MeOH due to its readily absorbed nature and irrespective of the route of exposure, and it is rapidly distributed to tissues according to the distribution of body water. MeOH is metabolized primarily in the liver by sequential oxidative steps to formaldehyde, formic acid and carbon dioxide and its metabolite formic acid. Formaldehyde in human is metabolized relatively slower and cause for the toxicity observed in the literature is entirely focused on the formaldehyde. The metabolism of formate is mediated through a tetra hydro folate dependent pathway (Eells et al., 1982). Humans (and non-human primates) are uniquely sensitive to MeOH poisoning because of their low liver folate content (Johlin et al., 1987). Hence, it is essential to know about MeOH metabolism.

Among alcohols, MeOH shows the highest reactivity in reactions in which the breaking of the O-H bond is involved. The interaction of MeOH, formaldehyde, and formate with endogenic components of the organism is determined by the chemical structure. The formaldehyde molecule has a strongly polarized carbonyl group. This is the reason for the above compounds react more readily than do corresponding compounds situated higher in the homologous series (Morrison and Boyd, 1983).

MeOH metabolism

After uptake and distribution, most of the MeOH is metabolized in the liver to carbon dioxide (96.9%), while a small fraction is excreted directly to the urine (0.6%) and through the lung. In all mammalian species studied, MeOH is metabolized in the liver by sequential oxidative steps to form formaldehyde, formic acid and CO₂. However, there are profound differences in the rate of
formate oxidation in different species, which determine the sensitivity to MeOH (Palese and Tephly, 1975, McMartin et al., 1977; Eells et al., 1981, 1983). Two enzymes are involved in the oxidation of MeOH to formaldehyde which makes the species difference (figure-2). In non-human primates and humans, alcohol dehydrogenase mediates this reaction (Makar et al., 1968a). In rats and other non-primate species, this reaction is mediated by catalase. Formaldehyde is oxidized to formate by several enzyme systems including a specific formaldehyde dehydrogenase. In the reaction catalysed by this enzyme, formaldehyde combines with reduced glutathione to form S-formyl glutathione, which is hydrolysed in the presence of thiolase to formate and reduced glutathione (Uotila and Korvusalo, 1974).

Formate is oxidized to CO$_2$ in vivo in mammalian species primarily by a tetrahydrofolate-dependent pathway. Formate enters this pathway by combining with tetrahydrofolate (H$_4$folate) to form 10-formyl-H$_4$folate in a reaction catalysed by formyl-tetrahydrofolate synthetase. 10-Formyl-H$_4$folate may then be further oxidized to CO$_2$ and H$_4$folate by formyl-H$_4$folate dehydrogenase (Kutzbach and Stokstad, 1968). Formate metabolism in rats and monkeys has been shown to be mediated by the folate-dependent pathway (Makar et al., 1968b; Palese and Tephly, 1975). Tephly et al. (1961) also proved that a catalase system is also one of the major pathways of MeOH oxidation in rat hepatocytes.

Folate determines the toxicity

Tetrahydrofolate is derived from folic acid in the diet and is the major determinant of the rate of formate metabolism (McMartin et al., 1975). The folate-mediated oxidation of formate proceeds about twice as slowly in non-human primates and humans as in rats. This explains the susceptibility of primates to the accumulation of formate, which is seen to occur at doses of MeOH greater than
Scheme for the metabolism of methanol. Major enzymes for primates (left) and rodents (right) are noted. Species differences in methanol toxicity are due primarily to the metabolic conversion of formate to carbon dioxide which is rapid in rodents but slow in primates (Medinsky and Dorman, 1994).

Metabolism of formate by folate – dependent factors (Eells et al., 1981)

DHF – Dihydrofolate. THF – Tetrahydrofolate
0.5 g·kg (Tephly and McMartin, 1984). Humans (and non-human primates) are uniquely sensitive to MeOH poisoning because of their low liver folate content (Johlin et al., 1987).

**Formic acid metabolism**

It is the rate of metabolic detoxification, or removal of formate that is vastly different between rodents and primates and this forms the basis for the dramatic differences in MeOH toxicity observed between rodents and primates. Two pathways have been suggested for the disposition of formic acid. They are oxidized through the catalase-per oxidative system or through the one carbon pool (folate dependent mechanism). The catalase system appears to be poor in rat and monkey probably due to their low level of peroxidative capacity of the hepatic systems and the low activity of peroxide-generating oxidizes (Goodman and Tephly, 1970).

An alternative pathway for the metabolism of formic acid is a tetrahydrofolic acid (THF) dependent one carbon pool (figure-3). Formic enters this pool by combining with tetrahydrofolate to 10-formyl tetrahydrofolate. The ATP-dependent reaction is catalyzed by 10-formyl-Tetrahydrofolate synthetase, a ubiquitous enzyme in mammalian tissues (Whiteley, 1960). However, rats metabolize formic acid at about twice the rate of that seen in monkeys (McMartin et al., 1977). Hepatic tetrahydrofolate levels in humans and monkeys are only half of those in rats (Black et al., 1985). The activity of 10-formyl-THF dehydrogenase, the enzyme catalyzing the final step of formic acid oxidation to Carbondioxide, is markedly smaller in monkey and human liver, being 20 – 25% of that in rat liver (Johlin et al., 1987). However, in animals that readily metabolize formate, the consequences of central nervous system depression is the only cause behind the cause of death as no metabolic acidosis is observed in them.
Formate toxicity

Formate is accumulated in high quantities in the tissues and body fluids of MeOH intoxicated monkeys and humans (Eells et al., 2000; Hantson et al., 2000; Langan, 2001). In such cases, a decrease in the level of bicarbonate and in blood pH by even 0.3 units (Fig. 6) can cause metabolic acidosis. Progressing acidosis is likely to cause circulatory insufficiency and histotoxic hypoxia as well as lactic acidosis (Jacobsen and McMartin, 1986).

