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1.1: Mitochondria

In cell biology, a mitochondrion is a membrane-enclosed organelle found in most eukaryotic cells (Henze and Martin, 2003). Mitochondria are sometimes described as "cellular power plants" because they generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy (Campbell et al., 2006). Several characteristics make mitochondria unique. The number of mitochondria in a cell varies widely by organism and tissue type. Many cells have only a single mitochondrion, whereas others can contain several thousand mitochondria (Voet et al., 2006). A single somatic cell can contain from 200 to 2000 mitochondria (Veltri et al., 1990), while human germ cells such as spermatozoa contain a fixed number of 16 mitochondria and oocytes have up to 100,000 (Szewczyk and Wojtczak, 2002). The largest number of mitochondria is found in the most metabolically active cells, such as skeletal and cardiac muscle and the liver and brain (Cohen and Gold, 2001).

The organelle is composed of compartments that carry out specialized functions (Fig.1.1.A). These compartments or regions include the outer membrane, the intermembrane space, the inner membrane, and the cristae and matrix. Mitochondrial proteins vary depending on the tissue and the species. Although most of a cell's DNA is contained in the cell nucleus, the mitochondrion has its own independent genome. Further, its DNA shows substantial similarity to bacterial genomes (Andersson et al., 2003). Mitochondria produce more than 90% of our cellular energy by OXPHOS (Chance et al., 1979). Energy production is the result of two closely coordinated metabolic processes- the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC). The TCA cycle converts carbohydrates and fats into some ATP, but its major job is producing the coenzymes nicotinamide dinucleotide (NADH) and flavin adenine dinucleotide (FADH) so that they, too, are entered into the ETC.

1.1.1 Functions

The most prominent roles of mitochondria are to produce ATP (i.e., phosphorylation of ADP) through respiration, and to regulate cellular metabolism (Voet et al., 2006). The central set of reactions involved in ATP production is collectively known as the Krebs Cycle (TCA cycle). However, the mitochondrion has many other functions in
Fig. 1.1. A. Structure of mitochondria

![Mitochondria Diagram](image)

Fig. 1.1. B. Major functions of mitochondria

![Mitochondrial Functions Diagram](image)
Fig. 1.2 A. Tricarboxylic acid cycle
addition to the production of ATP such as signaling, cellular differentiation, as well as the control of the cell cycle and cell growth (McBride et al., 2006) (Fig.1.1.B). Mitochondria have been implicated in several human diseases, including mitochondrial disorders (Gardner and Boles, 2005) and cardiac dysfunction (Lesnefsky et al., 2001), and may play a role in the aging process. Mitochondria play a vital role in cellular homeostasis. They house the OXPHOS machinery, and multiple metabolic pathways, such as β-oxidation of fatty acids and the TCA and urea cycles. In addition, mitochondria have important biosynthetic activities, control intracellular Ca\(^{2+}\) metabolism and signaling, regulate thermogenesis, generate cellular reactive oxygen species (ROS) and serve as the gatekeeper of the cell for programmed cell death (apoptosis) (Scheffler, 2001). Again, mitochondria are primarily responsible for meeting the enormous energy demands of the ‘fight and flight response’ in vital tissues, by oxidizing the large amounts of substrates that are made available by stress hormone-induced mobilization from energy storages.

A rapidly expanding body of literature also suggests that mitochondrial dysfunctions play pivotal roles in neurodegenerative disorders ranging from Parkinson’s to Huntington’s to Alzheimer’s diseases. Mitochondrial DNA mutations, whether inherited or acquired, cause impaired respiratory chain functioning. This, in turn, leads to decreased production of ATP, formation of free radicals, and alterations in cellular calcium handling. These events may initiate damages to mitochondrial DNA, proteins, and lipids, and opening of the mtPTP transition pore, an event linked to apoptotic cell death. Mitochondria are also targets for drugs such as antidiabetic sulfonylureas, immunosuppressants, some antilipidemic agents, etc.

1.2 The TCA cycle

The TCA cycle is frequently called the Krebs cycle because Sir Hans Krebs first formulated its reactions into a cycle. The most common name for this pathway, the tricarboxylic acid or TCA cycle, denotes the involvement of the tricarboxylates citrate and isocitrate. It is also called the “citric acid cycle” because citrate was one of the first compounds known to participate. This cycle is of central importance in all living cells that use oxygen as part of cellular respiration. In eukaryotic cells, the citric acid cycle occurs in the matrix of the mitochondrion (Fig.1.2). The major pathways of fuel oxidation generate acetyl CoA, which is the substrate for the TCA cycle.
In the first step of the TCA cycle, the acetyl portion of acetyl CoA combines with the 4-carbon intermediate oxaloacetate to form citrate (6 carbons), which is rearranged to form isocitrate. In the next two oxidative decarboxylation reactions, electrons are transferred to NAD to form NADH, and 2 molecules of electron-depleted CO2 are released. Subsequently, a high-energy phosphate bond in GTP is generated from substrate level phosphorylation. In the remaining portion of the TCA cycle, succinate is oxidized to oxaloacetate with the generation of one FAD (2H) and one NADH. The net reaction of the TCA cycle, which is the sum of the equations for individual steps, shows that the two carbons of the acetyl group have been oxidized to two molecules of CO2, with conservation of energy as three molecules of NADH, one of FAD (2H), and one of GTP.

1.2.1. Dehydrogenases of TCA cycle and their importance

1.2.1.1. Isocitrate dehydrogenase (ICDH)

Isocitrate dehydrogenase (EC 1.1.1.42) and (EC 1.1.1.41), also known as ICDH, is an enzyme that participates in the citric acid cycle. It catalyzes the third step of the cycle: the oxidative decarboxylation of isocitrate, producing alpha-ketoglutarate (α-ketoglutarate) and CO2 while converting NAD+ to NADH. This is a two-step process, which involves oxidation of isocitrate (a secondary alcohol) to oxalosuccinate (a ketone), followed by the decarboxylation of the carboxyl group beta to the ketone, forming alpha-ketoglutarate. Mammalian tissues contain two isoforms of this enzyme. One exists entirely in mitochondria, and the other exists in mitochondria and also in cytosol. Both the forms catalyze the same reaction (Corpas et al., 1999). ICDH provides the first connection between the TCA cycle and the electron transport pathway and OXPHOS, via its production of NADH.

Isocitrate + NADP+ Mg2+ (metal ion) → alpha-ketoglutarate + NADPH + H+ + CO2

1.2.1.2. Alpha-Ketoglutarate dehydrogenase (α-KGDH)

α-ketoglutarate dehydrogenase complex is an enzyme complex, composed of three components (1) oxoglutarate dehydrogenase (EC 1.2.4.2) (2) dihydrolipoyl succinyltransferase (EC 2.3.1.61) and (3) dihydrolipoyl dehydrogenase (EC 1.8.1.4) that employs five different coenzymes
Fig. 1.3. Generation of ROS by α-KGDH.

Subunit composition of α-KGDH and generation of ROS in the physiological forward reaction of the enzyme, when substrates and cofactors and oxygen are present (a). ROS generation in the forward reaction by α-KGDH is enhanced in the absence of NAD+ (b). ROS generation by the E3 subunit induced by NADH in the absence of substrates (c). E1, α-KGDH; E2, dihydrolipoamide succinyltransferase; E3, dihydrolipoamide dehydrogenase; TPP, thiamine pyrophosphate.
This enzyme catalyzes the oxidative decarboxylation of an alpha-keto acid, releasing the citric acid cycle’s second CO$_2$ and NADH.

\[
\alpha\text{-ketoglutarate} + \text{NAD}^+ + \text{CoA} \rightarrow \text{Succinyl CoA} + \text{CO}_2 + \text{NADH}
\]

There are a number of features that makes this enzyme distinct from other enzymes important in the bioenergetic processes. First of all, it is highly regulated and is the primary site of control of the metabolic flux through the Krebs cycle (Hansford, 1980). The mammalian enzyme is inhibited by its end products, succinyl-CoA and NADH (Smith et al., 1974). $\alpha$-KGDH could be a crucial target of ROS in cells and, being an important regulatory site in the mitochondrial metabolism, could play a key role in the bioenergetic deficit evolving in oxidative stress. On the other hand, it has been revealed recently that the enzyme itself is able to generate ROS (Starkov et al., 2004; Tretter and Adam-Vizi, 2004), therefore could contribute to the induction of oxidative stress (Fig. 1.3). $\alpha$-KGDH appears to be more sensitive to disturbed homeostatic factors than other enzymes (Mastrogiacomo et al., 1993).

1.2.1.3. Succinate dehydrogenase (SDH)

This enzyme (EC 1.3.99.1) catalyzes stereospecific dehydrogenation of succinate to fumarate. The enzyme is strongly inhibited by malonate, a structural analog of succinate and an example of competitive inhibitor. The enzyme contains an FAD, the enzyme’s electron acceptor. SDH is part of the succinate-coenzyme Q reductase of the ETC. In contrast with all of the other enzymes of the TCA cycle, which are soluble proteins found in the mitochondrial matrix, SDH is an integral membrane protein tightly associated with the inner mitochondrial membrane. SDH is a dimeric protein, with subunits of molecular masses 70 kD and 27 kD. It is the only enzyme that participates in both the citric acid cycle and the electron transport chain (Oyedotun and Lemire, 2004).

1.2.1.4. Malate dehydrogenase (MDH)

Malate dehydrogenase (EC 1.1.1.37) (MDH) catalyzes the conversion of malate into oxaloacetate (using NAD$^+$) and vice versa (this is a reversible reaction).

\[
\text{L-Malate} + \text{NAD}^+ \leftrightarrow \text{oxaloacetate} + \text{NADH} + \text{H}^+
\]

Several isozymes of MDH exist, depending on where they are localized in the cell and their specific dependence on NAD$^+$ or NADP$^+$ (only in chloroplasts). There
are two main isoforms in eukaryotic cells (Minarik et al., 2002). One is found in the mitochondrial matrix participating as a key enzyme in the citric acid cycle that catalyzes the oxidation of malate. The other is found in the cytoplasm, assisting the malate-aspartate shuttle with exchanging reducing equivalents so that malate can pass through the mitochondrial membrane to be transformed into oxaloacetate for further cellular processes (Musrati et al., 1998).

1.3. The Electron Transport chain and Oxidative phosphorylation

The Electron Transport Chain (ETC) and the Oxidative phosphorylation (OXPHOS) system is made up of five complexes (complex I-V) and consists of approximately 90 subunits, of which only 13 are encoded by the mtDNA. Complexes I-IV comprises the ETC. Oxidation of carbohydrates in the TCA cycle and lipids via \( \beta \)-oxidation generate the electron carriers NADH and FADH\(_2\), which donate electrons to the ETC. In the ETC, the transport of electrons is coupled to the generation of a proton gradient across the inner mitochondrial membrane, which is further used by the fifth enzyme complex to synthesize ATP from ADP+P\(_\cdot\) (Saraste, 1999).

1.3.1. Complex I (NADH: ubiquinone oxidoreductase; EC 1.6.99.3)

This is the largest of the enzyme complexes of the OXPHOS system consisting of 45 subunits in bovine heart, of which seven are encoded in mitochondria. The enzyme contains multiple prosthetic groups, one flavinmononucleotide (FMN) and eight iron-sulphur clusters (Carroll et al., 2006) (Fig. 1.4.A). Another common name for this enzyme complex is NADH dehydrogenase. NADH in the inner membrane space is oxidised to NAD\(^+\) transferring two electrons to the FMN moiety of complex I. The electrons are then further transferred via a series of iron-sulphur clusters to the matrix side of the inner membrane to reduce ubiquinone to ubiquinol. This transfer is coupled to the translocation of four protons across the inner membrane into the intermembrane space.

