (300 mg daily) or placebo by systematic randomization, and were followed-up for 8 weeks. Fasting blood glucose, post prandial glucose, and GPx activity were assessed. ALA caused significant reduction in fasting as well as post prandial blood glucose levels and increased the GPx activity compared with placebo group supporting the use of ALA in diabetes/diabetic patients.

Gu, et al., (2010) evaluated the efficacy and safety of high-dose ALA in the treatment of diabetic polyneuropathy with regards to sensory symptoms and nerve conduction velocity. A total of 236 diabetics with symptomatic polyneuropathy were enrolled for the study. ALA 1800 mg daily (n = 117) or matching placebo (n = 119) were administered for 12 weeks. HbA1c decreased at the end of trial after ALA treatment. Oral treatment with high-dose ALA for 12 weeks improved symptoms in patients with diabetic polyneuropathy.

Ametov, et al., (2010) estimated the effect of ALA (600 mg/day i.v.) in patients with symptoms of myodiabetic polyneuropathy for 3-weeks. The neuropathic symptoms reduced within 8 weeks after initiation of ALA therapy.

Chun-jun, et al., (2009) investigated whether ALA attenuates mitochondrion-dependent myocardial apoptosis in diabetic cardiomyopathy. Oxidative stress is widely considered to be one of the major factors underlying the pathogenesis of diabetic cardiomyopathy. Streptozotocin 45 mg/Kg was administered intravenously to induce diabetes in rats. ALA (100 mg/Kg i.p./day) effectively attenuated the
mitochondria-dependent cardiac apoptosis and exerts a protective role against the development of diabetic cardiomyopathy.

- **In dermatology**

Davis, et al., (2009) investigated the radioprotective role of ALA on murine skin fibroblasts exposed to a single dose of 2, 4, 6 and 8 Gy gamma-radiation. Irradiation of fibroblasts significantly increased ROS, nitric oxide and lipid peroxidation which substantially decreased with 100 μM ALA treatment. ALA was found to inhibit hydroxyl radical production at 100-μM concentrations. Dose-dependent depletion of antioxidant enzymes such as catalase and GR was observed in irradiated fibroblasts, while treatment with ALA restored the levels of antioxidant enzymes (Davis, et al., 2009). Thirunavukkarasu, et al., (2004) studied the effect of ALA on the content and characteristics of the protein collagen from skin of high fructose fed rats. The rats were divided into 4 groups of 6 each. Two groups of rats were fed with a high fructose diet (60 g/100 g diet) and administered with ALA (35 mg/kg b.w., i.p) for 45 days. The other 2 groups were fed with control diet containing starch (60 g/100 g diet) and administered with saline. The rats were maintained for 45 days and then sacrificed. Collagen was isolated from skin and the physicochemical properties of collagen were studied. Fructose administration caused accumulation of collagen in skin. Administration of ALA to fructose-fed rats had a positive influence by preventing collagen accumulation in skin and improved variables such as solubility, shrinkage temperature and glycation in high fructose-fed rats and thereby delayed the diabetic complications of skin.

- **Cataracts**

The common complication of diabetes is cataract which is mediated via OS (Wolff, et al., 1991). Buthionine sulfoximine is reported to induce cataracts by decreasing lens
ascorbate, tocopherol and GSH (Maitra, et al., 1995). ALA decreased the incidence of buthioninesulfoximine induced cataract formation by protecting the lens ascorbate, tocopherol and GSH. Also, in vitro diabetic cataractogenesis in rat lens cell cultures exposed to high concentrations of glucose was prevented by ALA (Kilic, et al., 1995).

- **Ischemic-reperfusion Injury**

Ischemic-reperfusion injury occurs when a burst of free radicals are produced during reoxygenation of tissues under hypoxia. Agents that prevent ischemia-reperfusion injury are important in the treatment of cardiac infarct, during open-heart surgery and in the treatment of stroke and other conditions that cause interruption of blood flow to the brain.

Serbinova, et al., (1992) reported the protective effect of ALA against ischemic-reperfusion injury induced in an isolated perfused Langendorff heart system. ALA preserved Vit E in heart tissue, improved post ischemic left ventricular functional recovery, and decreased lipid peroxidation and lactate dehydrogenase leakage.

Boveris, et al., (1994) reported that administration of ALA prevented rat intestinal short-term ischemic-reperfusion-induced overshoot of chemiluminescence, which is indicative of an increased rate of lipid peroxidation. Boveris also observed that ALA inhibits xanthine oxidase activity, which is postulated to play an important role in the mechanism of ischemic-reperfusion injury.