Despite its toxicity at elevated concentrations, formate is normally present in mammals (Smith and Taylor, 1982) and has been recognized as a major source of one-carbon units in mammalian systems (Appling, 1991). The recognition that formate is a major source of one-carbon units, add further insight to formate toxicity. Formate has been hypothesized to produce toxicity by disrupting mitochondrial electron transport and energy production (Hayreh et al., 1980; Sharpe et al., 1982) by inhibiting cytochrome oxidase activity, a component of the electron transport chain involved in ATP synthesis, at concentrations between 5 and 30 mM both in vitro and in vivo (Nicholls, 1976; Wallace et al., 1997). Formic acid apparently penetrates mitochondria where it exerts its toxic effect by inhibiting cytochrome oxidase (Nicholls, 1975). As a moderate inhibitor of cytochrome c oxidase, formate may cause tissue oxygen utilization to be impaired leading to anaerobic respiration with subsequent increased lactate production, which may further contribute to the acidosis (IPCS, 2001).

Metabolic acidosis results from an accumulation of formate, which inhibits mitochondrial respiration (Nicholls, 1976), increases lactate production, and lowers blood pH (Jacobsen, and McMartin, 1986). Formaldehyde also reacts easily with proteins, and these reactions result in a decreased number of free amino groups (Kitamoto and Maeda, 1980). It is known that this aldehyde also
reacts with cysteiny1, methionyl, arginyl, tyrosyl residues and, to a smaller extent, with the other amino acid residues as well as with peptide bonds (Tome and Nault, 1981). In fact, the treatment of MeOH toxicity is largely aimed at treating the metabolic acidosis and accelerating the elimination of MeOH from the body by dialysis, and by slowing down the oxidation of MeOH to formic acid (McMartin et al., 1980).

**Differences in MeOH Toxicity among Species**

The amount of oxidized formate is determined by the dose of MeOH and by the animal species involved. Formate is metabolized twice as fast in the rat as in the monkey (McMartin et al., 1978), results in the accumulation of formate in the monkey and the human; however, such accumulation does not occur in the rat. Rats and rabbits are able to oxidize formate almost completely, whereas in dogs a small amount of formate accumulation is observed (Roe 1982). All these species metabolize formate primarily through a folate-dependent pathway, but differences in the abilities of the animals to metabolize formate by means of the folate system are observed. However, these species that accumulate formate have no tendency to accumulate the most reactive MeOH metabolite, formaldehyde.

**Rodent model modified for MeOH toxicity**

The severe acidosis observed in human cases of MeOH poisoning cannot be induced in rodents because they are capable of rapidly converting formate to carbon dioxide. However, the primates are capable of using the folate-dependent metabolism of formic acid, the rate of this pathway is much slower compared with that in rodents (Kruse, 1992), leading to formic acidosis. Therefore, rats made marginally folate deficient can serve as better models akin to human studies. Based on the reports that rodents do not develop metabolic acidosis in MeOH poisoning owing to their high liver folate content to create similar to human being
and only, folate deficient rodents are required to accumulate formate and acidosis in MeOH poisoning (Lee et al., 1994b; Eells et al., 2000).

In this study also to mimic the human situation, a folate deficiency was induced. There are many other methods available to induce folate deficiency. It can be by administering folate deficient diet (Lee et al., 1994a) and in his study the methodology adapted was described by Reeves et al. (1993). There are chemicals that interfere with tetrahydrofolate regeneration such as nitrous oxide exposure (Eells et al., 1982; Eells, 1991; Murray et al., 1991) or methotrexate treatment (MTX) (Barford et al., 1980; Dorman et al., 1994; Schalinske and Steele, 1996). MTX is highly potent competitive inhibitor of dihydrofolate reductase enzyme. Dihydrofolate reductase catalyzes the reduction of dihydrofolate to tetrahydrofolate (Bertino et al., 1965). Since MeOH itself is toxic and by administrating, more and more chemicals may modify the free radical generation, the deficient diet provision was selected.

Toxicity due to MeOH in brain

Blindness and serious visual impairment are well-known effects of human MeOH poisoning (Eells, 1992). The minimum dose causing permanent visual defect is unknown, although blindness has been reported after ingestion of as little as 4 ml (53 mg/kg) of MeOH. Single oral moderate to large doses of MeOH (400 - 1000 mg/kg bw) are known to cause blindness (Darwish, 2002). Visual dysfunction was measured as reductions in the flash-evoked cortical potential and electroretinogram, which occurred coincident with blood formate accumulation. Alterations in the electroretinogram occurred at formate concentrations lower than those associated with other visual changes and provide functional evidence of direct retinal toxicity in MeOH poisoning (Eells, 1991). Ocular toxicity may be related to the selective accumulation of formate in the retina and vitreous humor.
compared with other regions of the central nervous system (Wallace et al., 1997). Moreover, due to the limited capacity of the retina to oxidize, thus accumulate formate (Eells et al., 1996).

