INTRODUCTION
"When meditating over a disease, I never think of finding a remedy for it, but instead, a means of preventing it."

LOUIS PASTEUR (1884)

The aerobic life style has great advantages but is full of danger. Oxygen, the essential life supporting molecule, can be one of the most destructive reagents in nature. Oxygen supplied at concentrations greater than those in normal air has long been known to damage plants, animals and aerobic bacteria. In 1954, Rebecca Gershman and Daniel Gilbert proposed that many of the damaging effects of oxygen could be attributed to the formation of oxygen radicals\(^1\) This hypothesis was developed by Fridovich, in 1978, into a superoxide theory of oxygen radical which, \textit{in vivo}, plays a major role in the toxic effects of oxygen\(^2\).

1.1 FREE RADICALS

A free radical may be defined as any molecular species that has one or more unpaired electrons. This broad definition includes the hydrogen atom which has one unpaired electron, most transition metals and the oxygen molecule itself\(^3\). The presence of one or more unpaired electrons causes the species to be slightly attracted to a magnetic field and sometimes makes the species highly reactive. Free radicals can be formed by the loss of a single electron by a non-radical, or by the gain of a single electron by a non-radical. They can also be formed when a covalent bond is broken and if one electron from each of the pair shared remains with each atom. This process is known as homolytic fission\(^4\).
The different cytotoxic oxygen species are given in Table 1.1.

Table 1.1: Cytotoxic oxygen species

<table>
<thead>
<tr>
<th>Species</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_2^-$</td>
<td>Superoxide free-radical</td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>OH$^-$</td>
<td>Hydroxyl free-radical</td>
</tr>
<tr>
<td>$^1$O$_2$</td>
<td>Singlet oxygen</td>
</tr>
<tr>
<td>R-O$^*$</td>
<td>Peroxyl free-radical</td>
</tr>
<tr>
<td>R-OO$^*$</td>
<td>Alkoxy free-radical</td>
</tr>
<tr>
<td>ROOH</td>
<td>Hydroperoxide</td>
</tr>
<tr>
<td>R-Lipid</td>
<td>Lipid hydroperoxide</td>
</tr>
</tbody>
</table>

There are free radicals other than the ones associated with oxygen. Metals in the first row of the d-block of the Periodic Table contain unpaired electrons and can qualify as radicals. As observed by Fenton in 1894, a mixture of hydrogen peroxide and an Fe$^{2+}$ salt reacts with many organic molecules. The reactivity is most likely due to the formation of the hydroxy radical

$$\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Intermediate complex} \rightarrow \text{Fe}^{3+} + \text{OH}^+ + \text{OH}^-$$

Similarly, copper salts(Cu$^+$) are thought to react with H$_2$O$_2$ to make hydroxyl radicals.

$$\text{Cu}^+ + \text{H}_2\text{O}_2 \rightarrow \text{Cu}^{2+} + \text{OH}^+ + \text{OH}^-$$
Free radicals are also associated with sulphur compounds. For example, thiol (R-SH) compounds oxidize in the presence of a transition metal ion to form thyl radicals (RS'). Thyl radicals can also be formed by the homolytic fission of disulphide bonds in proteins.

Carbon centred radicals are formed in many biological systems during the detoxification of chlorinated hydrocarbons. During the metabolism of carbon tetrachloride by liver microsomes the trichloromethyl radical is formed.

\[
\text{Cytochrome P450 system} \quad \begin{array}{ccc} \text{CCl}_4 \rightarrow \text{CCl}_3 + \text{Cl}^- \end{array}
\]

Nitrogen and Phosphorus centred radicals are also seen.\(^3\)

All the above mentioned free radicals have a single unpaired electron in its outer orbit. These free radicals are highly unstable and the energy created by this unstable configuration is released through reactions with molecules in the cell membranes and nucleic acids. Also these free radicals initiate autocatalytic reactions whereby molecules with which they react are themselves converted into free-radicals to propagate a chain of damage.\(^6\) Within the cells, free radicals can be initiated in different ways as shown below.