- **Neurodegenerative diseases**

Central nervous system is vulnerable to oxidative stress because of its high rate of oxygen consumption and high mitochondrial density. Mitochondria inevitably produce free radicals as byproducts of normal oxidative metabolism (Boveris and Chance, 1973), and these free radicals damage the mitochondrial DNA (Nohl and
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Jordan, 1986). The defective proteins coded by the damaged DNA synthesis damaged protein leading to greater free radical production (Takeshige and Minakami, 1979; Saggu, et al., 1989) and more mitochondrial damage in a vicious cycle. Such a vicious cycle may be responsible in part for neurodegenerative diseases. In mice, ALA (100 mg/kg body weight for 15 days) improved performance in an open-field memory test than the control animals. The ALA treated animals performed better than young animals 24 h after the first test (Stoll, et al., 1993). The ALA treated animals exhibited decreased age-related N-methyl-D-aspartate (NMDA) receptor deficits compared to controls. The authors concluded that ALAs free radical-scavenging ability improved NMDA receptor density, leading to improved memory. Excitotoxins induce lesions in the striatum affecting NMDA receptors (Greenamyre, et al., 1994) which may lead to calcium influx and generation of nitric oxide and other free radicals. Administration of ALA and DHLA treatment decreased the area of lesion by 50% (Choi, 1988).

- **Heavy Metal Poisoning**

The possible chelating effects of ALA, together with its antioxidant effects, make it a good candidate for the treatment of heavy-metal poisoning. ALA is effective in arsenite, cadmium and mercury poisoning.

Grunert, (1960) demonstrated that, administration of ALA completely protected mice and dogs from arsenite poisoning. Exposure of isolated hepatocytes to ALA or DHLA resulted in the amelioration of cd^{2+} induced membrane damage, lipid peroxidation, and depletion of cellular GSH (Müller and Menzel, 1990).

Sumathi, et al., (1994) reported that administration of ALA 30 mg/Kg completely prevented cadmium-induced lipid peroxidation in brain, heart and testes. In this study, it completely abolished cadmium induced reduction in the activities of Ca^{2+}, Na^{+} and Mg^{+} ATPases in the above organs. At low doses, ALA prevented Hg^{2+} poisoning in
mice. ALA administration in rats increased biliary excretion of injected Hg\(^{2+}\) by 12 to 37 fold (Gregus, et al., 1992).

ALA is found to increase the rate of elimination of radiomercuty in rabbits (Cardinale and De Simone, 1968). Hence, ALA may be of clinical value in the treatment of mercury and cadmium poisoning.

**N-ACETYL CYSTEINE**

![Chemical structure of NAC](image)

(R)-2-acetamido-3-sulfanylpropanoic acid

N-acetylcysteine (NAC) or Acetyl cysteine or N-acetyl-L-cysteine, is an acetylated derivative of the amino acid L-cysteine. It is an endogenous product of the sulfur-containing aminoacid cysteine, which is a non-essential amino acid. It is a precursor of GSH, which is involved in the detoxification process and possesses direct free radical scavenging action (Vendemiale, et al., 2001). NAC is a potent antioxidant which plays a key role in the neutralization of reactive oxygen molecules and other free radicals. NAC was used primarily as a mucolytic agent in chronic respiratory illnesses as well as in the management of paracetamol toxicity (Geier, et al., 2006). NAC due to its antioxidant property finds its application in the treatment of cancer, heart disease, human immunodeficiency virus (HIV) infection, heavy metal toxicity and other diseases characterized by oxidative damage.

**Mechanism of action**

NAC’s effectiveness is primarily attributed to its ability to reduce extracellular cystine to cysteine, and it acts as a source of sulphhydryl groups. NAC is rapidly metabolized
to intracellular GSH, which acts as a powerful antioxidant and detoxifies chemicals into less harmful compounds. NAC enhances GSH-S-transferase activity, promotes liver detoxification by inhibiting xenobiotic biotransformation. It is a powerful nucleophile capable of scavenging free radicals (De Vries and De Flora, 1993; De Flora, et al., 1985).

GSH, a predominant anti-oxidant in the cytoplasm of cells, 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 and breaks mucus into smaller, less viscous units in lung. By reducing the chain length of mucus and thinning the mucus, it improves conditions such as bronchitis and flu. NAC also acts as an expectorant by stimulating ciliary action and the gastro-pulmonary vagal reflex, thereby clearing mucus from the airways (Ziment, 1988).