1.3.2. Complex II (Succinate ubiquionone oxidoreductase; EC 1.3.5.1)

This is the only exclusively nuclear encoded complex, oxidizes succinate to fumarate in the TCA cycle, and donates electrons to the ETC. Complex II consists of a catalytic subunit, SDH, and two membrane subunits, anchoring the complex into the inner mitochondrial membrane (Capaldi et al., 1977). Electrons from succinate are donated
Fig. 1.4.A. Reactions in Complex I

Fig. 1.4.B. Reactions in Complex II

Mitochondrial intermembrane space

Mitochondrial matrix

Fig. 1.4.C. Reactions in Complex III

Fig. 1.4.D. Reactions in Complex IV
to the covalently bounded FAD of SDH, reducing it to FADH₂. The electrons are then further transported via a number of iron/sulphur clusters to ubiquinone, reducing it to ubiquinol (Fig. 1.4.B). Complex II feeds electrons to the ETC without translocating protons across the membrane (Lancaster and Kroger, 2000). This enzyme has a mass of approximately 100 to 140 kD and is composed of four subunits: two Fe-S proteins of masses 70 kD and 27 kD, and two other peptides of masses 15 kD and 13 kD. Also known as flavoprotein 2 (FP2), it contains an FAD covalently bound to a histidine residue, and three Fe-S centers: a 4Fe-4S cluster, a 3Fe-4S cluster, and a 2Fe-2S cluster. When succinate is converted to fumarate in the TCA cycle, concomitant reduction of bound FAD to FADH₂ occurs in succinate dehydrogenase. This FADH₂ transfers its electrons immediately to Fe-S centers, which pass them on to ubiquionone (UQ). Electron flow from succinate to UQ,

1.3.3.Complex III (Ubiquinol-cytochrome c oxidoreductase; EC 1.10.2.2)

Complex III catalyzes the transfer of electrons from ubiquinol, to cytochrome c. It consists of a homodimer, with each monomer composed of eleven subunits, of which one is encoded by the mitochondrial genome (Yu et al., 1998). Ubiquinol is a two-electron carrier, whereas cytochrome c is a single electron carrier. Ubiquinol donates its two electrons consecutively to complex III, releasing two protons at the inner membrane space. One electron is transferred to cytochrome c via the Rieske iron-sulphur protein, while the second electron is transferred back to the matrix side, to cytochrome b of complex III. Cytochrome b is able to accept two electrons, which in turn, are donated to ubiquinone at the matrix side, generating ubiquinol. The reduction of ubiquinone to ubiquinol via cytochrome b also requires the removal of two protons from the matrix side, thus adding to the proton gradient of the respiratory chain. Due to the recycling of ubiquinone this process is also termed the Q-cycle (Darrouzet et al., 2001) (Fig. 1.4.C).

1.3.4.Complex IV (Cytochrome c oxidase; Ferrocytochrome c- oxidoreductase; EC 1.9.3.1)

Complex IV is a water-soluble protein that donates electrons on the cytoplasmic side of the inner mitochondrial membrane to complex IV, the final step in the respiratory chain. Complex IV is composed of 13 subunits of which three are encoded by the mtDNA. It catalyzes the transfer of electrons from the reduced cytochrome c pool to
Fig. 1.5. A. Reactions in Complex V

Fig. 1.5. B. Overview of ETC and complexes

Fig. 1.5. C. Major inhibitors on ETC
molecular oxygen, reducing it to water. In this step, four electrons have to be donated from complex IV to two molecules of oxygen, without generating any ROS (Fig. 1.4.D). This is achieved by complex IV storing the four electrons on haem and copper atoms, before releasing them only in the presence of two molecules of oxygen and four protons at the matrix side of the mitochondrial inner membrane. Additionally, four protons are translocated across the inner mitochondrial membrane during this reaction (Schultz and Chan, 2001).

1.3.5. Complex V (ATP synthase; F1F0-ATPase):

The overall outcome of the action of the ETC is the removal of protons from the matrix side, transferring them to the inner membrane space side; thus rendering the matrix side negatively charged, while storing protons on the cytoplasmic side of the inner mitochondrial membrane. This electrochemical gradient is finally utilised by the fifth component of the OXPHOS system, the Complex V, which drives the generation of ATP from ADP and P· (Saraste, 1999). Complex V is composed of a membrane-bound subcomplex (F0), a large extra-membranous complex (F1) that resides in the matrix space, and a stalk connecting the two complexes (Fig. 1.5.A). Protons from the intermembrane space are allowed to enter complex V through the F0 complex leading to subunit rotation within the enzyme complex. The energy from this rotation is then used for ATP synthesis, which takes place in the F1 complex (Schultz and Chan, 2001).

Even though the structure of each individual OXPHOS complex is fairly well known, it is unclear how the complexes are organized in relation to one another. It has been suggested that the enzyme complexes of the OXPHOS system exists in super complexes, where several complexes are organized and function together. These super complexes have been reported in bacteria, yeast and mammals (Stroh et al., 2004). Whereas ATP made in glycolysis and the TCA cycle is the result of substrate-level phosphorylation, NADH-dependent ATP synthesis is the result of OXPHOS. Electrons stored in the form of the reduced coenzymes, NADH or (FADH₂), are passed through an elaborate and highly organized chain of proteins and coenzymes, the so-called ETC, finally reaching O₂ (molecular oxygen), the terminal electron acceptor (Fig 1.5.B). Each component of the chain can exist in (at least) two oxidation
states, and each component is successively reduced and reoxidized as electrons move through the chain from NADH (or FADH₂) to O₂.

1.4. Inhibitors of Oxidative Phosphorylation

The unique properties and actions of an inhibitory substance can often help to identify aspects of an enzyme mechanism. Many details of electron transport and OXPHOS mechanisms have been gained from studying the effects of particular inhibitors. Figure 1.5.C. presents the structures of some electron transport and OXPHOS inhibitors.

1.4.1. Inhibitors of Complexes I, II, and III Block Electron Transport

Rotenone is a common insecticide that strongly inhibits the NADH-UQ reductase. Ptericidin, Amytal, and other barbiturates, mercurial agents, and the widely prescribed painkiller Demerol also exert inhibitory actions on this enzyme complex. All these substances appear to inhibit reduction of coenzyme Q and the oxidation of the Fe-S clusters of NADH-UQ reductase. 2-Thenoyltrifluoroacetone and carboxin and its derivatives specifically block Complex II, the succinate-UQ reductase. Antimycin, an antibiotic produced by *Streptomyces griseus*, inhibits the UQ-cytochrome c reductase by blocking electron transfer between b₅₆ and coenzyme Q in the Qₐ site. Myxothiazol inhibits the same complex by acting at the Qₚ site.

1.4.2. Inhibitors of Complex IV

Complex IV, the cytochrome c oxidase, is specifically inhibited by cyanide (CN⁻), azide (N₃⁻), and carbon monoxide (CO). CN⁻ and N₃⁻ bind tightly to the ferric form of cytochrome a₃, whereas carbon monoxide binds only to the ferrous form. The inhibitory actions of CN⁻ and N₃⁻ at this site are very potent, whereas the principal toxicity of CO arises from its affinity for the iron of hemoglobin.

1.4.3. Inhibitors of ATP Synthase

Inhibitors of ATP synthase include dicyclohexylcarbodiimide (DCCD) and oligomycin. DCCD bonds covalently to carboxyl groups in hydrophobic domains of proteins in general, and to a glutamic acid residue of the c subunit of F₀, the proteolipid forming the proton channel of the ATP synthase, in particular. If the c subunit is labeled with DCCD, proton flow through F₀ is blocked and ATP synthase activity is inhibited. Likewise, oligomycin acts directly on the ATP synthase. By
binding to a subunit of F0, oligomycin also blocks the movement of protons through F0.

1.5. Mitochondrial DNA

One of the unique features of mitochondria is that they contain their own genome known as mitochondrial DNA (mtDNA). The mammalian mitochondrial genome is a double-stranded, circular DNA molecule encoding 37 genes: 13 protein-encoding genes, 22 tRNA, and 2 rRNA genes, all of which are needed to form the mitochondrial ETC protein synthesis system (Linnane et al., 1998) (Fig. 1.6.A). The remainder of the ETC enzymes and other mitochondrial components are encoded by nuclear DNA (nDNA). Seven of the 13 mitochondrial proteins are subunits (ND1-6 and ND4L) of complex I, one is a subunit (cytochrome b) of complex III, three are subunits (CO I-III) of complex IV and two are subunits (ATPase 6 and 8) of complex V (Anderson et al., 1981) are encoded by mtDNA. Each cell contains multiple copies of mtDNA with up to 1000-5000 copies in somatic cells, organised in DNA-protein complexes called nucleoids (Wang and Bogenhagen, 2006). Replication of mtDNA takes place in the mitochondrial matrix and is independent of the cell cycle. In contrast to nDNA, mtDNA can be replicated several times or not at all during a cell cycle (Bogenhagen and Clayton, 1977).

1.6. Role of mitochondria in programmed cell death

Apoptosis, a special form of programmed cell death that plays an indispensable role in the development and homeostasis of multicellular organisms. Mitochondria contain several pro-apoptotic molecules that activate cytosolic proteins to execute apoptosis, block anti-apoptotic proteins in the cytosol and directly cleave nuclear DNA. Disruption of electron transport has been recognized as an early event of cell death. Mitochondrial permeability transition (MPT), a phenomenon characterized by mitochondrial swelling, uncoupling and inner membrane permeabilization to solutes of molecular mass up to 1500 Da plays an important role in initiating both apoptotic and necrotic cell death (Kim et al., 2004). MPT is implicated in lethal cell injury from anoxia, ischemia/reperfusion, and oxidative stress to many cell types including heart (Kim et al., 2003). ATP depletion caused by uncoupling of OXPHOS after the MPT leads to necrotic cell killing, where as cytochrome c release caused by mitochondrial swelling and outer membrane rupture after the MPT initiate apoptosis (Fig. 1.6.B)
Fig. 1. 6. A. Mitochondrial DNA
(adapted from MITOMAP, a web site maintained by Dr. Doug Wallace)

Fig. 1. 6. B. Cellular signals resulting in apoptosis

Fig. 1. 6. C. Role of mitochondria in programmed cell death (Apoptosis)
(Kim et al., 2004). MPT associated with changes in changes in mitochondrial membrane potential ($\Delta \Psi_{mt}$).

The intrinsic pathway of apoptosis is mediated by the activation of proapoptotic members of the Bcl-2 family proteins ie. Bax, Bak (Fig. 1.6.C). Activation of either Bax or Bak is required for apoptosis. Bax, a monomeric protein found in the cytosol, oligomerises on the outer mitochondrial membrane following apoptotic stimulus and causing mitochondrial membrane permeabilization. While Bak is an oligomeric integral mitochondrial membrane protein forms larger aggregate during apoptosis. Another Bcl-2 family protein, Bid provides crosstalk between the extrinsic (ie. death receptor pathway) and intrinsic pathways. Following this mitochondrial dysfunction, several apoptotic factors including cytochrome c (Cyt c), second mitochondria derived activator of caspase (Smac) are released from the mitochondrial intermembrane space into cytosol. Cyt C binds to the adapter apoptotic protease activating factor 1 (Apaf-1) that eventually results in the activation of cysteinyl aspartate specific proteases, caspases. Apoptosis-inducing factor (AIF), a proapoptotic mitochondrial protein is also released from mitochondria whereupon it can translocate to nuclei and stimulates chromatin condensation and incomplete 50 kb DNA fragmentation (Stage I of apoptosis; caspase independent apoptotic stage). Smac contributes to caspase activation by binding and inactivating the endogenous inhibitor of caspases, IAPs. p53 can promotes apoptosis by up regulation of death receptor and death ligands including TNF-related apoptosis inducing ligand (TRAIL-R1), Fas and Fas L (Liu et al., 2004). A transcriptional independent p53 mediated mitochondrial dysfunction and associated apoptotic mechanism has also been described. An increase in cellular production of ROS is often observed in intrinsic (mitochondria mediated) pathway of apoptotic process triggered by various stimuli.

1.7.Free Radicals

A free radical is a molecule with one or more unpaired electrons in its outermost orbital. The reactivity of free radicals results from their desire to attain an electron of opposing spin direction (Assmann et al., 2000). The ability of radicals and other reactive species to induce cellular damage has been demonstrated in a variety of experimental systems. Numerous studies have shown that free radicals have the potential to produce most of the tissue changes associated with the expression of a
variety of toxicities and disease processes (Kehrer, 2000). Therefore, free radicals have been implicated as contributors to a wide range of disorders (Valko et al., 2007).