MeOH chronic exposures damage the CNS often in the form of lesions in basal ganglia especially the putamen, which may result in long term neurological deficits ranging from moderate polyneuropathy to tremors, rigidity, spasticity and hypokinesis as well as Parkinsonian-like extrapyramidal syndrome with mild dementia (Fujita et al., 2004; Fontenot and Pelak, 2002). MeOH poisoning led to acute hemorrhagic necrosis of the basal ganglia and resulted in acute Parkinson's syndrome within 10 days of the ingestion. However, the patient after MeOH exposure developed respiratory muscle stiffness/weakness, which responded poorly to anti-Parkinsonian drug therapy (Reddy et al., 2007). After chronic MeOH intoxication with methotrexate and MeOH the serotonin increased and dopamine decreased in its turnover rate, and resulted in changes in the amino acid balance (Gonzalez-Quevedo et al., 2003)

MeOH is teratogenic in rodents and MeOH enzymatic biotransformation leads to formaldehyde (CH₂O) and formic acid causing increased biological reactivity and toxicity and all these produced a significant depletion of GSH in cultured rat embryo and formaldehyde toxicity is potentiated by GSH depletion (Harris et al., 2004). Formic acid is considered to be the key toxicant; and in animal species with a poor ability to metabolise this product (primates and humans) fatal toxicity may occur from metabolic acidosis and neuronal toxicity (IPCS, 1997). Undissociated formic acid readily crosses the blood brain barrier leading to CNS toxicity, aggressive alkaline therapy is required to maintain formic acid in the dissociated form (IPCS, 2001). Despite evidence that formate inhibits the mitochondrial electron transport chain, only certain tissues, including the
retina, optic nerve, and basal ganglia (Sharpe *et al*., 1982; Roe, 1955), appear sensitive to its toxic actions.

**Free radicals**

Oxygen is indispensable to the lives of most organisms on earth; with the mitochondrial ATP production that is linked to the reduction of O$_2$ to H$_2$O being the primary energy-producing pathway of the cell and one that few organisms can live without indefinitely. Although more than 90% of the O$_2$ taken up by the human body is used by mitochondrial cytochrome oxidase which adds four electrons onto each O$_2$ molecule to form water (Halliwell, 1992), oxygen is also used as a substrate by numerous other enzymes. Many of these enzymatic reactions generate reactive oxygen species (ROS) as their products, including superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$).

A free radical or ROS is a compound with one or more unpaired electrons in its outer orbital (Jesberger and Richardson, 1991). Such unpaired electrons make the species very unstable and therefore quite reactive with other molecules, as they try to pair their electrons and generate a more stable compound. In addition, ROS are derived from other cellular activities including the autooxidation of various small molecules (e.g. flavins, catecholamines, hydroquinones), the microsomal cytochromes P450 and b5, microsomal flavoprotein reductases, and superoxide leakage from the electron transport chain (Kleinveld *et al*., 1989). Oxygen radicals can attack proteins, nucleic acids and lipid membranes, thereby disrupting cellular functions and integrity. Brain tissue contains large amounts of poly unsaturated fatty acids, which are particularly vulnerable to free radical attacks (Gutteridge, 1995). The rate of ROS generation is closely related to oxygen consumption and proportional to the amount of mitochondria in the tissue. It has been estimated that about 1-4% of consumed
oxygen is converted into O2 and H2O2 at the mitochondrial level due to electron leaks (Turrens et al., 1982; Konstantinov et al., 1987)

**Free radical functions**

Devasagayam et al. (2004) emphasizing the important beneficial role of free radicals as follows

1. Generation of ATP (universal energy currency) from ADP in the mitochondria. Oxidative phosphorylation
2. Detoxification of xenobiotics by Cytochrome P450 (oxidizing enzymes)
3. Apoptosis of effective or defective cells
4. Killing of micro-organisms and cancer cells by macrophages and cytotoxic Lymphocytes
5. Oxygenases utilize peroxides (e.g., COX: cyclo-oxygenases, LOX: lipoxygenase) for the generation of prostaglandins and leukotrienes, which have many regulatory functions.

In recent years, it has become increasingly clear that the ROS, such as O2·− and H2O2 may act as second messengers. Further observations made some twenty years ago had suggested that ROS may play a role in modulating the cellular functions. From the foregoing, it is clear that free radicals must be within the limits to have a normal functions.

**Types of free radicals generated**

The superoxide radical is the most well-known oxygen derived free radical (Yu, 1994) and can be led to the formation of additional reactive species. Superoxide and hydrogen peroxide are relatively unreactive and long-lived in
biological systems but their danger lies in the fact that they readily give rise to highly reactive hydroxyl radicals (•OH) which are involved in numerous forms of damage to cellular macromolecules (Kleinveld et al., 1989).

\[
\begin{align*}
O_2 + e^- & \rightarrow O_2 \quad \text{superoxide} \\
O_2 + e^- + 2H^- & \rightarrow H_2O_2 \quad \text{hydrogen peroxide} \\
H_2O_2 + e^- + H^+ & \rightarrow H_2O + •OH \quad \text{hydroxyl radical} \\
•OH + e^- + H^+ & \rightarrow H_2O
\end{align*}
\]

*In-vivo* much of the hydroxyl radical production comes from the reduction of $H_2O_2$ by superoxide (the Haber-Weiss reaction) which is, in fact, a two-step process catalyzed by transition metals ($Fe^{3+}$, sometimes $Cu^{1+}$) and involving the Fenton reaction

\[
\begin{align*}
Fe^{3+} + O_2 & \rightarrow Fe^{2+} + O_2 \\
Fe^{2+} + H_2O_2 & \rightarrow Fe^{3+} + OH^- + •OH \quad \text{Fenton reaction} \\
O_2 + H_2O_2 & \rightarrow O_2 + OH^- + •OH \quad \text{Haber-Weiss reaction}
\end{align*}
\]

**Free radicals and brain**

It is well known that intensive stress response results in creation of reactive oxygen species (ROS), e.g. hydrogen peroxide ($H_2O_2$), hydroxyl radical (•OH) and superoxide anion radical ($O_2^-$) that cause lipid peroxidation, especially in membranes and can play an important role in tissue injury. Since lipid peroxidation is a self-propagating chain reaction the initial oxidation of only a few lipid molecules can result in significant tissue damage and disease. The high susceptibility of Na⁺/K⁺-ATPase to oxidative stress leading to inactivation in various tissues and particularly in brain neurons has also been reported (Lehotsky et al., 1999). Thus, the LPO level indicates not only the membrane changes but
also alteration in the cellular functions. Injury of the substantia nigra by free radicals reduces the concentration of its main neurotransmitter, dopamine (Grahnen et al., 1992; Kurth et al., 1993). The membrane injury causes disruption of the tissue integrity (Bagchi et al., 1999). Brain is the target for different stressors because it contains large amounts of polyunsaturated fatty acids, which are particularly vulnerable to free radical attacks (Gutteridge, 1995).