- **Absorption of radiant energy**: Ionising radiation, such as ultraviolet light and X-rays passing through water produces hydrogen atoms, hydrated electrons and hydroxyl radicals. These, in the presence of dissolved oxygen, secondarily yield superoxide radical and hydrogen peroxide.\(^2,7\)
- **Metabolism of toxic chemicals and drugs:** Free radicals are produced during metabolism of toxic compounds. For example, during the metabolism of halogenated hydrocarbons such as carbon tetrachloride, a highly toxic free-radical (CCl$_3^-$) is formed$^8$.

- **Normal metabolism:** During the reduction-oxidation reactions that occur during normal metabolic processes, free radicals are formed. For example, metabolic generation of superoxide radical has been shown to occur in stimulated neutrophils$^9$.

- **Transition metals:** Some of these metals can donate or accept free electrons during intracellular reactions and catalyze free radical formation. For example iron, in Fenton’s reaction, catalyses the formation of hydroxyl radical$^{10}$ In a similar kind of reaction, cobalt can also react with hydrogen peroxide to generate hydroxyl radical$^{11}$.

- **Nitric oxide:** This is an important chemical mediator generated by endothelial cells, macrophages and neurons. This molecule can act as a free radical and can also be converted to highly reactive peroxynitrite anion$^{12}$.

These reactive species have been implicated in various diseases such as rheumatoid arthritis, cancer, diabetes, epilepsy and atherosclerosis$^{3,13,14,15,16,17}$.

### 1.2 EFFECTS OF REACTIVE OXYGEN SPECIES

The effects of these reactive species are wide ranging, but four reactions are particularly relevant to cell injury.
- **Lipid Peroxidation**: The polyunsaturated fatty acids of cell membranes are attacked repeatedly and severely by reactive oxygen species to yield highly destructive lipid hydroperoxy radicals and lipid hydroperoxides. This reaction is termed lipid peroxidation. The lipid peroxides are decomposed by transition metals such as iron. Lipid peroxidation is propagated to other sites causing widespread membrane damage and destruction to organelles\textsuperscript{18}.

- **Oxidation of Proteins**: Oxygen-derived free radicals cause cell injury by oxidation of protein macromolecules of the cells, cross linking of labile amino acids as well as by fragmentation of polypeptides directly. The end result is degradation of cytosolic neutral proteases and cell destruction\textsuperscript{18,19}.

- **DNA damage**: Free radicals cause breaks in the single strands of the nuclear and mitochondrial DNA. This results in cell injury. This DNA damage has been implicated in cell aging and in malignant transformation of cells\textsuperscript{20,21}.

- **Cytoskeletal damage**: Reactive oxygen species are also known to interact with cytoskeletal elements and interfere in mitochondrial aerobic phosphorylation and thus cause ATP depletion\textsuperscript{22}.

These changes cause cell injury. Cell injury is reversible up to a certain point, but if the stimulus persists or is severe enough from the beginning, the cell reaches a point of no return and suffers irreversible cell injury and cell death. There are two principal patterns of cell death, necrosis and apoptosis\textsuperscript{23}. Necrosis refers to a spectrum of morphologic changes that follow cell death in living tissue, largely
resulting from the progressive degradative action of enzymes on the lethally injured cell\textsuperscript{6}.

Apoptosis, otherwise called as programmed cell death, is a type of cell death in which an individual cell changes from an intact metabolically active state into a number of shrunken remnants retaining their membrane bound identity\textsuperscript{23}. Reactive oxygen species have been known to activate apoptosis\textsuperscript{24,25}.