1.7.1. Reactive Oxygen Species (ROS)

Oxygen, an abundant molecule in biological systems, is invariably associated with aerobic existence. Reactive oxygen species (ROS) include free radicals (hydroxyl radical, $\text{HO}^\cdot$ and superoxide ion radical, $\text{O}_2^\cdot$), non-radicals (hydrogen peroxide, $\text{H}_2\text{O}_2$, and singlet oxygen $^1\text{O}_2$), short lived lipid peroxidation products (peroxyl radical, $\text{ROO}^\cdot$, and alkoxyl radical $\text{RO}^\cdot$), and long lived secondary products (malondialdehyde, and 4-hydroxyalkenals). The most biologically relevant ROS, $\text{O}_2^\cdot$, peroxide ($\text{O}_2$), and $\text{OH}^\cdot$, are produced in vivo through partial reduction of triplet-state molecular oxygen in normal metabolic processes. Univalent reduction of ground state triplet oxygen leads to the formation of $\text{O}_2^\cdot$ anion. $\text{H}_2\text{O}_2$ reacts with reduced transition metals such as $\text{Fe}^{2+}$ to produce highly reactive $\text{OH}^\cdot$ by the Fenton reaction. Initially, oxidation of $\text{O}_2^\cdot$ by $\text{Fe}^{3+}$ generates molecular oxygen and $\text{Fe}^{2+}$. The resultant $\text{Fe}^{2+}$ initiates the Fenton reaction and regenerates $\text{Fe}^{3+}$ to propagate the reaction (Kehrer, 2000). Additional pathways of ROS generation include mitochondrial electron transport, peroxisomal fatty acid metabolism, and cytochrome P450 reactions (Ames et al., 1993). Mitochondria consume approx 90% of the oxygen, and about 1-2% of the oxygen metabolized by mitochondria is converted to $\text{O}_2^\cdot$ at several sites in the respiratory chain and matrix (Chance et al., 1979).

1.7.2. Reactive Nitrogen Species (RNS)

Studies on reactive nitrogen species (RNS) often focus on nitric oxide, but RNS include several other reactive moieties that are important in modulating cellular oxidative stress. RNS are derived from the oxidation of the guanido nitrogen of L-arginine. Production of the nitric oxide (NO) radical is catalyzed by a family of NO synthases, which includes constitutively expressed isoforms (neuronal and endothelial NO synthases) and an inducible form. NO has a wide range of biological roles, including regulation of vascular tone, memory formation, and inflammation (Moncada and Higgs, 1993). However, other important functions of NO are phagocytosis and mediation of cellular injury in pathological processes such as reperfusion injury and rheumatoid arthritis (Mazzetti et al., 2001).
1.7.3. Generation of free radicals in the body

Formation of ROS and RNS can occur in the cells by two ways: enzymatic and non-enzymatic reactions. Enzymatic reactions generating free radicals include those involved in the respiratory chain, the phagocytosis, the prostaglandin synthesis and the cytochrome P450 system (Halliwell and Gutteridge, 2007). Free radicals can be produced from non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiations. The nonenzymatic process can also occur during oxidative phosphorylation (i.e. aerobic respiration) in the mitochondria (Valko et al., 2007). ROS and RNS are generated from either endogenous or exogenous sources. Endogenous free radicals are generated from immune cell activation, inflammation, mental stress, excessive exercise, ischemia, infection, cancer, aging. Exogenous ROS/RNS result from air and water pollution, cigarette smoke, alcohol, heavy or transition metals (Cd, Hg, Pb, Fe, As), certain drugs (cyclosporine, tacrolimus, gentamycin, bleomycin), industrial solvents, cooking (smoked meat, used oil, fat), radiation (Halliwell and Gutteridge, 2007; Valko et al., 2007). After penetration into the body by different routes, these exogenous compounds are decomposed or metabolized into free radicals.

1.7.4. Mitochondrial generation of ROS

It is firmly believed that mitochondrial ETC is a rich source of ROS (Loschen et al. 1973). Large number of studies using different substrates supporting the respiration and different inhibitors of the respiratory complexes established that ROS can be produced at complex I (Starkov and Fiskum, 2003) and the Q-cycle of the respiratory chain (Starkov and Fiskum, 2001). Independent studies have demonstrated that α-KGDH could be an important source of ROS (Starkov et al., 2004; Tretter and Adam-Vizi, 2004).

Approximately 90-95 percent of cellular oxygen (O_2) is reduced to water stepwise via electron carriers of the mitochondrial respiratory chain. Approximately 1-5 % of total O_2 consumption gives rise to potentially cytotoxic ROS such as O_2•^- and H_2O_2 (Fig. 1.7.A). Based on O_2 consumption of 6.4 l/kg/day, 80 kg man would produce some 215-430 mmol and a 60 kg woman would produce some 160-320 mmol of O_2•^- each day from mitochondrial respiration alone (Cadenas and Davies, 2000). Most of the electron carriers are thermodynamically capable of reducing oxygen to
Fig. 1. 7. A. Generation of ROS by the mitochondria

![Diagram showing the generation of ROS by the mitochondria](image)

Fig. 1. 7. B. Sites of ROS generation in mitochondria

![Diagram showing the sites of ROS generation in mitochondria](image)

Fig. 1. 7. C. Free radical mediated abnormalities in body

![Diagram showing free radical mediated abnormalities in body](image)
While it had been demonstrated that the isolated mitochondria generate O$_2$•−-derived H$_2$O$_2$, the concept of H$_2$O$_2$ generation from O$_2$•− is induced in the presence of respiratory chain inhibitor e.g. inhibition of complex III by antimycin A. Any factors adjusting the function of the respiratory chain affect ROS production. Later, it has been proved that a high mitochondrial membrane potential (ΔΨmt), except in aging where membrane potential declines, is one of the triggers that release H$_2$O$_2$ from O$_2$•− (Korshunov et al., 1997). According to Kadenbach and Arnold (1999), the dephosphorelation, that inhibits cytochrome oxidase, the terminal electron acceptor of the respiratory chain, increases mitochondrial membrane potential and thus induces the O$_2$•− generation.

It has been demonstrated that mitochondrial ROS is produced by electron leakage from ETC complexes during normal respiration particularly in complex I and III (Fig. 1.7B) (Liu et al., 2002). O$_2$•− generation is enhanced at complex III during hypoxia via autooxidation of ubiquinone on both sides of mitochondrial inner membrane. O$_2$•− is the major source of intracellular oxygen radicals under physiological conditions (Cadenas and Davies, 2000). The risk of ROS formation due to leakage of electron from ETC has been mainly observed under pathophysiological rather than physiological conditions, where alteration of membrane fluidity is one of the many reasons that eventually results in the generation of ROS. Mutations in genes that encode mitochondrial proteins could compromise mitochondria by altering components of the ETC, resulting in inefficient electron transport and increased O$_2$•− production. Low level of nitric oxide (NO), produced by mitochondrial-specific nitric oxide synthase (mt NOS) partially inhibits respiratory chain. This partial inhibition increases mitochondrial ROS production in short term. O$_2$•− and NO are readily converted either by enzymatic or non-enzymatic chemical reaction to non-radical species such as $^{1}$O$_2$, H$_2$O$_2$ or ONOO−.

A wide variety of medicinally useful drugs can generate ROS when they undergo redox cycling with the mitochondrial ETC such as the most widely studied drugs is an antitumor anthracyclin antibiotic, doxorubicin. Other quinonoid compounds such as daunorubicin, rubidazone and aclacinomycin cause cytotoxicity because of the ROS produced during the mitochondrial and cytochrome P-450 dependent redox cycling (Davis and Doroshow, 1986).
1.8. Damages produced by the free radicals

Free radicals such as ROS and RNS are attractive species to blame as mediators of toxicities. They have been extensively studied in recent years, but with few exceptions their roles in the etiologies of specific disorders remain largely undefined. At low or moderate concentrations, ROS and RNS are necessary for the maturation process of cellular structures and can act as weapons for the host defense system.

The reactive nature of these species makes all cellular macromolecules potential targets, and a vast array of changes has been identified that could provide mechanistic explanations for the observed injury, cause protein oxidation, DNA damage, and lipid peroxidation and thereby leads to different diseases and abnormalities in body (Fig. 1.7.C). Free radicals induced DNA damage can be described both chemically and structurally and shows a characteristic pattern of modifications. The forms of DNA damage produced by free radicals include modification of all bases, production of base-free sites, deletions, frame shifts, strand breaks, DNA–protein cross-links, and chromosomal rearrangements. The endogenous reactions that are likely to contribute to ongoing DNA damage are oxidation, methylation, depurination, and deamination (Ames, 1989). Double strand break is the most serious type of DNA damage caused by free radicals because neither strand is able to provide physical integrity or information content.

Oxidization of proteins occurs through multiple reactions, including side chain alterations and backbone cleavage, and disrupts protein structure, causing denaturation, aggregation, and susceptibility to degradation (Dean et al., 1997). Oxidized proteins are functionally inactive and the consequent unfolding in some cases make them susceptible to proteinases. Alterations of protein structure and function induced by ROS and RNS may contribute to carcinogenesis. ROS produced from various sources can directly damage DNA, activate transcription factors, kinases, or genes, inactivate the same factors, or modulate signal transduction pathways. ROS and RNS react with proteins to modify amino acid residues by oxidation, nitrosation, nitration, and halogenation. Tyrosine residues in protein react with various RNS to form 3-nitrotyrosine (NTYR) (Blanchard-Fillion et al., 2001). Myeloperoxidase (MPO) and Eosinophil peroxidase (EPO) can also nitrate tyrosine to form NTYR using $\text{H}_2\text{O}_2$ and nitrite ($\text{NO}_2^-$) as substrates (Eiserich et al., 1998).
Fig. 1.8. A. Oxidative stress mediated abnormalities in the various organs

- CHD, Cardiac Fibrosis, Hypertension, ischemia, Myocardial infarction
- Skin Ageing, Sunburn, Psoriasis, Dermatitis, Melanoma
- Diabetes, Aging, Chronic Fatigue
- Multi-organ
  - Cardiovascular: Atherosclerosis, Endothelial Dysfunction, Hypertension
  - Renal: Kidney disease, Renal Graft, Nephritis
  - Rheumatologic: Rheumatoid, Osteo-Arthritis, Psoriasis
- Neurological: Alzheimer, Parkinson, OCD, ADHD, Autism
  - Musculoskeletal: Migraine, Stroke, Trauma, Cancer

Fig. 1.8. B. Oxidative stress mediated mitochondrial damage

- Defects in ETC and OXPHOS
  - Enzyme activity
  - Ischemia/reperfusion
  - Hypoxia
  - Mitochondrial dysfunction
- mDNA damage
- mDNA mutations
- Oxidative stress
- Even higher ↑ in ROS production
- ↓ ETC activity Mito dysfunction
- Cell Death

Fig. 1.8. C. The vicious cycle theory of aging

1.9. Antioxidant defenses in body

Anti-oxidants are substances capable to mop up free radicals and prevent them from causing cell damage. Halliwell, a leading scientist in the field of antioxidant research, formulated the definition of an antioxidant as: “any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate” (Halliwell et al., 1995). Antioxidants cause protective effect by neutralizing free radicals, which are toxic byproducts of natural cell metabolism. The human body naturally produces antioxidants but the process is not 100 percent effective in case of overwhelming production of free radicals and that effectiveness also declines with age (Sies, 1991).

1.9.1. Enzymatic antioxidants

1.9.1.1. Superoxide dismutases (SOD) EC 1.15.1.1

O$_2^{-}$ is one of the most frequently generated ROS in the cell. The first line of defense against O$_2^{-}$ is SODs (Fridovich 1995). Three types of SOD have been identified based on their structure, localization, inducibility, and metal ion requirements (Rahman, 2006). (1) Copper–zinc superoxide dismutase (CuZn-SOD) is localized in the cytosol that requires both Cu and Zn at its active site for its activity. (2) Manganese-superoxide dismutase (Mn-SOD) is considered to be one of the most important antioxidant components of a cell. MnSOD constitutes about 10-15% of the total SODs and is localized in the mitochondria (3) Extracellular superoxide dismutase (ECSOD), which is the major extracellular SOD of the pulmonary fluids and interstitial spaces of the lungs.

All the forms of SOD act by a common mechanism of dismutation of the O$_2^{-}$ radical O$_2^{-}$ to the less potent H$_2$O$_2$ as shown in the following equation:

\[
2 \text{O}_2^{-}\text{+} 2\text{H}^+ + \text{SOD} \rightarrow \text{H}_2\text{O}_2 \text{+} \text{O}_2
\]

1.9.1.2. Catalase (CAT) EC 1.11.1.6

This antioxidant enzyme is a homotetrameric protein (mol. wt. 240 kDa) that catalyzes the conversion of hydrogen peroxide to water and oxygen (Rahman, 2006):

\[
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\]
CAT is present in most aerobic cells in animal tissues and is especially concentrated in the liver and erythrocytes. The brain, heart, and skeletal muscle contain only low amounts. Mammalian CAT is a hemeprotein, which reduces $H_2O_2$ to water by utilizing electrons from either $H_2O_2$ (catalase reaction) or from other small molecules such as methanol or ethanol (peroxidase reaction) (Calabrese, 1989). CAT is found in peroxisomes and cytoplasm and is especially localized in the alveolar type II pneumocytes and macrophages.