**Free radical scavenging systems**

Antioxidant and free radical scavenging systems exist in the cell to protect it against the damaging effects of free radicals produced as a part of normal cell respiration and other cellular processes (Kaplowitz et al., 1996). Biological systems have evolved with endogenous defense mechanisms to help protect against free radical induced cell damage. In order to neutralize ROS, the body uses enzymatic copper, zinc-superoxide dismutase (Cu,Zn-SOD), catalase (CAT) and selenium dependent glutathione peroxidase (Se-GSH-Px) and non-enzymatic (reduced glutathione) antioxidants. Glutathione, ascorbic acid, alpha-tocopherol, betacarotene, bilirubin, selenium, NADPH, butylhydroxyanisole (BHA), mannitol, benzoate, histidine peptide, the iron-bonding transferrin, dihydrolipoic acid, reduced CoQ10, melatonin, uric acid, and plasma protein thiol, etc., as a whole play a homoeostatic or protective role against ROS produced during normal cellular metabolism and after active oxidation insult. Oxygen radicals can attack proteins, nucleic acids and lipid membranes, thereby disrupting cellular functions and integrity.

The first line of defence against these products is Super oxide dismutase (SOD). The function of this enzyme is to convert two superoxide radical molecules into oxygen and hydrogen peroxide (Fridovich, 1975). Hydrogen peroxide is handled by several mechanisms. Catalase causes direct break down of
hydrogen peroxide to oxygen and water. Another mechanism of \( \text{H}_2\text{O}_2 \) detoxification is its reduction by reduced GSH catalyzed by the enzyme GP\( \text{x} \). This enzyme not only protects against hydrogen peroxide, but also has the ability to react with lipid peroxides (Comporti et al., 1973). The increased SOD activity is, therefore, an indication that the brain’s antioxidant machinery is activated in response to excessive generation of free radicals (Bønnmister et al., 1987). Rosenbaum et al. (1994) reported a similar increase in hypoxic ischemic cells and suggested a protective role for SOD.

GP\( \text{x} \) is a major antioxidant enzyme in many tissues and speculated to play a major role in the antioxidant mechanism of the brain (Barlow-Walden, 1995). The effectiveness of GP\( \text{x} \) activity is more due to its placement in both mitochondria and cytosol. According to Dringen and Hamprecht (1997) reported that catalase and GP\( \text{x} \) can substitute for each other, whereas according to Aebi (1984) the limitation of CAT activity is due to its location at microperoxisomes.

**Glutathione system**

The tripeptide glutathione (GSH, g-L-glutamyl-L-cysteinylglycine) is the cellular thiol that can function both as reductant in the metabolism of hydrogen peroxide and organic hydro peroxides (Cotgreave and Gerdes, 1998). GSH reacts directly with radicals in non enzymatic reactions (Sac\( \text{r} \) et al., 1990, Winterbourn and Metodiewa, 1994) and is also an electron donor in the reduction of peroxides catalyzed by GP\( \text{x} \) (Chance et al., 1979). During glutathione peroxidase-catalysed metabolism of hydro peroxides, GSH serves as an electron donor and GSSG formed in the reaction is subsequently reduced back to GSH by glutathione reductase. When an individual is exposed to high levels of xenobiotics, more glutathione is utilized for conjugation making it less available to serve as an antioxidant. It also maintains ascorbate (vitamin C) and alpha-tocopherol
(vitamin E), in their reduced form, which also exert an antioxidant effect by quenching free radicals.

Glutathione directly quenches ROS such as lipid peroxides. It appears that this glutathione system is mostly provided by the astroglial cells. Even at a cellular ratio of 1 astroglial cell to 20 neurons a significant protection against H₂O₂ toxicity towards neurons has been observed (Desaghe et al., 1996) Neurons in culture become damaged by extracellular ROS (Drukarch et al., 1998) which can be detoxified by astroglial cells. Since the analysis of whole brain tissue was taken for the current study, it is clear that noise stress also could affect the glial cell functions.

When oxy radicals are present in large amounts, GSSG formation exceeds its clearance and the ratio of reduced to oxidized glutathione (GSH/GSSG) decreases. Due to this, inter relation it is always better to consider the GSH and GSSH together to draw a meaningful conclusion. Hence, this ratio is frequently used as an indicator of the level of oxidative stress in cells. Storey (1996) also suggests that the ratio of reduced / oxidized glutathione in the cell is a good indicator of the level of oxidative stress.