**Therapeutic applications of NAC**

- **In Cystic fibrosis**

A single-centre, randomised, double-blinded, placebo-controlled phase II clinical study examined the safety and efficacy of low-dose (700 mg/day) or high-dose (2800 mg/day) of NAC in patients suffering with cystic fibrosis. NAC decreased the levels of inflammatory cells, TNF-alpha (Tumour necrosis factor-α), IL-8 and increased the concentrations of extracellular GSH in sputum and blood. High-dose NAC is considered as well tolerated and safe medication for therapy in patients with cystic fibrosis (Dauletbaev, et al., 2009).
• **In Bronchitis**

A review of 39 clinical trials of NAC found that 400 to 600 mg NAC per day was a safe and effective treatment for chronic bronchitis (Stey, et al., 2000). NAC supplementation was found to reduce the number of aggravations of the illness in almost 50% of people taking the supplement, compared with only 31% of those under placebo.

Smokers were also benefited by NAC (Boman, et al., 1983). In addition to its mucolytic action, NAC reduced the elevated bacterial counts that are often seen in the lungs of smokers with chronic bronchitis (Riise, et al., 1994). In another double-blind study, people with chronic bronchitis who were administered with NAC showed an improved ability to expectorate along with reduction in cough severity (Jackson, et al., 1984).

These benefits may result from NAC’s capacity to reduce the viscosity of sputum (Tattersall, et al., 1983). NAC also protects lung tissue through its antioxidant activity (Schayck, et al., 1998).

• **In Angina**

NAC enhanced the effects of nitroglycerin in people with angina (Marchetti, et al., 1999). People with unstable angina who took 600 mg of NAC three times daily in combination with a nitroglycerin transdermal patch for four months had significantly lower rates of subsequent heart attacks than people who used either nitroglycerin alone or placebo (Ardissino, et al., 1997).

• **In Gastritis**

In a double blind trial, 200 mg of NAC four times daily offered significant benefit in people with NSAID induced gastritis (Salim, et al., 1993). In a preliminary trial, NAC 1-4 grams/day was reported to alleviate gastritis (Farinati, et al., 1997).
• **In HIV and AIDS**

NAC is found to inhibit the replication of HIV *in vitro* (Roederer, et al., 1990). Supplementation of NAC 800 mg per day slowed the rate of decline in immune function in people with HIV infection. NAC promotes the synthesis of GSH by which it is believed to be protective in people with HIV infection and acquired immunodeficiency syndrome (AIDS) (Herzenberg, et al., 1997).

Human immunodeficiency virus positive individuals generally exhibit low GSH and cysteine levels. NAC enhances T cell immunity by stimulating T cell colony formation (Wu, et al., 1989) and by blocking Nuclear factor kappa B (NF kappa B) expression (Breithaupt, et al., 1996; Droge, et al., 1992). In a double-blind placebo controlled trial, NAC increased the plasma cysteine levels and CD4+ lymphocyte cell counts (Akerlund, et al., 1996). Thus, NAC was suggested to prevent the progression of AIDS.

• **In Heavy metals poisoning**

Heavy metals like arsenic, mercury and lead are detoxified 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 by gold, silver, mercury, lead, etc. It also increases the excretion of zinc and other essential minerals when consumed over an extended period. It is therefore necessary to supplement zinc, copper and other trace minerals during administration of NAC (Ziment, 1988).

• **Against respiratory illness**

NAC is effective against various types of respiratory illnesses. NAC is found to reduce cough severity (Jackson, et al., 1984) and diaphragm fatigue (Hida, et al.,
Administration of NAC (600 mg) thrice daily for 12 weeks resulted in improvement of pulmonary function and GSH levels in patients with fibrosing alveolitis, which is a condition 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 the treatment of Cancer**

Oxidative stress is involved in the etiology of carcinogenesis. ROS derived from environmental sources, cigarette smoke or cellular metabolism, constantly attack macromolecules within the cell. The damaged lipids and proteins can be replaced, whereas oxidative Deoxy ribonucleic acid (DNA) damage needs to be repaired. Damages exceeding DNA repair capacity might lead to permanent mutations.

Baumeister, et al., (2009) found that supplementation with NAC reduced DNA damage and increased the viability of the cell in patients with cancer of upper aerodigestive tract. NAC also reduced the proliferation of cells lining the colon and thus decreased the risk of colon cancer in people with recurrent polyps. Its action as an antioxidant and a GSH precursor contributes to its protective effect against cancer.