### 1.9.1.3. Glutathione peroxidase (GPx)

GPxs are a family of selenium-dependent and-independent antioxidant enzymes and can be divided into two groups: cellular and extracellular. In general, GPx is a tetrameric protein (mol. wt. 85 000 kDa). It requires four atoms of selenium (Se) bound as seleno-cysteine moieties that confer the catalytic activity. GPx reduces $H_2O_2$ to $H_2O$ by oxidizing GSH as shown (Brigelius-Flohe, 2006).

$$H_2O_2 + 2GSH \rightarrow GSSG + 2H_2O$$

Re-reduction of GSSG is then catalyzed by glutathione reductase through the glutathione cycle.

$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$$

Currently, five GPxs are known in mammals, two of which are present in liver cells (Rahman, 2006): cytosolic GPx (GPx1) and phospholipid hydroperoxide GPx (GPx4). About 75% of the total cellular GPx1 enzyme activity is located in the cytosol and 25% in mitochondria (Flohe, 1982). Potentially relevant GPx for the liver is plasma GPx (GPx3) (Brigelius-Flohe, 2006). All GPx enzymes contain selenium in the form of selenocysteine (Forstrom et al., 1978). Selenium is critical for the catalytic function of the enzyme (Flohe, 1982). GPx reduces hydrogen peroxide to water, utilizing reducing equivalents from GSH. In addition to $H_2O_2$, the enzyme can metabolize a wide variety of organic peroxides (Flohe, 1982).

### 1.9.2. Low-molecular weight antioxidants

This category includes $\alpha$-tocopherol (vitamin E), $\beta$-carotene, ascorbate (vitamin C), and GSH as most relevant examples (Sies, 1993). These compounds have two major objectives. First, they intercept radical chain reactions and prevent the further formation of damaging radicals, and second, they transfer the radical function away
from sensitive targets, for example, from hydrophobic membranes to the aqueous phase (Sies 1993).

The most effective chain-breaking compound in membranes is $\alpha$-tocopherol (Chow, 1991). It effectively reduces lipid peroxyl radicals to the hydroperoxide, which can be reduced by GPx4 to the alcohol (hydroxy fatty acid). $\alpha$-Tocopherol prevents the propagation of the radical chain by avoiding the formation of new alkyl radicals by forming a relatively stable tocopherol radical (Chow, 1991).

GSH, $\gamma$-glutamyl-cysteinyl-glycine, is the most important water-soluble antioxidant. GSH is an essential cofactor for various GPxs and GSH transferases; it is involved in maintaining protein sulfhydryl groups, and it is important as a direct scavenger of ROS and ONOO$^-$ in intra- and extracellular compartments. The key function of GSH is the sulfhydryl group of cysteine, which has a lower susceptibility for autoxidation in GSH compared to the isolated amino acid (Deleve and Kaplowitz, 1990).

The antioxidant enzymes, Mn SOD and GPx are recognized as primary defense against $O_2^•$ and $H_2O_2$ in eukaryotic cell mitochondria. However, presence of a heme-containing CAT in the rat heart mitochondrial matrix was demonstrated by Radi et al. (1991). The CAT activity in the mitochondria of rat liver, brain, and skeletal muscle has been previously reported (Savitha et al., 2005; Salvi et al., 2007; Srividhya et al., 2009).

1.10. Oxidative Stress

The rate of free radical production and scavenging capacity of the antioxidants are essentially constant or balanced under normal homeostasis. However, under certain conditions, especially in pathological conditions, the production of ROS increased more strongly and persistently.

There are mainly three kinds of oxidative stress, namely, (1) Dietary Oxidative Stress which is defined as ‘‘a substance in foods that significantly decreases the adverse effects of reactive species, such as ROS, RNS, on normal physiological function in humans.’’ (2) Physiological Oxidative Stress, which results by the uneven distribution of oxygen and its metabolites in organs and in subcellular organelles of body. The mitochondria are considered to be a site of physiological
oxidative stress. (3) **Photooxidative Stress** in which the generation of oxidants resulting by the electronic excitation through impinging light. The wavelength ranges of biological importance are ultraviolet B (UVB) and ultraviolet A (UVA), but visible light and even infrared (IR) are known to generate photobiological responses. (4) **Radiation-Induced Oxidative Stress**, here the oxidative stress results by the oxidants generated by radiation (Sies, 1991).

Oxidative stress involves an increased production and/or a decreased elimination of ROS in cells and has been shown to contribute to the cell damage caused by ischaemia/reperfusion, trauma, aging as cancer, diabetes, inflammation, arthritis and several neurodegenerative diseases (Fig.1.8.A) (Halliwell and Gutteridge, 2007).

### 11.1. Oxidative stress and mitochondrial damage

Damages to mitochondrial macromolecules are exaggerated during oxidative stress as is observed especially in pathological conditions (Fig.1.8.B). The pathological symptoms may result either from ROS and RNS mediated damage of macromolecules or from the changes in the gene expression. The generated ROS have very short life span and can readily react with the macromolecules such as lipids, proteins and nucleic acids of mitochondria.

#### 1.11.1. Mitochondrial lipid peroxidation

The excessive generation of free radicals leads to peroxidative changes that ultimately result in enhanced lipid peroxidation (LPO). Cardiolipin, an important phospholipid that serves as a cofactor for a number of critical mitochondrial transport proteins and retains cytochrome c at inner mitochondrial membrane through electrostatic interaction, declines due to either oxidative damage. The oxidized cardiolipin is not only removed from the membrane but may also be decreased in level because of decreased *de novo* synthesis. Loss of cardiolipin, coupled with oxidation of critical thiol groups in key proteins, may adversely affect transport of substrates and cytochrome c oxidase activity that is necessary for mitochondrial function. Peroxidation of cardiolipin releases cardiolipin to execute the apoptotic cell death (Ott et al., 2002).
1.11.2. Mitochondrial protein functional alteration

Proteins may differ strongly in their susceptibility to damage by oxidants. Practically all amino acids can serve as targets for oxidative attack while some of them such as tryptophan, tyrosine, histidine, and cysteine are particularly sensitive to ROS. ROS-induced oxidative modification of many enzyme proteins inside mitochondria results in structural alteration and their functional inactivation. Deformation of enzyme structures may also lead to altered $K_m$ for the substrates. Oxidants may also cause increased damage or use of critical metabolites such as ubiquinone or small molecular weight antioxidants. Cadenas and Davies (2000) reported that activities of NADH dehydrogenase, NADH oxidase, SDH, succinate oxidase and ATPase were rapidly inactivated by $\cdot$OH. Moreover, $O_2^-\cdot$ can be a highly efficient inactivator of NADH dehydrogenase, NADH oxidase, and ATPase. ONOO- was found to be an inactivator of aconitase, an iron containing enzyme in the Krebs cycle. It has been demonstrated that under oxidative stress conditions the oxidized proteins instead of undergoing proteolytic digestion, aggregated by cross linking with one another and affect the normal cellular functions (Babusíková et al., 2004).

1.11.2. Damage to mitochondrial DNA

Since 1988, when the first mutations in mtDNA were described, more than 400 mutations in mtDNA have been identified as being responsible for respiratory chain and OXPHOS diseases. Oxidative damage to mtDNA such as strand breaks and base modifications can occur either directly from ROS or from ROS derived lipid-hydroperoxide. For instance, $\cdot$OH mediated modification of deoxyguanosine base of DNA to 8-hydroxydeoxyguanosine has been demonstrated. Gradual accumulation of somatic mtDNA mutations can finally cause mitochondrial dysfunction and loss of cellular energy production. mtDNA is highly susceptible to oxidative damage probably due to the (1) close proximity to the site of ROS/RNS production for being inside mitochondria, and that $O_2^-\cdot$ generated inside mitochondria is not permeable to cytosol (2) mtDNA lacks histone proteins (which protects nuclear DNA from oxidative damage); and (3) mitochondrial polymerases lack specificity for base excision repair, which is the major pathway eliminating oxidative DNA base lesions. Many of these DNA damages are mutagenic, contributing to human diseases including cancer, aging, cardiovascular and neurodegenerative diseases. Thus,
mtDNA damage is a good biomarker of oxidative stress. Interestingly, oxidative damage to mtDNA in the heart and brain is inversely related to maximum life span of mammals (Barja and Herrero, 2000).

1.12. Role of mitochondria in aging

Aging is a syndrome of changes that are deleterious, progressive, universal and thus far irreversible. Aging, the process of growing old, is defined as the gradual biological impairment of normal function, probably as a result of changes made to cells (mitotic cells, such as fibroblasts and post-mitotic cells, such as neurons) and structural components (such as bone and muscle). These changes would consequently have a direct impact on the functional ability of organs (such as the heart, kidney and lungs), biological systems (such as the nervous, digestive and reproductive system) and ultimately the organism as a whole (Strehler, 1962). It was hypothesized that these deficits might be due to defects of the mitochondrial OXPHOS responsible for the maximum output of ATP in the cell (Linnane et al., 1989). Aging damage occurs to molecules such as DNA, proteins, lipids etc, to cells and to organs. Aging is generally accompanied by a gradual decline in biochemical and physiological functions of most organs, ultimately leading to an increase in the susceptibility to age-associated disorders.

Many serious degenerative diseases might also develop such as prostatitis, osteoporosis, diabetes, cancer, atherosclerosis, heart disease, Alzheimer's disease, Parkinson's disease, etc. Under very rare abnormal circumstances, people suffer from certain accelerated aging diseases such as Werner's syndrome, Cockayne syndrome, Hutchinson-Gilford Progeria syndrome and so on. Such symptoms are sometimes used as models to study the mechanisms of aging. In the process of exploring the mechanisms of aging, more than 300 theories have been proposed. However, it's important to note, many of the theories of aging are not mutually exclusive, but intrinsically related and sometimes supportive of each other. The major among them are (1) Telomerase theory of aging, (2) Hayflick limit theory of aging, (3) Cross-linking theory of aging, (4) The neuroendocrine theory, (5) The membrane theory of aging, (6) The free radical theory etc. But the most recent theory in this type is Mitochondrial Vicious Cycle Theory of Aging.
2.12.1 Mitochondrial theory of aging and mitochondrial vicious cycle theory of aging

The mitochondrial theory of aging (MTA) was first proposed in 1972 by Denham Harman, the "father" of the free radical theory of aging (FRTA) (Harman, 1972). The MTA was further refined and developed by Jaime Miquel (Miquel et al., 1980). There is such a strong connection between the MTA and the FRTA that they are often discussed together as if the MTA was just one form or specific development of the FRTA (Beckman and Ames, 1998). Yet the MTA concerns far more than free radicals. The MTA involves three other major biological topics as well: genetics, membranes, and bioenergetics. There is strong evidence that age induces alterations in the mitochondrial genome that lead to defects in mitochondrial function, especially in post-mitotic tissues with high energy requirements such as the heart, brain and skeletal muscle (Ojaimi et al., 1999).

The very feature that makes mitochondria unique among the various cell organelles - having their own DNA - gives rise to a major problem. It is also well demonstrated that oxidative damage to mtDNA is much greater compared to nuclear DNA in various species and tissues examined (Barja and Herrero, 2000). ROS - induced damage to mtDNA is believed to be the primary source of mutagenesis in mitochondria (Hamilton et al., 2001), giving rise to both mtDNA mutations and deletions. To make matters worse, the mitochondrial ETC is the main source of cellular free radicals/oxidants, especially O$_2^•^-$, H$_2$O$_2$ and •OH (Shigenaga et al., 1994). mtDNA is at least transiently attached to the inner mitochondrial membrane, where the ETC is located and from which free radicals/oxidants are continuously released.

The mitochondrial ‘vicious cycle’ theory of aging can be considered as an extension and refinement of the free radical theory which was first put forward by Harman (1973). Its major premise is that mtDNA mutations accumulate progressively during life, as a side effect of respiration, and are directly responsible for a measurable deficiency in cellular OXPHOS activity, leading to an enhanced ROS production (Alexeyev et al., 2004). In turn, increased ROS production results in an increased rate of mtDNA damage and mutagenesis, thus causing a ‘vicious cycle’ of
exponentially increasing oxidative damage and dysfunction, which ultimately culminates in death (Fig. 1.8.C) (Miquel et al., 1980; Alexeyev et al., 2004).