**MeOH induces free radicals**

MeOH is metabolized by three enzyme systems, namely the alcohol dehydrogenase system, the catalase peroxidative pathway and the microsomal oxidizing systems. Among these, microsomal oxidizing system is reported to be responsible for free radical generation (Goodman and Tephyly, 1968). The metabolism of MeOH shows many similarities to the metabolism of ethanol. Oxygen free radicals are generated during MeOH exposure induced the liver injury, as similar to ethanol (Skrzydlewska et al., 2000). It is known that the metabolism of ethanol is accompanied by the formation of reactive forms of
oxygen (Kato et al., 1990) Formic acid is able to reduce Schiff bases to amines in vitro by means of a free-radical mechanism (Bianchi et al., 1997) confirmed that free-radical reactions may really take place in MeOH poisoning. This was substantiated by the demonstration of enhanced generation of free radicals during MeOH metabolism due their presence in the bile, liver, and urine of poisoned rats (Kadńska and Mason, 2000, Skrzyllew ska et al., 2000). The first ESR evidence of MeOH-derived free radical generation in an animal model of acute MeOH intoxication was reported by (Kadńska and Mason, 2000).

Exposure to MeOH causes oxidative stress by altering the oxidant/antioxidant balance in lymphoid organs of the rat (Parthasarathy et al., 2006). Inactivation of glutathione-related enzymes in liver, erythrocytes and serum of rats after MeOH intoxication was reported by Skrzyllew ska and Farbiszews ki (2004). It is also known that the toxicity of MeOH is partially due to its metabolite formate. Cell death due to formate is believed to result from inhibition of the mitochondrial electron transport chain which reduces ATP to levels too low to perform essential cell functions dependent upon aerobic metabolism, and which increases reactive oxygen species (ROS) secondary to electron transport chain blockade, subjecting cells to increased oxidative stress (Treichel et al., 2003).

This shows that MeOH affect all organs as it easily distributed throughout the body. The perusal of literature reveals little information on MeOH induced free radicals in brain. Hence the next member of the alcohol series ethanol was considered.

**Ethanol induced brain free radicals**

Alcoholic patients develop not only liver lesions but also brain damage, cardiomyopathy, and skeletal muscle myopathy (Fromenty and Pessayre, 1995; Neiman, 1998) and moreover ethanol-induced oxidative damage to mitochondrial...
proteins, lipids, and DNA may play an important role in the pathogenesis of these extra-hepatic alcoholic lesions. Acute exposure to ethanol inhibit cytochrome P-450 dependent mixed function oxidases, which are effective in metabolizing exogenous chemicals, or it may disturb the lipid bilayer membrane (Hoyumpa and Schenker, 1982). Chronic ethanol consumption leads to direct or indirect changes in the viability of central nervous system cells via oxidative stress and ethanol metabolism also causes oxidative degradation of the mitochondrial genome in brain, heart, and skeletal muscles. These effects could contribute to the development of (cardio-) myopathy and brain injury in some alcoholic patients (Mansouri et al., 2001).

An acute ethanol load has been reported to enhance lipid peroxidation in the cerebellum and further the ethanol-induced decrease in the main antioxidant systems (superoxide dismutase, α-tocopherol, ascorbate and selenium) is a likely contributor to the cerebellar oxidative stress (Nordmann et al., 1990). Agar et al. (2004) suggested that the cerebral hemispheres of adult rats are susceptible to the oxidative neurotoxic effects of ethanol as indicated by the increased level of lipid peroxidation and decrease GSH in both hemispheres. Ethanol metabolism also causes oxidative degradation of the mitochondrial genome in brain and vitamin E or melatonin efficiently prevented ethanol-mediated mitochondrial DNA depletion not only in mouse liver, but also in brain, heart, or skeletal muscles (Mansouri, 2001). This report supports the supplementation therapy that could be useful for such situations.

**Free radical induced damages**

The basic source of free radicals production is the respiratory chain and research done on free radical induced or mediated processes in the living systems observed that molecules having unpaired electrons on their outmost molecular
orbital may induce such oxido-reductive cascade mechanisms that can damage protein, nucleic acid and fat molecules of the living cells. These molecules are called free radicals (FR) (Del Maestro, 1980, Demopoulos, 1973; Pryor, 1973; Pacifici and Davies, 1991) The extent to which \( \cdot \mathrm{O}_2 \) and \( \mathrm{H}_2\mathrm{O}_2 \) cause tissue injury independently of their participation in \( \cdot \mathrm{OH} \) formation is uncertain. Free radical induced damages most easily occur in the fatty acids of the biological membranes especially in the polyunsaturated fatty acids (PUFA) During the oxidation of these, the structure of the membranes is partially or totally disrupted; cells undergo degeneration and may die while different catabolic enzymes and mediators are released into the interstitial space and to the circulation, which by reaching other organs can worsen the basic processes (Pryor, 1973, 1982). Free radical induced damage of membrane lipids is called lipid peroxidation

**Free radical damages in brain**

Free radicals are highly reactive molecules that essentially modify phospholipids, nucleic acids, proteins (Floyd and Carney, 1992) and involvement of free radicals in excitotoxicity and neuronal apoptosis have already been reported (Schulz et al., 1995; Andrieu-Abadie et al., 2001). The brain is especially vulnerable towards the accumulation of oxidative stress induced damage (Halliwell, 1992). Although the brain accounts for less than 2% of the body weight it consumes 20% of the basal oxygen uptake. High oxygen consumption is linked to leakage of electrons along the respiratory chain with subsequent radical formation. A second reason is the high amount of PUFAs present in neuronal membranes. These PUFAs are especially prone to undergo lipid peroxidation reactions resulting in the formation of cytotoxic aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (Smith et al., 1999).
Injury to brain cells may release iron ions, which lead to oxidative stress via the iron-catalysed formation of ROS (Bauer and Bauer, 2002). Alcohol-induced increases in the levels of free iron in the cell (i.e., iron that is not bound to various proteins), which can promote ROS generation. Chronic alcohol consumption has been shown to increase iron levels in the body not only when iron-rich alcoholic beverages, such as red wine, are consumed, but also because chronic alcohol consumption enhances iron absorption from food (Nanji and Hiller-Sturmhofel, 1997).