1.3 ANTI-OXIDANT ENZYMES

As a result of the above changes, cells have inherently developed multiple mechanisms to remove free radicals and thereby minimize injury. There are several enzymatic and non-enzymatic systems that contribute to inactivation of free-radical reactions (Fig.1.1a). There are a group of enzymes that act as free-radical scavenging systems and they break down hydrogen peroxide and superoxide anion. These are collectively called as anti-oxidant enzymes. They include the following:

(i) Catalase (CAT): This enzyme is present in virtually all mammalian cell types. Only one form is seen and it has a molecular weight of 240,000 in rat liver. It is made up of 4 subunits and it is generally located in the peroxisomes. This is a haem containing enzyme and this enzyme catalyses the following reaction\textsuperscript{26}.

\[
\text{Catalase} \quad 2\text{H}_2\text{O}_2 \xrightarrow{\text{Catalase}} 2\text{H}_2\text{O} + \text{O}_2
\]
Fig. 1.1a Antioxidant protection in a cell

Fig. 1.1b Oxidative stress
(ii) **Superoxide dismutase (SOD)**: This is a widely distributed enzyme and it exists in a variety of forms. There are three distinct types of SOD classified on the basis of metal co factor: the copper/zinc SOD (Cu/ZnSOD), the manganese SOD (MnSOD) and the iron SOD (FeSOD) isoenzymes. The MnSOD is found in the mitochondria of eukaryotic cells, Cu/ZnSOD is found in the cytoplasm and FeSOD is seen in the chloroplasts. This enzyme catalyses the dismutation of superoxide to hydrogen peroxide and oxygen.

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

(iii) **Glutathione peroxidase (GPx)**: These are selenium containing enzymes that reside in the cytosol and mitochondrial matrix. This enzyme is found in high activity in the liver and erythrocytes where it was first discovered. It catalyses the reduction of hydrogen peroxide and organic hydroperoxides including those derived from unsaturated lipids to alcohol at the expense of glutathione.

\[
\text{GPx} \quad \text{ROOH} + 2\text{GSH} \rightarrow \text{ROH} + \text{H}_2\text{O} + \text{GSSG}
\]

\[
\text{GPx} \quad \text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow 2\text{H}_2\text{O} + \text{GSSG}
\]

(iv) **Haem-peroxidase**: Examples of this enzyme include horse radish peroxidase, lactoperoxidase and other mammalian peroxidases. This enzyme catalyses the oxidation of a wide variety of electron donors with the help of hydrogen peroxide and thereby scavenges endogenous hydrogen peroxide.
Glutathione-s-transferases (GST): These enzymes are widely distributed among eukaryotes and are often present in relatively high concentrations intracellularly. These enzymes are dimeric in nature with a molecular weight between 40,000 and 50,000 Da. In rat liver alone, seven distinct forms of GST's have been separated. These enzymes are widely distributed in higher animals and perform several detoxification functions. They conjugate a variety of pharmacologically active compounds such as potential alkylating agents as well as reactive metabolites with glutathione\(^\text{33}\).

Glutathione reductase (GR). This enzyme is a flavoprotein containing one mole of flavine adenine dinucleotide per enzyme subunit. The prosthetic group is linked non-covalently to the enzyme. The function of this enzyme is to regenerate glutathione which has been oxidized.

\[
\text{GR} \quad \text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{NADP}^+
\]

The enzyme contains a cysteine moiety that undergoes reduction and oxidation during the catalytic cycle. It is highly active in liver, kidney and many other mammalian tissues\(^\text{34,35}\).

Glucose-6-phosphate dehydrogenase (G-6-PD): This is considered to be an antioxidant enzyme as it reduces NADP\(^+\). It catalyses the following reaction\(^\text{26}\).
1.4 CELLULAR ANTIOXIDANTS

Apart from antioxidant enzymes, the cells have antioxidants for fighting against free radicals. Antioxidants are endogenous or exogenous substances which inactivate free-radicals. The common cellular antioxidants are given below.

Glutathione: This is a tripeptide (γ-glutamyl cysteinyl glycine) and it is predominantly a thiol compound. It is synthesized by three precursor amino acids namely γ-glutamate, cysteine and glycine. It is mainly involved in detoxification reactions through conjugation reactions. It is also involved in thiol transfer, destruction of free-radicals and metabolism of various exogenous and endogenous compounds\(^{34}\).