1.13. Role of mitochondria in diseases

After the landmark discovery of the regulation of mitochondrial energy production by chemiosmosis (Mitchell and Moyle, 1967) many scientists considered the role and function of the mitochondrion. Mitochondria again took the spotlight in the 1980s, with the breakthrough that certain diseases are caused by mutations in mitochondrial DNA (mtDNA) (Wallace et al., 1988) as well as by the seminal findings of Liu et al. (1996) that mitochondria are key regulators of programmed cell death by apoptosis. These discoveries rekindled scientific interest in the mitochondrion, and in its potential role in a variety of diseases including cancer, cardiovascular disease, diabetes, aging and neurodegenerative diseases all of which have a significant mitochondrial component.

1.13.1. Role of mitochondrial oxidative stress in the pathogenesis of cardiovascular diseases

Heart diseases including myocardial infarction, arrhythmia and cardiomyopathy are the leading cause of morbidity and mortality in the world. Heart is vulnerable to damage induced by free radicals because of lower levels of antioxidant enzymes. The role of oxidative stress has been reported in certain cardiovascular disease states, including atherosclerosis, hypertension, and in heart failure (Tsutsui, 2001). Over the past few decades, the role of mitochondrial oxidative stress has been increasingly recognized in the pathophysiology of cardiovascular diseases (Fig.1.9.A). Experimental and clinical studies have detected an impaired respiratory function of cardiomyocyte mitochondria in heart failure. The myocardial cell mitochondria contribute a greater H$_2$O$_2$ production owing to high tissue mitochondrial density and exclusively aerobic metabolism of myocardium than other tissues (Radi et al., 1991). Therefore, the role of CAT to remove H$_2$O$_2$ especially under extensive GSH depletion and high H$_2$O$_2$ concentration is non-significant. Thus, mitochondria of cardiac myocytes cells become less efficient with increasing age, resulting in greater damage to DNA and proteins.
Fig. 1. 9. A. Role of mitochondria in cardiac diseases

Fig. 1. 9. B. Mechanism of APAP toxicity
Heart is dependent on O₂ and OXPHOS to provide high-energy compounds necessary for contraction, but this exposes the myocardium to harmful ROS that are generated continuously as normal by-products of the mitochondrial ETC. The mitochondria play a central role in different forms of heart failure. There is now a consensus view that mutations in mtDNA and abnormalities in mitochondrial function are associated with common forms of cardiac diseases such as ischemic heart disease (Corral-Debrinski et al., 1992) and dilated cardiomyopathy (Arbustini et al., 1998). All of these findings have provided only indirect evidence. Mitochondria are primarily involved in any myocardial ischemic event. When ischemia continues, the components of the mitochondrial respiratory chain are progressively impaired, beginning with a loss of cytochrome c which disconnects from its site at the outer surface of the inner mitochondrial membrane and the denaturation of Complex I (Rouslin, 1983). Ischemia causes anoxia, acidosis from anaerobic metabolism, and ATP depletion, whereas reperfusion produces reoxygenation, a return of physiologic pH, and possible recovery of ATP levels which leads to the formation of the MPT pore and is looked upon as a key factor in reperfusion (Bond et al., 1993). Although the mechanisms underlying cardiac decay are not clear, loss of mitochondrial function and a resultant increase in oxidative stress has been proposed to be one of the key factors in myocardial aging. Myocardial infarction induced by Isoproterenol (ISO) has been reported to show many metabolic and morphologic aberrations in the heart tissue of the experimental animals similar to those observed in human myocardial infarction (Nirmala and Puvanakrishnan, 1996).

1.13.2. Mitochondrial dysfunction during Isoproterenol induced MI

Isoproterenol (L-β-(3, 4-dihydroxyphenyl)-α-isopropylaminoethanol hydrochloride), a sympathomimetic β-adrenergic receptor agonist, causes severe stress to the myocardium resulting in an infarct like necrosis of heart muscle (Sushma et al., 1989). The rat model of ISO induced myocardial necrosis serves as a well accepted standardized model to evaluate several cardiac dysfunctions and to study the efficacy of various natural and synthetic cardioprotective agents (Rathore et al., 1998). ISO induced necrosis is maximal in the subendocardial region of the left ventricle and in the interventricular septum. Continuous infusion of ISO in rats elicits typical cardiac
gene expression similar to that observed in cardiac hypertrophy caused by pressure overload (Boluyt et al., 1995).

Several mechanisms for the cardiotoxic effects of high levels of ISO have been suggested. These mechanisms include: (1) functional hypoxia and ischemia, (2) coronary insufficiency, (3) alterations in metabolism, (4) decreased level of high-energy phosphate stores, (5) intracellular Ca\textsuperscript{2+} overload, (6) changes in electrolyte contents and (7) oxidative stress. Although these changes represent individual pathological states, they are known to affect each other and thus are interpreted as complex entities. The primary disturbances of ISO induced myocardial infarction has been reported to enhance adenyl cyclase activity, resulting in increased cAMP formation, which in turn would lead to the higher lipid accumulation in the myocardium (Dhawan et al., 1978). Several early events, such as ultrastructural changes, histological, biochemical, electrolyte and membrane changes, have been shown to occur within 48 h after the injection of ISO. Glycogen depletion and fat deposition have been reported.

Ischemia-reperfusion injury leads to mitochondrial dysfunction caused through oxidative stress (Becker, 2004). Previous reports shown MI induces a substantial inhibition of the mitochondrial respiratory chain in the heart (Zhou et al., 2006; Tsutsui et al., 2009). The production of ROS during ischemia impairs the expression of genes encoded by the mitochondrial DNA and that a down regulation of expression and activity of enzymes composing the respiratory chain complexes plays an important role in the progression of left ventricular remodeling and failure after MI (Ide et al., 2001). For example, the enzyme activity of ETC complex I, III, and IV decreased in mice subjected to MI (Ide et al., 2001). Paradies et al. (2004) reported that ROS accumulation in mitochondria could affect complex I and III activities through oxidative damage of cardiolipin, which is required for the functioning of these complexes. So, it is assumed that under ISO induced MI oxidative damage in mitochondria might have created blocks in electron flow along the respiratory chain in heart. Thus, the impairment of complex I and III activities due to the ROS-induced cardiolipin peroxidation may increase the electron leakage from the ETC, generating more O\textsubscript{2}•− and perpetuating a cycle of oxygen radical-induced damage to mitochondrial membrane constituents leading to MI (Paradies et al., 2004).
1.13.3. Role of mitochondrial oxidative stress in the pathogenesis of liver diseases

Over the past few decades, it has become increasingly recognized that inherited or acquired mitochondrial dysfunction plays a major role in many forms of acute and chronic liver disease. In recent years, however, mitochondrial dysfunction has been recognized as the second most important mechanism of drug-induced liver injury (Fromenty et al., 1997). Mitochondria occupy 20% of the cytoplasm of hepatocytes by volume. Therefore, it is not surprising that patients with defects in this system can present with liver cell dysfunction manifesting as mild abnormalities in liver biochemical tests or even acute liver failure. The primary mitochondrial hepatopathies are mtDNA depletion syndrome, Pearson’s syndrome, Alpers disease, and Navajo neurohepatopathy. The underlying toxicity to the mitochondria generally involves oxidative stress, lipid peroxidation, mtDNA oxidation or mutation, induction of pro-apoptotic cytokines, opening of the MPT pore, and impairment of OXPHOS can all be caused by excessive ROS associated by oxidative stress.

A growing body of evidence suggests that mitochondrion-mediated apoptosis is a key mechanism of injury in many liver diseases, namely alcohol, viral, and cholestatic liver diseases (Rust and Gores, 2000). It appears that oxidative stress leads to mitochondrial dysfunction and triggers opening of the MPT pore. This depletes the proton motive force by allowing protons out of the intermembrane space. In addition, the increased oxidative stress appears to upregulate transcription of Fas ligand within hepatocytes. This ligand can then bind to the death receptor (Fas) on neighboring hepatocyte membranes, inducing apoptosis (Pessayre et al., 2001).

The primary mechanism of drug-induced mitochondrial injury is impairment of β-oxidation of fatty acids, which typically manifests as microvesicular steatosis. Mitochondrial β-oxidation may be directly inhibited by binding of drugs and/or their metabolites to β-oxidation enzymes or to coenzyme A (CoA), a required cofactor for oxidation of fatty acids in the mitochondria. Both aspirin and valproic acid (VPA) can sequester CoA, while VPA, tetracyclines (high dose), glucocorticoids (high dose), nonsteroidal anti-inflammatory drugs (NSAIDS) such as ibuprofen and pirprofen, and amiodarone may inhibit β-oxidation enzymes. Other drugs upset the metabolic machinery by damaging mtDNA directly (ethanol), inhibiting mtDNA replication (nucleoside analogues) or transcription (interferon-α), or interfering with OXPHOS.
(amiodarone). The end result of mtDNA damage or depletion is a defect in OXPHOS. When OXPHOS is impaired, the β-oxidation cycle also slows down due to the requirement for oxidized nicotinamide adenine dinucleotide (NAD\(^+\)), generated during OXPHOS. This secondary impairment in β-oxidation again leads to microvesicular steatosis, the predominant expression of mitochondrial injury within the liver. Molecules with a carboxylic group or a protonatable amine group appear to be more likely to cause mitochondrial injury (Hassanein, 2004).

### 1.13.4 Mitochondrial damage by acetaminophen toxicity

Acetaminophen (N-acetyl-p-aminophenol, 4-hydroxyacetanilide, paracetamol) (APAP) is a widely used analgesic and antipyretic. At therapeutic levels, APAP is considered as an efficacious and safe drug even for susceptible individuals, for example, alcoholics (Kuffner et al., 2007). However, an acute or cumulative overdose can cause centrilobular necrosis and even induce liver failure. APAP hepatotoxicity is currently the most frequent cause of acute liver failure of any etiology (Larson et al., 2005).

APAP can either directly undergo phase II metabolism or is first metabolized by the P450 system and then is conjugated with glutathione. APAP is converted to a toxic reactive intermediate called N-acetyl-p-bezoquinoneimine (NAPQI) following metabolism by a number of isozymes of cytochrome P-450 (CYPs), i.e., CYP 2E1, CYP 1A2, CYP 2A6, CYP 3A4 and CYP2D6 (Nelson, 1990). The precise role of oxidative stress in APAP-induced liver injury is not fully understood. It has been shown that binding of NAPQI to GSH sulfhydryl group’s results in reduction of hepatic antioxidative capacity (Bessems and Vermeulen, 2001). Also, it has been postulated that NAPQI can lead to direct oxidative damage of numerous cell components (Ruepp et al., 2002) which eventually lead to oxidative stress and liver toxicity (Fig.1.9.B).

Morphological changes in mitochondria can be detected during the first hours after treatment with APAP, that is, well before any cell death occurs (Ruepp et al. 2002). Direct exposure of mitochondria to NAPQI can reproduce the respiratory changes (Meyers et al., 1988). In addition, mitochondrial dysfunction in vivo is dependent on the metabolism of APAP and can be prevented by treatment with N-acetyl cystein (NAC), which facilitates scavenging of NAPQI by GSH (Meyers et al.,
1988; Donnelly et al., 1994). These observations suggest that covalent binding of NAPQI to mitochondrial proteins may be responsible for inhibition of mitochondrial respiration. In direct support of this hypothesis, APAP protein adducts in mitochondria were identified (Qiu et al., 2001). The APAP treatment also affects the respiratory chain enzymes in the mitochondria. Studies shows that the NAPQI formed during APAP toxicity inhibits the respiration supported at complexes I and II in hepatic mitochondria in experimental animals (Ramsay et al., 1989). Furthermore, exposure of submitochondrial particles to NAPQI inhibited NADH dehydrogenase and SDH activities, associated with respiratory complexes I and II (Burcham and Harman, 1991). This proposal may be consistent with the finding that significant covalent binding of the reactive metabolite to mitochondrial proteins occurs during APAP hepatotoxicity in mice in vivo (Jollow et al., 1973). Studies show that the induction of hypoxia inducible factor -1 α (HIF-1 α) by the oxidative stress is one the major process during the APAP toxicity (Sparkenbaugh et al., 2011). The mitochondrial O₂ consumption and ATP generation were decreased progressively following activation of HIF1 α (Fig. 1.10.A).