In addition, those brain regions that are rich in catecholamines are exceptionally vulnerable to free radical generation. The catecholamine adrenaline, noradrenaline, and dopamine can spontaneously break down (auto-oxidise) to free radicals, or can be metabolized to radicals by the endogenous enzymes such as MAO (monoamine oxidases). One such region of the brain is the substantia nigra, where a connection has been established between antioxidant depletion (including GSH) and tissue degeneration (Perry et al., 2002).

The elevated release of ROS and RNS significantly contributes to the pathological changes observed in neurodegeneration (Halliwell, 1992). Neuropathologically, these are characterised by abnormalities of relatively specific regions of the brain and specific populations of neurons. Decreased levels of antioxidant enzyme activity have been reported inpatients with Parkinson’s disease (Spina and Cohen, 1989). Similar increase in markers of oxidative stress, have also been reported in Alzheimer’s disease and Huntington’s disease (Saggu et al., 1989). Adams et al. (1991) also reported that increase in free radical production is responsible for the cause of several neurological disorders such as Parkinson’s disease and Alzheimer’s disease.
Exogenous antioxidants: Contribution from the diet

Evidence supports that fruits and vegetables containing generous amounts of antioxidant nutrients are important for neurological function. Rats supplemented with strawberry, spinach, or vitamin E showed a significant enhancement in striatal dopamine release indicating that other nutrients present in fruits and vegetables, in addition to the well-known antioxidants, may be important for brain function (Martin et al., 2000). The most widely studied dietary antioxidants are vitamin C, vitamin E, and beta-carotene. Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids, as it is capable of neutralizing ROS in the aqueous phase before lipid peroxidation is initiated. Vitamin E is a major lipid-soluble antioxidant, and is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation. Beta-carotene and other carotenoids also provide antioxidant protection to lipid-rich tissues.

Unlike most other antioxidant nutrients which work in either the fatty parts of the body (including the outer layers of cells), or the watery parts (including the blood), lipoic acid works in both. From the fore going, it can, thus, be stated: “A single antioxidant is not an antioxidant” (Tulok and Matkovics, 1997). More over, the central nervous system (CNS) is extremely sensitive to free radical damage because of a relatively small total antioxidant capacity. Hence, the supplementation must be quite useful for the brain to prevent the oxidative stress and the choice is LA.

α-Lipoic acid

LA acid was discovered in 1951, it serves as a coenzyme in the Krebs cycle and in the production of cellular energy. In 1957, LA was isolated and characterized as a compound found in yeast extracts (Figure 4). LA is an
Lipoic acid or α-lipoic acid (alpha lipoic acid) has formula $C_8H_{14}S_2O_2$
Systematic name: 5-(1,2-dithiolan-3-yl)pentanoic acid.
Dihydrolipoic acid or reduced lipoic acid has formula $C_8H_{16}S_2O_2$
Systematic name 6,8-disulfanyloctanoic acid & also called lipoic acid.
Antioxidant found in certain foods, including red meat, spinach, broccoli, potatoes, yams, carrots, beets, and yeast. LA is readily absorbed from the diet, transported, taken up by cells, and reduced to dihydrolipoate in various tissues including brain (Panigrahi et al., 1996; Podda et al., 1994). LA exists as two enantiomers, the R-enantiomer and the S-enantiomer. Normally only the R-enantiomer [R (+)] of an amino acid is biologically active, but for lipoic acid the S-enantiomer can assist in the reduction of the R-enantiomer when a racemic mixture is given. The S-enantiomer [S (-)] in fact has an inhibiting effect on the R-enantiomer, reducing its biological activity substantially and actually adding to oxidative stress rather than reducing it. More importantly, studies show that R (+) is taken up into the tissues to a greater degree than the S (-) form. The vast tissue distribution of the reductive enzymes contributes also to the high clearance of LA.

Exogenously supplied LA is readily taken up by a variety of cells and tissues in which it is rapidly reduced by NADH- or NADPH-dependent enzymes to dihydrolipoate (DHLA) (Packer et al., 1995). Further, DHLA is known to regenerate major physiological antioxidants of lipid and aqueous phases, such as vitamin E, ascorbate, and GSH (Packer et al., 1995).

LA is an essential cofactor in the multienzyme complexes of α-ketoacid dehydrogenases such as pyruvate, α-keto-glutarate and branched chain α-ketoacid-dehydrogenases (Schmidt et al., 1965). LA also acts as a coenzyme for mitochondrial pyruvate dehydrogenase and a-ketoglutarate dehydrogenase (Hagen et al., 1999).

**Antioxidant property of LA**

In the late 1980s, researchers realized that alpha-lipoic acid had been overlooked as a powerful antioxidant. LA, a metabolic antioxidant, functions as a redox modulator of cells and has proven clinically beneficial effects. LA is also a
Dietary supplement and is readily absorbed. In cells, it is enzymatically reduced to dihydrolipoate (DHLA) which is a strong reductant. LA/DHLA can directly scavenge a number of free radicals and regenerate oxidized forms of vitamin E, C, glutathione and thioredoxin and hence, it is a strong antioxidant (Packer et al., 1995). Several qualities distinguish LA from other antioxidants, and Packer has described it at various times as the "universal," "ideal," and "metabolic" antioxidant as it neutralizes free radicals in both the fatty and watery regions of the cells. In other words, it can counter many different forms of oxidative stress and prevent the cellular damage they might cause. Both (LA/DHLA) forms of lipoic acid quench peroxynitrite radicals, an especially dangerous type consisting of both oxygen and nitrogen, according to a recent paper in HBS Letters (Whiteman et al., 1996). It also protects membranes by interacting with vitamin C and glutathione, which may in turn recycle vitamin E (Packer, 1992). It can interact with reactive oxygen species (ROS), such as superoxide radicals, hydroxyl radicals, hypochloric acid, peroxyl radicals, and singlet oxygen (Biewenga et al., 1997). LA is an antioxidant, which penetrates cell membrane and can act intracellularly (Bierhaus et al., 1997).