Ascorbic acid: (Vitamin C) The specific role of ascorbic acid is to protect the mammalian tissues against oxidation damage, both at the extracellular and intracellular levels. Ascorbic acid is a powerful reducing agent and therefore it can act as an antioxidant\(^{35,36}\).

α Tocopherol: (Vitamin E) Vitamin E refers to tocopherols in general, but it is α tocopherol that has antioxidant activity. This molecule is able to ‘repair’ oxidizing radicals directly and therefore is a chain-breaking antioxidant. It is evident that ascorbic acid and α tocopherol function together to protect lipids from damage\(^{37,38,39,49,41}\).
β Carotene: (Vitamin A) Carotenoids have been considered antioxidants because of their capacity to relieve oxidative stress. β Carotene is a scavenger of singlet oxygen Vitamin A has also been shown to potentiate carbon tetrachloride induced hepatotoxicity.²²,³³,⁴⁴

Haemoglobin: Oxyhaemoglobin present in erythrocytes acts as a scavenger of superoxide radical that is continuously generated in the erythrocytes.⁴⁵

1.5 EXTRACELLULAR ANTIOXIDANTS

In addition there are extracellular antioxidant defenses in human blood plasma. They are given in Table 1.2.

Table 1.2 The antioxidant defenses of human plasma

<table>
<thead>
<tr>
<th>Antioxidant defense</th>
<th>Mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transferrin/Lactoferrin</td>
<td>Binds to iron and prevents its participation in lipid peroxidation and non-catalysed OH⁺ radical formation.</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>Catalytically oxidizes Fe(II) to Fe(III) without release of oxygen intermediates. Also inhibits iron and copper dependant lipid peroxidation</td>
</tr>
<tr>
<td>Albumin</td>
<td>Binds to copper tightly and iron weakly and prevents their oxidative effects.</td>
</tr>
<tr>
<td>Haptoglobin/ Haemopexin</td>
<td>Binds free haemoglobin or haem which release iron readily on exposure to H₂O₂</td>
</tr>
<tr>
<td>Uric acid</td>
<td>Inhibits lipid peroxidation and scavenges radicals.</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Lipid soluble antioxidant which breaks chains by trapping peroxy and alkoxy radicals.</td>
</tr>
<tr>
<td>Glucose</td>
<td>A scavenger of OH⁺ radical</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>May protect albumin bound fatty acids from peroxidation.</td>
</tr>
</tbody>
</table>

Source: B Halliwell and JMC Gutteridge in 'Free radicals in Biology and Medicine'⁴."
1.6 OXIDATIVE STRESS

In aerobic biota, the oxygen free radicals are formed in normal cell metabolism from molecular oxygen. Despite tight antioxidant defenses, these oxygen free radicals cause constant damage to oxidizable molecules which are repaired or replaced in a dynamic equilibrium. The condition of cellular oxidative stress arises either from over production of oxygen free radicals or from the deficiency of antioxidant defenses or repair mechanisms and results in reversible or irreversible tissue injury (Fig.1.1b). As a result of oxidative stress, programmed cell death or apoptosis gets initiated as mentioned before.

1.7 OXIDATIVE STRESS INDUCED APOPTOSIS

Many of the chemical and physical stimuli capable of inducing apoptosis are known to evoke oxidative stress by increasing the steady state concentration of reactive oxygen species. These molecules are highly reactive and these non-specific molecules can induce and mediate the well co-ordinated and controlled changes that occur during apoptosis. Some of the agents that cause apoptosis may not be free radicals but they may induce reactive species production and may or may not decrease the reduction capacity of the cell\textsuperscript{25}. Apoptosis that results is a form of cell death that is genetically controlled\textsuperscript{46,47}.

1.8 MOLECULAR CONTROL OF APOPTOSIS

Mechanisms controlling apoptosis was first studied in \textit{Caenorhabditis elegans}. Their programmed pattern of cell growth followed by cell death has allowed the identification of specific genes. Exposure of cells to oxidizing agents
and radiation induces apoptosis by a mechanism that is initiated by DNA damage. 