Evidence also suggests that the formation of oxidative stress is the major mechanism of APAP toxicity (Bajt et al., 2004) and the formation of ONOO⁻ from O₂⁻ and nitric oxide (NO) is the early stages of APAP toxicity (Knight et al., 2001). ONOO⁻ is generated after GSH depletion mainly inside mitochondria. Thus, ONOO⁻ is a critical mediator of APAP-induced liver injury (Knight et al., 2002). Although, there is now solid experimental support for the presence and pathophysiological relevance of ROS and especially peroxynitrite, potential consequences of this oxidant stress are less clear. Since oxidant stress can induce opening of the MPT pores with consequent collapse of the ΔΨmt and depletion of ATP, this mechanism was investigated by confocal microscopy in primary cultured hepatocytes and the data indicate that the MPT and the resultant decline of ΔΨmt occurs about 4-5 h after APAP exposure (Kon et al., 2004), that is, after GSH depletion and after the onset of the mitochondrial oxidant stress but before the loss of plasma membrane integrity (cell necrosis) (Kon et al., 2004). The APAP-induced MPT could be inhibited by cyclosporine A, which binds to cyclophilin D, one of the MPT pore-forming proteins. However, the effect of cyclosporin A was transient, that is, there was a delay in cell death but not a complete prevention in vitro (Kon et al., 2004). The relevance of the
Fig. 1. 10. A. Role of mitochondria in APAP toxicity

Fig. 1. 10. B. Mechanism of Carbon tetrachloride toxicity

Carbon Tetrachloride (CCl₄)
  ↓
  Cytochrome P450
  ↓
  Trichloromethyl radical
  ↓
  Trichloromethylperoxy free radical
  ↓
  Attack polyunsaturated fatty acids
  ↓
  Lipid peroxidation
  ↓
  Decrease glutathione
  ↓
  Injury to membrane
  ↓
  Increase in permeability
  • Leakage of enzymes
  • Disruption of calcium homeostasis
  ↓
  Induction of calcium dependent degradative enzymes
  ↓
  Hepatic Damage (cell death, fibrosis)
  ↓
  Regenerative and proliferative changes
  ↓
  Cancer
MPT in APAP induced cell death was confirmed in vivo (Masubuchi et al., 2005). However, inhibition of the MPT in vivo appears to have a permanent rather than a transient effect. Nevertheless, the evidence is quite strong that the MPT, most likely triggered by mitochondrial oxidant stress and ONOO⁻ formation, is a critical event in APAP induced liver cell death (Burke et al., 2010).

Activation of c-Jun N-terminal kinase (JNK) was suggested to be another consequence of the mitochondrial oxidant stress (Hanawa et al., 2008). Because it requires the diffusion of the intramitochondrial ROS into the cytosol, the most relevant oxidant would be hydrogen peroxide. The proposed JNK involvement in the injury mechanisms included direct promotion of the MPT, induction of iNOS and promotion of mitochondrial bax translocation (Hanawa et al., 2008). Regarding bax translocation, this effect is critical for the early phase of injury (Bajt et al., 2008a). Mitochondrial DNA damage has also been shown in APAP hepatotoxicity (Cover et al., 2005). The nature of the damage remains unclear. During APAP toxicity, the rates of ATP synthesis decreased by 20-63% (Katyare and Satav, 1989). Additionally, 40-60% reduction in hepatic ATP content after treatment with a toxic dose of APAP in mice has been reported (Tirmenstein and Nelson, 1990). Interestingly, AAP also inhibits mitochondrial respiratory activity under in vitro conditions (Burcham and Harman, 1991; Strubelt and Younes, 1992). Thus, during the last decade, significant progress has been made in our understanding of the mechanisms of APAP-induced liver cell injury.

1.13.5. Mitochondrial damage by Carbon tetrachloride toxicity

Carbon tetrachloride (CCL₄) is a xenobiotics one of the oldest and most widely used toxins for induction of lives damage (Brattin et al., 1985). Through the investigation of acute CCL₄ induced liver damage in animal models, it is now generally accepted that CCL₄ toxicity results from bioactivation of CCL₄ into trichloromethyl free radical by cytochrome P-450 system in liver microsomes and consequently causes lipid peroxidation of membranes that leads to severe necrosis in the pericentral regions of the liver (Recknagel et al., 1989) (Fig.1.10.B). CCL₄ also induces hydropic degeneration, centrilobular necrosis, fatty changes, cirrhosis and hepatoma (Recknagel et al., 1989). CCL₄-induced damage also produces alteration in the antioxidant status of the tissues (Martin-Aragon et al., 2001).
At 24 h after CCl₄ administration, alterations observed involved the whole cell. At this time of poisoning by CCl₄ in the rat centrilobular hepatocytes nuclear karyorrhexis, karyolysis, breakdown of the nuclear membrane, and chromatin condensation around the nuclear membrane were observed. Mitochondria were found to be swollen and to have a less dense matrix; frequently their membranes were found to be broken and their cristae swollen. Peroxisomes were observed to be frequently altered and exhibiting a less dense matrix. They also had a prominent nucleoid and appeared more abundant. The plasma membrane was found to be frequently broken and organelles were seen within the lumen or the sinusoids. Lysosomes were increased in number and abundant lipid droplets were observed (Fujimoto and Iimuro, 2010).

Among the early biochemical features of CCl₄ intoxication were total liver fat accumulation, depression of liver microsomal glucose-6-phosphatase activity (Benedetti et al., 1979), polysome breakdown (Smuckler and Arcasoy, 1969) and a corresponding decrease in the protein synthesis activity (Castro and Diaz Gomez, 1976), and the destruction of liver microsomal cytochrome P450 (CYP) (Sohn et al., 1991) and of the microsomal Ca²⁺ pump (Srivastava et al., 1990). Another particularly relevant early change occurring in livers from CCl₄-poisoned animals is a rapid depletion of nicotinamide adenine dinucleotide phosphate (NADPH) (Slater, 1972).

Mitochondrial function, in contrast, did not appear to be seriously altered during 10–13 h after CCl₄ administration (Recknagel 1967). For example, Reynolds et al. (1962) found that mitochondrial oxidations of octanoate, glutamate, β-hydroxybutyrate, and succinate were all at control levels 10 h after poisoning. At 20 h after poisoning these reactions were significantly depressed. Further, Calvert and Brody (1958) reported that only as late as 20 h after hepatotoxicant was there activation of the liver mitochondrial Mg²⁺-dependent adenosine triphosphatase (ATPase). However, other authors reported a peculiar susceptibility of aged mitochondria from CCl₄-poisoned animals when exposed to hypotonic media at earlier times of intoxication (Christie and Judah 1954). The idea was that some subtle alteration existed in mitochondria. This was not expressed in terms of parameters determined at the time. Later studies revealed that in fact, even as early as 1 or 3 h after treatment with CCl₄, covalent binding of CCl₄-reactive metabolites to
mitochondrial proteins and lipids can be observed (Villarruel et al., 1987). Most of the attack by the reactive metabolites was on phospholipids and cholesterol esters.

The loss of mitochondrial Ca$^{2+}$ uptake and increased mitochondrial release of Ca$^{2+}$ has been observed during CCl$_4$ poisoning (Hemmings et al., 2002). This might also be related to NADPH destruction observed during the toxicity (Slater and Sawyer 1977). Further, studies shows that CCl$_4$ induced cirrhosis of the liver leads to a loss of hepatocytes which is paralleled by reduced oxygen uptake and reduced mitochondrial enzyme activities and mitochondrial ATP production rate (Krahenbühl et al., 1989)

Studies also showed that the CCl$_4$ hepatotoxicity is associated with the decrease in the rate of respiration, respiratory control ratio, activities of NADH dehydrogenase, SDH, cytochrome c oxidase and the rate of electron transfer through site I, site II and site III. Similarly, the decrease in the content of cytochrome aa$_3$ and the levels of phospholipids particularly cardiolipin and a significant increase in the lipid peroxide level happens during the CCl$_4$ challenge (Weber et al., 2003). Hence, mitochondrial malfunction by CCl$_4$ disrupts the function of cells and contributes to a wide range of liver diseases. Several studies have previously demonstrated that antioxidants prevent CCl$_4$ toxicity particularly hepatotoxicity, by inhibiting lipid peroxidation and increasing antioxidant enzyme activities in the hepatic mitochondria (Hernandez-Munoz et al., 1992; Ip et al., 1996; Padma and Setty, 1999; Tang et al., 2006).

1.14. Therapeutical interventions or antioxidants used to combat against oxidative stress and aging and resultant cellular damages

Mitochondrial oxidative damage is a major factor in many human disorders, including neurodegenerative diseases, ischaemia-reperfusion injury, aging and inflammatory damage (Ames et al., 1993; Wallace, 1995). Oxidative damage accumulates more in mitochondria than in the rest of the cell because electrons continually leak from the respiratory chain to form damaging ROS (Yan et al., 1997). This oxidative damage impairs mitochondrial ATP synthesis and calcium homeostasis and induces the MPT leading to necrotic or apoptotic cell death (Green and Reed, 1998). The lethal potential of this damage was dramatically illustrated by the premature death of mice lacking mitochondrial SOD (Lebovitz et al., 1996), while mice lacking cytosolic SOD developed normally (Reaume et al., 1996). Selective prevention of mitochondrial
oxidative damage should therefore be an effective therapy in a wide range of human diseases (Wallace, 1994; Adlam, et al., 2005).

Vitamin A/Carotenoids are longevity determinants (Cutler, 1984). The effects of postnatal administration of an excess of vitamin A on DNA, protein and metabolism and pyridine nucleotide-linked dehydrogenases in various organs including the brain have been studied by Shukla et al. (1984). Craft et al. (2004) reported an age-related reduction in carotenoids, xanthophylls (oxygenated carotenoids), retinol and tocopherol in the frontal lobe cortex of the human brain, which is vulnerable in Alzheimer’s disease, suggesting a role of these vitamins in the brain. Seidman (2000) inferred from his study using rats that treatment with antioxidants such as vitamins C and E would reduce the degree of age-related hearing loss.

Bourre (1991) reported that vitamin E protected membrane PUFA against radical peroxidation during cerebral aging, especially in cerebral capillaries and microvessels. Vitamin E protects against nerve terminal dysfunction caused by oxidative stress. Dietary vitamin E plus exercise training corrected the age-related deficit in SOD, GPx and CAT in rat cerebral cortex and hippocampus (Devi and Kiran, 2004). Vitamin E reduced the levels of MDA and lipofuscin and increased the activities of SOD and CAT in the brains of D-galactose-induced aging mice (Ling, 2004).

Recently, a great deal of attention has been focused on the antioxidant activities of α-lipoic acid (Packer, 1995; Biewenga et al., 1997; Bilaska and Włodek, 2005). α-lipoic acid is essential to cell energy metabolism, is a cofactor at entry to Krebs cycle, displays anti-oxidant effects by increasing the GPx activity and reducing oxidative and regulates calcium homeostasis (Bilaska and Włodek, 2005). Indeed several studies have shown that α-lipoic acid exerts multiple pharmacological actions able to prevent nerve degeneration in experimental in vitro models of Parkinson disease (Bharath et al., 2002), and Alzheimer diseases (Mohmmad Abdul and Butterfield, 2007). It also reduces damage from ischemia–reperfusion in animal models (Freisleben, 2000). Recent studies showed that it can decrease the age related decline of antioxidant status and cellular energy status and decrease the oxidative
stress in various animal models (Kumaran et al., 2005; Savitha et al., 2007; Tamilselvan et al., 2007).

Similarly of acetyl- L -carnitine (ALCAR) is another potent antiaging drug which has been studied in detail (Kumaran et al., 2005; Savitha et al., 2007; Tamilselvan et al., 2007). Fraschini et al. (1991) suggested that the anti-aging action of ALCAR might be attributed to increased melatonin synthesis. Carnitine may increase melatonin secretion, reduce lipid peroxidation and improve the antioxidant status.

Again, natural antioxidants such as *Centella asiatica*, grape seed etc have been shown to have potent antioxidant activities which protect against oxidative stress induced damages associated with aging (Balu et al., 2005; Subathra et al., 2005). Similarly, polyphenols, isoflavones, ginsenosides and flavonoids, extracted from medicinal plants, have antioxidant function and are proved to have protective effects on mitochondrial function. Green tea polyphenols are believed to be a strong antioxidant against HO·, NO· and lipid oxidation (Nanjo et al., 1996; Panickar et al., 2009).

1.15. Mitochondrial medicine

Targeting of biologically active molecules to mitochondria in living cells will open up avenues for manipulating mitochondrial functions. Increasing pharmacological and pharmaceutical efforts have been undertaken to find effective therapies for disorders associated with malfunctioning mitochondria thus leading to the emergence of ‘Mitochondrial Medicine’. The delivery of both, the small drug molecules and large macromolecules to and into mitochondria may provide the foundation for a large variety of future cytoprotective and cytotoxic therapies. The delivery of antioxidants may protect mitochondria from oxidative stress caused by a variety of insults; perhaps even contribute to slowing down the natural aging process.