Neuroprotective properties of the substance, e.g., a decrease of the effect of neurotoxins, have been demonstrated in cell culture systems (Loske et al., 1998; Packer et al., 1997). Acrylamide induced neurotoxicity by increased neural peroxidative status was reduced by LA (Anuradha and Varalakshmi, 1999). It is especially promising antioxidant acting against mitochondrial dysfunction, due to its intimate role in metabolism. Interestingly, α-lipoic acid, vitamin E, and coenzyme Q10 are being tried in the treatment of inborn or drug-induced mitochondrial cytopathies (Antozzi et al., 1997; de la Asuncion et al., 1998). Sonambi et al. (2000) suggest that the graded doses of lipoic acid effectively prevented a decrease in the renal antioxidant defense system and prevented an
increase in lipid peroxidation, platinum content induced by cisplatin and plasma creatinine concentrations in a dose-dependent manner

**Action at various organs**

Exhaustive exercise significantly increased thiobarbituric acid-reactive substance levels in the liver and red gastrocnemius muscle of the albino rat whereas LA supplementation protected against oxidative lipid damage in the heart, liver, and red gastrocnemius muscle (Khanna et al., 1999). Arend et al. (2000) reported that dietary LA may prevent the suppression of liver wound healing induced by n-3 PUFAs.

**LA and Glucose metabolism**

Glucose transporters GLUT-1 to GLUT-7 are reported (Ganong, 2001) to be responsible for the facilitated diffusion of glucose across membrane and each evolved for special task and hence differ from each other in their mode of action. Particularly a pool of GLUT-4 molecules is maintained in vesicles in the cytoplasm of insulin sensitive cells GLUT-4 is present as transporter in muscle in adipose tissue. LA also has been shown in cell culture experiments to increase cellular uptake of glucose by recruiting the glucose transporter GLUT-4 to the cell membrane, there by recommending its use in diabetes (Henriksen, 2006; Packer et al., 2001). Furthermore, the S-enantiomer was also found to reduce the expression of GLUT-4 in cells, responsible for glucose uptake, and hence reduce insulin sensitivity (Loffelhardt et al., 1995). This is further, strengthened by the Jain and Lim (1998) report. Insufficient Na⁺/K⁺ ATPase activity has been suggested as a contributing factor in the development of diabetic neuropathy. In an *in-vitro* study, LA supplementation has been found to be beneficial in preventing neurovascular abnormalities in diabetic neuropathy by significantly blocking the reduction in activities of Na⁺/K⁺ ATPases in the high glucose - treated RBCs.
There was a decrease in lipid peroxidation, and protein glycosylation was also observed in these RBCs (Jam and I.m. 1998, 2000)

According to Jacob et al (1995) insulin resistance of skeletal muscle glucose uptake is a prominent feature of Type II diabetes (NIDDM) and acute parenteral administration of I A to such diabetic patients resulted with a significant increase in insulin-stimulated glucose disposal Insulin resistance of muscle glucose metabolism is a hallmark of non insulin dependent diabetic mellitus.

Further, Jacob et al (1996) reported that Chronic I A treatment to obese Zucker rat (an animal model of muscle insulin resistance) increased both insulin-stimulated glucose oxidation (33%) and glycogen synthesis (38%) and it was also associated with a significantly greater (21%) in vivo muscle glycogen concentration. These adaptive responses after chronic I.A administration were also associated with significantly lower (15-17%) plasma levels of insulin and free fatty acids. No significant effects on glucose transporter (GLUT4) protein level or on the activities of hexokinase and citrate synthase were observed. All these clearly revealing the additional advantages of I.A apart being an antioxidant

Toxic levels and 1.D₅₀

Oxidative stress causes metabolic and structural abnormalities during cerebral ischemia-reperfusion and LA may act in a dual way, protecting from ischemia at lower concentrations (3.03 mM) and worsening this process at higher doses (60.06mM) (Cardoso do Vale et al., 2003). Oral LA produced hepato cellular toxicity and maximum tolerated dose was < 30 mg/kg in cats as LA is 10 times more toxic in cats than reported in humans, dogs or rats (Hill et al., 2004). The lowest reported dosage to exhibit toxic side-effects was 0.83 mg/kg (total dose less than 1/20 gram). The oral LD₅₀ for experimental rodents was found to be between 500 and 1000 mg/kg.
Other defensive system in the Brain

Heat Shock Protein (Hsp)

Heat shock proteins represent several families of cellular stress-response proteins, some of which are expressed constitutively and others expressed largely under conditions of stress (Welch, 1992). Heat-shock proteins are so-called chaperones. This term is used to classify these proteins because they help the long chains of amino acids correctly fold into shape as functioning proteins so they do not clump together. Hsp also reported to play a role in the assembly and/or disassembly of other proteins, as well as to facilitate the translocation and insertion of other proteins into membranes of cytoplasmic organelles (Cheng et al., 1989; Chirico et al., 1988, Deshaies et al., 1988). The well-characterized program of gene expression, leading to the synthesis of Hsps exerts cytoprotective functions (Sorger, 1991). Hsp70 gene encodes a major stress-inducible heat shock protein (Hsp70), which plays an important role in protecting cells from deleterious stresses. The well-characterized program of gene expression, leading to the synthesis of Hsps exerts cytoprotective functions (Sorger, 1991).