*P53*, a tumour suppressor gene product accumulates when DNA is damaged and arrests cell cycle at the G1 phase. This gives the cell additional time for repair. However if the repair process fails, *p53* triggers apoptosis. Another important molecular regulator of apoptosis in mammalian cells is the *c-myc* proto-oncogene. *c-myc* is involved in coupling the response to proliferation. There are also genes involved in inhibition of apoptosis. The first gene to be clearly associated with inhibition of apoptosis was *Bcl-2*. It was cloned from the break point of the 14:18 translocation found in the majority of follicular lymphomas. *Bcl-2* can block apoptosis in a variety of ways. It is observed that

(i) increased synthesis of extra cellular *Bcl-2* blocks the increase in reactive oxygen species associated with apoptosis.

(ii) *Bcl-2* inhibits reactive oxygen species induced apoptosis and

(iii) *Bcl-2* can act as an antioxidant.

It has been suggested that oxidative stress induced apoptosis involves alterations in the above said genes.

1.9 CARBON TETRACHLORIDE AS A TOXICOLOGICAL MODEL

A large number of methods to produce liver cirrhosis in laboratory animals have been introduced. Most of them are difficult to standardize and their reproducibility is often low. Carbon tetrachloride is known to have a selective hepatotoxic action and a number of investigators have utilized this chemical to produce liver cirrhosis in experimental animals.
The toxic effect of CCl₄ is due to its activation by cytochrome (CYP)2E1, CYP2B1 or CYP2B2 and possibly CYP3A, to form the trichloromethyl radical CCl₃⁺. This free radical produced locally causes auto-oxidation of polyenic fatty acids present within the membrane of phospholipids.

The CCl₃⁺ radical can also react with oxygen to form the trichloromethyl peroxyradical CCl₃OO⁺ which is a highly reactive species. This radical initiates the chain reaction of lipid peroxidation which attacks and destroys polyunsaturated fatty acids, in particular those associated with phospholipids. This affects the permeabilities of mitochondrial, endoplasmic reticulum and plasma membranes, resulting in the loss of cellular calcium sequestration and homeostasis, which can contribute heavily to subsequent cell damage. Also, these free radicals and the promoted lipid peroxidation process of liver microsomal lipids are able to attack DNA bases guanine, cytosine and thymine to give altered bases. These can function as initiators of liver carcinogenesis.

In short, there is rapid breakdown of the structure and function of the endoplasmic reticulum due to decomposition of lipids. Usually within thirty minutes of administration of CCl₄, there is a decline in hepatic protein synthesis. Within a couple of hours, there is swelling of smooth endoplasmic reticulum and dissociation of ribosomes from the rough endoplasmic reticulum. Lipid export from the hepatocytes is reduced because of their inability to synthesize apoprotein to complex with triglycerides and thereby facilitate lipoprotein secretion. The result is the fatty liver characteristic of CCl₄ poisoning. Mitochondrial injury occurs after this, followed by progressive swelling of the cells due to increased permeability of
the plasma membrane. Damage to the cell membranes is supposed to be caused by relatively stable fatty aldehydes, which are produced by lipid peroxidation in the smooth endoplasmic reticulum. This event is followed by massive influx of calcium and cell death\textsuperscript{56,57,58}. This is schematically represented in Figure 1.2.

1.10 HERBAL MEDICINE

Herbal medicines are the synthesis of therapeutic experiences of generations of practicing physicians of indigenous systems of medicine for over hundreds of years. Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They are also in great demand in the developed world for primary health care because of their efficacy, safety and relatively lower side effects. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. They also offer therapeutics for age-related disorders such as memory loss, osteoporosis and immune disorders for which no modern medicine is available. India is one of the twelve mega biodiversity centers having over 45,000 plant species\textsuperscript{59}. There are many plant extracts which have been shown to protect against oxidative stress.

In this study \textit{Crataegus oxyacantha} L, was used for evaluation of its antioxidative effects.