Attempts to achieve cell protection using antioxidants have already successfully been undertaken, many of them utilizing the avid reactivity of fullerene compounds with free radicals. The increase of mitochondrial concentrations of antioxidant drugs by selective targeting antioxidants to mitochondria in living cells should therefore be an effective therapy for a wide range of human diseases (Galley,
Two free radical scavengers, 4-hydroxy-2,2,6,6-tetramethylpiperidin-N-oxide (TEMPOL) and Salen-Mn(III) complex of o-vanillin (EUK-134) have been successfully synthesized and partially tested in term of their antioxidant and antiapoptotic properties (Dessolin et al., 2002).

The mitochondria targeted version of vitamin E protected mitochondria from oxidative damage induced by iron/ascorbate far more effectively than vitamin E itself, as measured by the level of both, lipid peroxidation (thiobarbituric acid reactive species) and protein damage (protein carbonyls) (Smith et al., 1999). Some antioxidants such as vitamin E, ubquinol and N-acetyl cysteine have been shown to decrease mitochondrial oxidative damage, but because these compounds were not accumulated within mitochondria their effectiveness was limited (Matthews et al., 1998; Sokol et al., 1998). Thus, antioxidants which can stay long inside mitochondria will be more effective. Recently, mitochondrially targeted versions of vitamin E (MitoE) and ubiquinone (MitoQ) were synthesized and characterized and found that they can selectively accumulate inside mitochondria and protect it from oxidative damage far more effectively than vitamin E and ubiquinone itself (Smith et al., 2008). Co-enzyme Q10 (CoQ10) and L-acetyl-carnitine can be considered to be safe adjunct to standard therapies in cardiovascular and neurological diseases (Fosslien, 2003). Carvediol, cardiovascular drug has been proved to be effective in heart failure probably mediated through its potential antioxidant and antiapoptotic activities (Feuerstein et al., 1998). Recently studies have been doing for the development of effective mitochondria-targeted antioxidants composed entirely of natural constituents. Lyamzaev et al. (2011) synthesized novel mitochondria-targeted antioxidants containing plant electron carrier and antioxidant plastoquinone conjugated by nonyloxy carbonylmethyl residue with berberine or palmatine, penetrating cations of plant origin.

This suggests that therapies designed to interfere with oxidative stress could be prevent from mitochondrial damages associated with oxidative stress and aging. Though many of the drugs used in the treatment of cardiovascular diseases, mainly statins are proved to be antioxidants, data from well-designed randomized trials to issue the general recommendation for people to take antioxidant supplements in order to prevent the oxidative stress induced disease is insufficient (Ajith et al., 2006; Ajith
et al., 2008). Thus, the area of sub-cellular, i.e. mitochondria-specific delivery of drugs is still in its infancy.

1.16. Importance of fungi

Higher fungi (mushrooms) have been used by mankind for millennia. Basidiomycetous fungi (mushrooms) can be defined as “macrofungi” with distinctive fruiting bodies that are large enough to be seen by the naked eye and to be picked by hand (Chang, 1996). Many mushrooms have long been valued as tasty, nutritious food by different societies worldwide. To the ancient Romans they were “the foods of the Gods” resulting from bolts of lightning thrown to the earth by Jupiter during thunder storms; the Egyptians considered them as “a gift from the God Osiris”; while the Chinese viewed them as “the elixir of life”. It is estimated that there are approximately 1.5 million species of fungi in the world of which approximately 70,000 species are described (Hawksworth, 2001). About 14,000 of the known species belong to the macro fungi, of which about 5,000 species are edible and over 1,800 species are considered to have medicinal properties (Chang, 1995). More varieties of mushrooms have been isolated and identified, and the number of mushrooms being cultivated for food or medicinal purposes has been increasing rapidly (Chang, 1995).

1.17. Ganoderma lucidum

*Ganoderma lucidum* species belong to the kingdom of Fungi, the division of Basidiomycota, the class of Homobasidiomycetes, the order of Aphyllophorales, the family of Polyporaceae (Ganodermataceae) and the genus of *Ganoderma* (Fig. 1.11) (Chang, 1995). *Ganoderma* species are among those fungi that can thrive under hot and humid conditions and are usually found in subtropical and tropical regions (Moncalvo and Ryvarden, 1997). *Ganoderma* species are not classified as edible mushrooms, as the fruiting bodies are always thick, corky and tough and do not have the fleshy texture characteristic of true edible mushrooms such as the common white button mushroom, *Agaricus bisporus*. Although they are not classified as edible, several types of *Ganoderma* products are available on the market (Jong and Birmingham, 1992).
Fig. 1.11. *Ganoderma lucidum* fruting body growing on the *Caesalpinia coriaria* tree
1.17.1. History

The Chinese and Koreans know it as Ling Zhi (mushroom of herb and immortality), whereas the Japanese call this mushroom reishi or mannentake (10,000 year mushroom). In Chinese, the mushroom is called "Ling Zhi", in Japanese “Reishi, Mannentake or Sachitake”, and “Youngzhi” in Korean. Chinese tradition proclaims that *Ganoderma* is also called “miraculous zhi”, or “auspicious herb” and is usually considered to “symbolise happy augury, and to bespeak good fortune, good health and longevity, even immortality” (Wasson, 1968).

According to the two famous Chinese herb medical books, *Shen Nong Ben Cao Jing* (25-220 A.D., Eastern Han Dynasty) and *Ben Cao Gang Mu* (1590 A.D., Ming Dynasty), there were six known species of *Ganoderma* (Ling Zhi) in China at that time, whereas now more than 250 species have been described (Moncalvo and Ryvarden, 1997) including: *G. adspersum*, *G. applanatum*, *G. australe*, *G. boninense*, *G. cupreum*, *G. incrassatum*, *G. lipsiense*, *G. lobatum*, *G. lucidum*, *G. oerstedii*, *G. oregonense*, *G. pfeifferi* *G. platense*, *G. resinaceum*, *G. sessile*, *G. sinense*, *G. tornatum*, *G. tsugae* and *G. weberianum*, to name a few. However, the majority of reports in the literature appear to be on the one species, *G. lucidum*.

1.17.2. Medicinal importance of *Ganoderma lucidum*

A number of reviews have described the bioactive substances, medicinal effects and health benefits of *Ganoderma* species (Sliva, 2004; Paterson, 2006). Medicinal uses of *G. lucidum* in ancient Far East countries included the treatment of neurasthenia, debility from prolonged illness, insomnia, anorexia, dizziness, chronic hepatitis, hypercholesterolemia, mushroom poisoning (antidote), coronary heart disease, hypertension, prevention of altitude sickness, treatment of “deficiency fatigue,” carcinoma, and bronchial cough in the elderly (Chang and Buswell, 1999; Zhou and Gao, 2002; Gao et al., 2004; Wasser, 2005).

1.17.3. Bioactive Substances in *Ganoderma* Species

Many bioactive compounds have been found in *G. lucidum* which contain properties conducive for normalizing and balancing the body, and as a result, they can enhance health and aid in relief of a multitude of diseases. Polysaccharides, triterpenes, sterols, lectins and proteins are some of the major active constituents that have been isolated
from *G. lucidum* and its closely related species, with the first two compounds being the most extensively investigated. These substances may be useful as starting materials for the development of chemical therapeutic agents in cancer treatment and for other ailments (Mizuno, 1995). Many investigations are currently underway to maximize the production and the utilization of these functional molecules (Fang and Zhong, 2002; Sliva, 2003). In addition, the modes of action of these compounds are being investigated (Eo et al., 2000; Sanodiya et al., 2009).

Over 300 reports have been published concerning the chemical constituents of *G. lucidum* and related species. The fruiting body, mycelia, and spores of *G. lucidum* contain approximately 400 different bioactive compounds, which mainly include triterpenoids, polysaccharides, nucleotides, sterols, steroids, fatty acids, proteins, peptides, and trace elements (McKenna et al., 2002; Smith et al., 2002). More than 200 polysaccharides have been isolated from the fruiting bodies, spores, mycelia and cultivation broth of Lingzhi (Wasser, 2002; Peng et al., 2005).

### 1.17.3.1. Polysaccharides

Polysaccharides, usually extracted from Lingzhi using the water extract and alcohol precipitating method, are the most important components, and represent a class of structurally-diverse biological macromolecules with wide-ranging physiochemical properties (Chen et al., 1998; Bao et al., 2001). The importance of polysaccharides (including protein/peptide bound polysaccharides) in pharmaceuticals has a long history, and has received considerable attention in recent years (Lin et al., 2005). For many years, Ganoderma polysaccharides (Ganopoly) had been the emphasis in Lingzhi research (Table 1.1).

The major bioactive polysaccharides isolated from *Ganoderma* species are glucans, β-1-3 and β-1-6 D-glucan. The basic structure is β-1-3 D-glucopyranan with 1 to 15 units of β-1-6 monogluicosyl side chains (Mizuno, 1995) (Fig. 1.12.A). Other antitumour polysaccharides of *G. lucidum* are heteropolysaccharides, glycoproteins (polysaccharides connected to proteins), or a group of polysaccharides known as ganoderans A, B and C (Lindequist, 1995).
Fig. 1.12.A Common structure of *Ganoderma lucidum* polysaccharides

Fig. 1.12.B Common structure of *Ganoderma lucidum* triterpenes

Ganoderic acid C (1): \( R_1, R_2 = 3-OH, R_3 = H, R_4 = 2-OH \)
Ganoderic acid B (2): \( R_1 = R_2 = 3-OH, R_3 = H, R_4 = O \)
Ganoderic acid AM (3): \( R_1 = 3-OH, R_2 = R_3 = O, R_4 = H \)
Ganoderic acid K (4): \( R_1 = R_2 = 3-OH, R_3 = \beta-OAC, R_4 = O \)
Ganoderic acid H (5): \( R_1 = 3-OH, R_2 = R_3 = O, R_4 = \beta-OAC \)

Fig. 1.12.C Mode of action of *Ganoderma lucidum* polysaccharides and triterpenes

Polysaccharides and Triterpenes from *Ganoderma lucidum*

- Macrophage
- NK cell
- T Lymphocyte
- Mast cell
- B cell
- TNF
- IL-1
- IL-2
- IL-5
- IL-6
- IL-12
- ICAM-1/ICAM-2/CTC
- TNF-alpha
- IL-6
- IL-12
- IFN-gamma
- TGF
- GM-CSF
- Histamine
- IgG

- Tumor regression
- Immunosurveillance
- Antiviral
- Antibacterial
- Anti-histamine
- Anti-inflammatory
- Anti-aging
<table>
<thead>
<tr>
<th>Name of the Polysaccharides</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-glucans</td>
<td>Inhibition of tumor growth of sarcoma –180 ascites in mice</td>
</tr>
<tr>
<td>Polysaccharide extracts</td>
<td>Inhibition of tumor growth of Lewis lung carcinoma in mice</td>
</tr>
<tr>
<td>Sulfated D-glucans</td>
<td>Inhibition of tumor growth of Ehrlich ascites carcinoma in mice</td>
</tr>
<tr>
<td>Polysaccharide extracts</td>
<td>Prevention of cancer chemically induced colon cancers in rats</td>
</tr>
<tr>
<td>Polysaccharides (GLPS)</td>
<td>Mucosal healing chemically induced gastric ulcers in rats</td>
</tr>
<tr>
<td>Polysaccharide (GLP)</td>
<td>Hepatoprotective effects against hepatic inflammation in mice</td>
</tr>
<tr>
<td>Protein-bound polysaccharides</td>
<td>Hepatoprotective effects</td>
</tr>
<tr>
<td>Glycans (ganoderan A and B)</td>
<td>Hypoglycemic activity in alloxan-induced diabetic mice</td>
</tr>
<tr>
<td>Polysaccharides (Gl-PS)</td>
<td>Hypoglycemic activity in alloxan-induced diabetic mice</td>
</tr>
<tr>
<td>Polysaccharide extracts</td>
<td>Antihypertensive effects in rabbits and rats</td>
</tr>
<tr>
<td><strong>Preclinical studies</strong></td>
<td></td>
</tr>
<tr>
<td>Ganopoly (polysaccharide extracts)</td>
<td>Stimulation of immune system, increased plasma concentration of IL-2, IL-6, IFN-γ advanced-stage cancer of lung, colon, breast, liver, prostate, bladder and brain</td>
</tr>
</tbody>
</table>
1.17.3.2. Triterpenes/Triterpenoids

Triterpenes/triterpenoids is one of the most important biologically active components of *G. lucidum*. The groups of triterpenes have received considerable attention because of their well-known pharmacological activities. These fat-soluble components in Lingzhi were heavily studied during the 1980s. Up to 2000, the total of triterpene compounds were summarized to 112 kinds and represented 24 structures (Lin, 2001). Currently, about 136 triterpene structures have been isolated (Kim and Kim, 1999; Huie and Di, 2004; Gao et al., 2005; Boh et al., 2007). Since the first isolation of two new triterpenes, ganoderic acids A and B, from the dried epidermis of *G. lucidum* by Kubota et al. (1982), more than 130 oxygenated triterpenes (mostly lanostane-type triterpenes) have been isolated from the fruiting bodies, spores, mycelia and culture media of Lingzhi (Huie and Di, 2004). These triterpenes could be divided into the C30, C27 and C24 compounds according to the number of carbon atoms and based on the structure and the functional groups (Luo and Lin, 2002; Gao et al., 2005) (Fig. 1.12.B). In general, triterpenoids have molecular weights ranging from 400–600 kDa and their chemical structures are more complex than the group of lanostanes because of their highly oxidized state (Table 1.2).