Several reports suggest that inducible Hsp70 has a protective function in the CNS (Lowenstein et al., 1991; Rordorf et al., 1991) and over expression of Hsp70 has been shown to be neuroprotective (Akbar et al., 2001). Specifically, over expression of Hsp70/72 in transgenic mice (Plumier et al., 1995) and in rats using viral vectors (Yenari et al., 1998) was related to the increased resistance to ischemic injury. In addition, augmenting the expression of Hsp70 in cell culture increased the tolerance to glutamate toxicity (Lowenstein et al., 1991) and stress-induced apoptosis (Mosser et al., 1997).
Marker of Apoptosis

Many mitogen activated protein (MAP) kinases are highly expressed within the central nervous system, particularly neurons, suggesting an important role in brain function. Three subclasses MAP kinase that plays an essential role within a number of intracellular signal transduction pathways (Davis, 1994; Davis, 2000; Pulverer et al., 1991) Vertebrate cells contain multiple MAP kinase pathways, of which the three best characterized terminate in the extra cellular-signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38MAP kinases. Individual MAP kinase subfamilies are activated by distinct stimuli; the ERK pathway is stimulated primarily by growth factors and trophic signals, whereas the JNK and p38 pathways are activated most potently by inflammatory cytokines and a variety of chemical and radiant stresses (Davis, 1994, Pulverer et al., 1991).

Powerful and rapid induction of selected MAP kinase phosphatases during neuronal differentiation (Camps et al., 1998) or following excitotoxic injury (Boschert et al., 1997, 1998) is also consistent with an important role for MAP kinases controlling brain functions under both normal and pathological conditions.

Neuronal apoptosis is regulated by a complex series of molecular events, which in many cases culminate in activation of the conserved mitochondrial pathway of death characterized by the activation of the apoptosome. Numerous signals, which activates this conserved death pathway. Increasing evidence indicates that one such regulatory signal may involve activation of the c-Jun N-terminal kinases (JNKs) (Tournerier et al., 2000). JNK isoforms encoded by different genes, JNK1, JNK2 are present in most tissues, whereas JNK3 is selectively expressed in the nervous system and in the heart (Martin et al., 1996; Gupta et al., 1996). C-Jun activation has a clear role in proliferation (Johnson et al., 1993) and differentiation (Hilberg et al., 1993). In neuronal systems, a large
and growing body of evidence suggests that the JNK/c-Jun pathway can function in a pro-apoptotic manner. The functional diversity of JNK isoforms and suggested that JNK3 may have a preferential role in stress-induced neuronal apoptosis (Kuan et al., 2003).

JNK3 is predominately expressed in the brain and is most consistently associated with neuronal death. The first reports demonstrating the importance of c-Jun in neuronal death in sympathetic neurons induced by nerve growth factor. It is important to emphasize that in some instances of neuronal injury, sustained c-Jun induction/activation does not necessarily lead to death (Herdegen et al., 1998, Raivich et al., 2004). Activation of JNK was shown to mediate excitotoxic and ischemic apoptosis in cerebral neurons (Kuan et al., 2003; Borsello et al., 2003). c-Jun amino terminal kinase (JNK) is one of the kinds, which targets specific transcription factors such as c-Jun, ATF-2 and NFkB (Crowe and Shemirani, 2000). Therefore, JNK is believed to be associated with the cellular machinery of stress activated responses that, under certain conditions, can induce cell death. Dual-specificity phosphatases (Xia et al., 1995), and other JNK repressors that can also be induced by the apoptosis inducing agents. Cell death response is provided by the recent observation that JNK is involved in the coupling of DNA damage to mitochondrial cytochrome c release in fibroblasts (Tourner et al., 2000).

A complex of proteins, describing the coupling and activation centre, has been conceptualised as an 'apoptosome', and contains the precursors of the proteolytic enzymes (caspases) that cleave key cellular proteins to generate the apoptotic morphology (Chinnaiyan and Dixit, 1997). Hippocampal neurons in Jnk3 knockout mice do not undergo excitotoxin-induced apoptosis (Yang et al., 1997). According to MacKeigan et al. (2000) drug induced apoptosis may partly
due to tilting of both sides of the balance, by both activating death signals and inhibiting survival signals.

**Regulation**

JNK signaling also targets the mitochondria and regulates the release of cytochrome c (Tourner et al., 2000, Kharbanda et al., 2000; Lee et al., 2002). Activated JNK translocates into nuclear and phosphorylates the transcription factor c-Jun. The phosphorylation leads to increase of activator-protein 1 (AP-1) transcription activity to modulate transcription of a number of genes, which lead to apoptosis (Guan et al., 2006). An antioxidant reagent, effectively inhibited JNK3 activation at 30 min reperfusion, which suggested that 30 min activation of JNK3 was related to the generation of free radicals during the early reperfusion (Tian et al., 2003). The JNK pathway is also negatively regulated by HSPs. Furthermore, apoptosis itself may act as a signal for JNK activation in some circumstances (Mosser et al., 1997).

The alterations in enzymatic and non-enzymatic antioxidant defense systems along with protein oxidation and lipid peroxidation are markers in the brain to understand the free radical changes. There are several studies that are related to the effects of stress on the antioxidant system and induction of lipid peroxidation in brain after various stress exposure models (Madrigal et al., 2001). Manoli et al. (2000) and Baek et al. (1999) concluded in their study that the vulnerability to oxidative stress in the brain is region specific. Based on this different brain regions were chosen for this study instead of whole brain changes as whole brain analysis may mask this regional specific action of MeOH.