NAC possesses a prophylactic potential against the treatment of certain types of cancer, including lung, skin, head and neck, breast and liver cancer (De Flora, et al., 1992). *In vitro* and *in vivo* studies have demonstrated that NAC exhibits anti-mutagenic and anti-carcinogenic activity (De Flora, et al., 1986). NAC administration in cell cultures and animal studies showed that NAC selectively protects normal cells, but not malignant ones, from chemotherapy and radiation toxicity (De Flora, et al., 1996). It was also observed that NAC inhibits cell growth and proliferation of human
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- **Against Viral Hepatitis**
  Interferon-alpha (INF-α) is the first line of treatment for hepatitis C infection. However, the virus develops resistance against the drug within a short period of time. In a pilot study, NAC supplementation (600 mg thrice daily) for six months enhanced the response to INF-α therapy in chronic hepatitis C patients resistant to INF-α, with normalization of serum alanine aminotransferase (ALT) in 41 percent of patients (Beloqui, et al., 1993).

- **Against cardiovascular disorders**
  NAC was reported to reduce plasma homocysteine levels by 45% (Wiklund, et al., 1996) and decrease lipoprotein (a) by 70% (Gavish and Breslow, 1991). NAC by augmenting tissue GSH level is useful in treating ischemia and reperfusion associated with acute myocardial infarction (Ceconi, et al., 1988). In addition, NAC appears to potentiate nitroglycerine’s coronary dilating and anti-platelet properties and hence is effective as combination therapy in patients with unstable angina pectoris and myocardial infarction (Winniford, et al., 1986; Chirkov, et al., 1996).

- **In acetaminophen and other poisonings**
  Historically the most prevalent and well-accepted use of NAC was as an antidote for acetaminophen or paracetamol poisoning. N-acetyl-p-benzoquinonimine, a metabolite of paracetamol is responsible for hepatotoxicity (Hazai, et al., 2001). N-acetyl-p-benzoquinonimine depletes hepatocytic GSH resulting in oxidative damage, mitochondrial dysfunction, alterations in membrane permeability, apoptosis, centrilobular necrosis followed by hepatocellular death (Manov, et al., 2002). Administration of NAC intravenously or orally within 10 hours of acetaminophen
poisoning is found to be effective in reversing hepatotoxicity. However improvement is most appreciable if the treatment is initiated within 8-10 hours of acetaminophen overdose. NAC’s effectiveness declines when treatment is delayed beyond 10 hours, with the risk of increased mortality (Smilkstein, et al., 1988; Wang, et al., 1997; Perry, et al., 1998). NAC is also effective against poisoning by carbon tetrachloride, acrylonitriles, halothane, paraquat, acetaldehyde, coumarin and interferon (Ziment, 1988).

- **Cataract**

  Injection of acetaminophen (350 mg/Kg body weight) into C57BL/6 mice produced acute cataract and other ocular tissue damage. NAC was reported to offer protection against acetaminophen induced cataract in C57BL/6 mice by increasing cellular cysteine level and GSH synthesis, which in turn improves GSH conjugation and subsequent detoxification of N-acetyl-p-benzoquinonimine (Zhao and Shichi, 1998).

- **Oxidative stress**

  Priya, et al., (2011) evaluated the hepatoprotective and antioxidant properties of NAC on dimethylnitrosamine induced hepatotoxicity in rats. Dimethylnitrosamine (5 mg/Kg i.p.) caused a significant increase in the levels of the serum hepatic marker enzymes SGOT, SGPT, ALP, LDH and GGT indicating hepatocellular damage. Dimethylnitrosamine enhanced lipid peroxidation and decreased SOD, catalase, Vit C and Vit E confirming oxidative stress. Oral administration of NAC (50 mg/Kg) for 7 days significantly prevented the above alterations in the status of the marker enzymes of hepatotoxicity and antioxidant parameters. Thus, NAC offers hepatoprotective activity by ameliorating dimethylnitrosamine induced oxidative stress and this protective effect was attributed to its antioxidant and free radical scavenging properties.
Several studies have indicated the involvement of oxidative stress in the development of diabetic neuropathy. Kamboj, et al., (2010) explored the effect of NAC in streptozotocin induced diabetic neuropathy. There was an increase in lipid peroxidation in sciatic nerve of diabetic animals along with decrease in phospholipid levels, while NAC treatment attenuated lipid peroxidation and restored phospholipids near to that of normal. NAC treatment significantly ameliorated the antioxidant defense thereby, reversed the alterations induced by streptozotocin in sciatic nerve of diabetic rats.

Çağlıkülekiç, et al., (2004) investigated the effects of NAC on liver and renal tissue inducible nitric oxide synthase, and liver tissue lipid peroxidation in lipopolysaccharide induced obstructive jaundice. NAC (100 mg/Kg) prevented the deleterious effects of lipopolysaccharide in obstructive jaundice by reducing inducible nitric oxide synthase expression in liver and renal tissue.