The fruiting bodies of *G. lucidum* contain some intensely bitter compounds (Lindequist, 1995) and it has been suggested that the fruiting body (and not the mycelium) of *G. lucidum* is the only species to contain bitter triterpenoids (Mizuno, 1995). It has also been suggested that the bioactivity is related to the bitterness (i.e. the more bitter, the greater the bioactivity), although the relationship is not fully understood (Mizuno, 1997).

1.17.3.3. Other Steroidal Constituents

Sterols, compounds closely related to triterpenoids, are also found in *Ganoderma* (Yokokawa and Mitsukaishi, 1981). They have been isolated from the fruiting body and mycelium (Strigina et al., 1971; Hirota et al., 1987; Chen and Yu, 1999).

2.17.3.4. Proteins

One of the most important proteins isolated from the mycelium of *G. lucidum* is Ling Zhi-8 (LZ-8) (Kino et al., 1989). LZ-8 is a polypeptide consisting of 110 amino acid residues with an acetylated amino terminus and has a molecular mass of 12 kDaltons.
<table>
<thead>
<tr>
<th>Name of the triterpene</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ganoderic acids U, V, W, X, Y</td>
<td>Cytotoxic against hepatoma cells</td>
</tr>
<tr>
<td>Ganoderic acids A, B</td>
<td>Inhibition of farnesyl protein transferase</td>
</tr>
<tr>
<td>Ganoderic acid F</td>
<td>Prevention of invasion of metastatic cells <em>in vivo</em></td>
</tr>
<tr>
<td>Ganoderic acid β</td>
<td>Inhibition of HIV-1 protease</td>
</tr>
<tr>
<td>Ganolucidic acid A</td>
<td>Inhibition of HIV-1 protease</td>
</tr>
<tr>
<td>Ganodermic acid S</td>
<td>Inhibition of Ca2+ mobilization in platelets</td>
</tr>
<tr>
<td>Ganoderic aldehyde A</td>
<td>Cytotoxic against hepatoma and nasopharyx carcinoma cells</td>
</tr>
<tr>
<td>Lucilaldehyde B, C</td>
<td>Cytotoxic against Lewis lung carcinoma (LLC), mouse sarcoma Meth-A, sarcoma 180, and breast cancer cells T-47D</td>
</tr>
<tr>
<td>Lucidimol A, B; Ganodermanondiol, Ganodermanontriol, Ganoderiol F</td>
<td>cytotoxic against LLC and Meth-A cancer cells</td>
</tr>
<tr>
<td>Ganodermanol, Ganodermadiol</td>
<td>cytotoxic against LLC, Meth-A, sarcoma 180, and T-47D cells</td>
</tr>
<tr>
<td>Ganodermanontriol, Ganoderiol F</td>
<td>HIV-1 inhibitors</td>
</tr>
<tr>
<td>Lucidimol B, Ganodermanondiol, Ganodermanontriol</td>
<td>Inhibition of HIV-1 protease</td>
</tr>
</tbody>
</table>
The native form of LZ-8, with a molecular mass of 24 kDaltons is a homodimer of the LZ-8 polypeptide (Tanaka et al., 1989). This protein has been shown to have mitogenic activity \textit{in vitro} and immunomodulating activity \textit{in vivo} (Kino et al., 1989; Haak-Frendscho et al., 1993; Van Der et al., 1995).

### 1.17.4. Pharmacological Functions

The extracts from the spore, mycelium and fruit bodies of Lingzhi have long been considered for good health. It can help enhance body’s immune system and improve metabolic functions (Fig.1.12.C) (Pero et al., 2005). Biological activities and pharmacological functions reported for \textit{Ganoderma species} include aspects of various effects on physiological function of human organs and systems including anti tumor effects, anti diabetic activity, hepatoprotective effects, cardioprotective effects and anti aging effects the major pharmacological activities have been summarized in Table 1.3.

#### 1.17.4.1. Antioxidant function of \textit{Ganoderma lucidum}

The majority of the studies concerning the \textit{G. lucidum} relate to the antitumour and antiviral effects, while the antioxidant properties associated with this fungus have only recently become apparent (Yen and Wu, 1999; Zhu et al., 1999; XiaoPing et al., 2009; Liu et al., 2010). Protein bound polysaccharides from \textit{G. lucidum} (Lee et al., 2001; Yuhong and Zhibin, 2002; Liu et al., 2010), methanolic extracts from \textit{G. tsugae} (Yen and Wu, 1999) and ethanolic extracts from \textit{G. lucidum} (Lakshmi et al., 2004) have exhibited superoxide and hydroxyl radical scavenging activity. A hot water extract of \textit{G. lucidum} has exhibited antioxidative effect on mouse liver and kidney lipid peroxidation (Shieh et al., 2001).

Studies showed that \textit{G. lucidum} have significant 2,2-diphenyl-1-picrylhydrazil (DPPH), 2, 2’-azinobis (3-ethylbenzothiazolin-6-sulphonic acid) (ABTS), superoxide and hydroxyl radical scavenging activities, ferric reducing power and lipid peroxidation inhibition properties (Liu et al., 1997; Joseph et al., 2009; Karaman et al., 2010). \textit{G. lucidum} polysaccharides isolated in China exhibited significant the antioxidant potential in \textit{in vitro} assays, and could enhance the antioxidant enzyme activities (SOD, CAT and GPx) (XiaoPing et al., 2009). Again, recently two low-
<table>
<thead>
<tr>
<th>Pharmacological activity</th>
<th>References</th>
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<tr>
<td>Anti-Tumor</td>
<td>Liu et al., 2005; Zhou et al., 2007; Chang et al., 2009; Hsieh and Wu, 2011; Xu et al., 2011</td>
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<td>Antidiabetic</td>
<td>Hikino and Mizuno, 1989; Hikino et al., 1989; Gao et al., 2004; He et al., 2006; Ma et al., 2007; Zhou et al., 2007; Seto et al., 2009; Chen et al., 2011; Teng et al., 2011</td>
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<tr>
<td>Hepatoprotection</td>
<td>Lin et al., 1995; Kim et al., 1999; Shieh et al., 2001; Lin et al., 2002; Zhang et al., 2002; Zhou et al., 2002; Sheena et al., 2005; Lakshmi et al., 2006; Lin and Lin, 2006; Yang et al., 2006; Wang, et al., 2007; Shi et al., 2008; Yan-ling et al., 2008</td>
</tr>
<tr>
<td>Cardioprotection</td>
<td>Lee and Rhee, 1990; Sheena et al., 2005; Lasukova et al., 2008; Wong et al., 2004; Chu et al., 2011</td>
</tr>
</tbody>
</table>
molecular-weight polysaccharide were isolated from *G. lucidum* having potent antioxidant activity (Liu et al., 2010)

**1.17.4.2. Anti aging function of *Ganoderma lucidum* **

Antiaging agents could be, in a certain sense, attentive to the well-being of the aged. There are quite a lot of medicinal plants and prescriptions recorded in Chinese medical literatures aimed at the well-being of the aged as well as the prevention of diseases and prolongation of life-span. Mushrooms are a whole different category of food that has different nutrients and substances that can use to keep us young. There are a number of medicinal plants which have been used as or related to the antiaging agents such as *Herba Epimedii, Fructus Lycii, Radix Polygoni multiflori, Radix Cynanchi auriculati, Ginseng, Radix Astragali seu Hedysari, Radix Angelicae Sinensis, Herba Epimedii, Cordyceps, G. lucidum, seu Japonicum, Radix Polygoni Multiflori, Radix Acanthopanacis Senticosi, Rhizoma Polygonati, Fructus Lycii, and Poria* in China for centuries (Xiao et al., 1993; Chen and Li, 1994). The oriental fungus, *G. lucidum* (Lingzhi) has been used for centuries by Asian people to promote health and increase longevity (Wasser and Weis, 1991).

In a clinical trial with 30 elderly people *G. lucidum* extract (GLE) was given oral 1.5 g 3 times daily for 30 days. Interleukin-2 and interferon (IFN) production by peripheral mononuclear cells (PBMC) and NK cell activity *in vitro* were respectively measured. Production of IL-2 and IFN were significantly increased after GLE treatment. Such results could suggest that GLE is a possible treatment to raise the cellular immunological activity in aging people (Tao and Feng, 1991; Tao, 1993). The methanol and chloroform extracts of *G. lucidum* exhibited antioxidative activities. The chloroform extract showed significant superoxide scavenging activity. The polysaccharides, polysaccharide–peptide complex (and phenolic components) of *G. lucidum* have been also shown to have significant antioxidant activities.

Again, a randomized, double-blind, placebo-controlled study in the Chinese population has shown that *G. lucidum* can effectively reduce the clinical score of fatigue (Tang et al., 2005). Another clinical study done in Hong Kong has shown that there was no significant changes in routine coagulation screen, fibrinogen concentration, von Willebrand ristocetin cofactor activity, platelet function and thrombelastography activity in healthy volunteers receiving *G. lucidum* orally for 4
weeks (Kwok et al., 2005). Therefore, this study suggests that *G. lucidum* has no adverse effect on healthy people and that it is safe for clinical trial.

*G. lucidum* has been reported to attenuate Aβ-induced synaptotoxicity by preserving a synaptic density protein, synaptophysin, in cultured neurons. It has been shown that Aβ peptide, triggers activation of caspase-3 resulting in neuronal apoptosis (Harada and Sugimoto, 1999) and is the main reason behind Alzheimer's disease (AD). Thus *G. lucidum* is a potent drug against the A. D which is a major age related neurological disease. Another study shows that *Ganoderma lucidum* can prevent age-related deteriorations in learning and memory ability in the brain of SAMP8 mice by the enhancement of enzymatic antioxidant defenses such as activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd) of RBC, brain and liver and decreased amyloid β -peptide accumulation of brain in aged SAMP8 mice (Wang et al., 2004).

A *G. lucidum* polysaccharide GLB7 decreased the production of oxygen free radicals and antagonised the respiratory burst induced by PMA in murine peritoneal macrophages (Li and Lei, 2000). Recently, two novel ergosterol derivatives, ganodermasides A and B, hydroxylated at C-15 were isolated from *G. lucidum*, showed to extend the replicative life span of *Saccharomyces cerevisiae*, yeast of K6001 strain. Based on results of the investigation of the mechanism of biological activity, ganodermasides A and B regulated UTH1 expression, a yeast-aging gene that has been ascribed to the stress resistance and longer life span of mutants (Camougrand et al., 2004), in order to extend the replicative life span of yeast (Weng et al., 2010). Such observations could imply that the polysaccharide-induced inhibition of oxygen free radicals in murine peritoneal macrophages play an important role in the anti-aging effect of *G. lucidum* extracts. However detailed studies are necessary to evaluate the exact anti aging mechanism of *G. lucidum*

1.18. Relevance of the study

*G. lucidum* has been traditionally used as a popular folk medicine for the promotion of health in the Orient. *G. lucidum* has been shown to be safe and prevents many chronic diseases in clinical practice. Previous investigations from south India showed that *G. lucidum* occurring in South India possessed significant antioxidant, antitumor, anti-inflammatory, antimutagenic, and radioprotective properties (Jones and
Janardhanan, 2000; Lakshmi et al., 2003; Pillai et al., 2008). But there are no studies related to the mitochondrial protective effect of this miracle mushroom against oxidative stress. This prompted us to evaluate the effect of *G. lucidum* on the oxidative stress induced declined cellular energy status in various models.