1.10 CRATAEGUS OXYACANTHA

\textit{Crataegus oxyacantha} L. is commonly called as HAWTHORN. It is a member of the Rosaceae family. It is a multi branched shrubby tree that can reach a
CCL₄

CCL₃⁻

Lipid radicals

LIPID PEROXIDATION

Membrane damage to RER

Polysome detachment

Decreased apoprotein synthesis

FATTY LIVER

Release of products of lipid peroxidation

Damage to plasma membrane

Increased permeability to Na⁺, Ca²⁺, H₂O

Cell swelling

Massive influx of Ca²⁺

Inactivation of mitochondria, cell enzymes and denaturation of proteins

CELL DEATH

Fig. 1.2 CCL₄ toxicity in the liver
height of up to 10 metres (Plate 1.1). It is native to Europe, West Asia and North Africa. Hawthorn berries are also used in Homeopathic medicine. It acts on the muscles of the heart and is a heart tonic. Traditionally, the berries are used for their astringent properties in heavy menstrual bleeding and in diarrhoea. Both the flowers and berries act as diuretics and can be used to treat kidney problems and dropsy.

The total extracts of both the flowers and berries have been recommended to treat cardiac failure, arteriosclerosis, hyperlipidaemia, hypertension, angina pectoris and a variety of geriatric conditions. Clinical studies have found that standardized extracts of hawthorn show promise as adjunctive agents for the treatment of left ventricular dysfunction. The extract has also been shown to have hypolipidemic action. The extract also lowered levels of beta-lipoprotein. Similarly, rats fed on an atherogenic diet showed that *Crataegus* extract increased the LDL receptor-binding capacity in the liver.

Antioxidant effects have been already demonstrated in some species of *Crataegus*. *Crataegus laevigata* and *Crataegus monogyna* plants subjected to drought and cold stress treatments have been assayed for antioxidant capacities and have been found to contain the same. Similarly, extracts of *in vitro* callus and cell suspension cultures of *Crataegus monogyna* have been shown to have antioxidant activities. Also tincture of *Crataegus* has a protective effect on oxidative stress in experimental atherosclerosis in rats.

*Crataegus* extract contains amygdalin, Crategolic acid, the alkaloid crataegin, a mixture of saponins, triterpene acids such as oleanolic acid and ursolic
Crataegus berries

Crataegus Plant

Plate 1.1 Crataegus oxyacantha L.
acid, purine and flavonoid glycosides\textsuperscript{64}. Some \textit{Crataegus} constituents are predicted to be good antioxidants. The flower and fruit constituents responsible for free radical scavenging activity are epicatechin, hyperoside and chlorogenic acid. The phenolic compounds it contains also have antioxidant activity. In \textit{Crataegus monogyna} the antioxidant activity was related to the phenolic contents of flowers\textsuperscript{71}. There has been no toxicity directly related to \textit{Crataegus} preparations. The acute parenteral toxicity (LD\textsubscript{50}), tested in different animals, was found to be in the range of 18-34ml/kg\textsuperscript{74}. Thus, it was chosen as a candidate suitable to evaluate its antioxidant effects.

1.11 RATIONALE AND OBJECTIVES

The normal functions of the body result in cell damaging molecules called free radicals. These molecules are formed as a result of oxidative reactions in the cell. Ultraviolet radiation from the sun, pesticides, tobacco smoke, fumes and other air pollutants can also lead to free radical formation. These free radicals can damage cells and tissues. Such damage may be one factor in the development of chronic diseases. Antioxidants can prevent some of this damage by squelching free radicals and rendering them inactive.

It is evident that the three major antioxidant nutrients namely, vitamin C, vitamin E and $\beta$ carotene play a crucial role in preventing or delaying the onset of major degenerative diseases. It has also been demonstrated that antioxidants may be beneficial in enhancing the immune system as well as aiding in the prevention and treatment of pulmonary as well as inflammatory diseases such as rheumatoid arthritis.
As such, researchers are concentrating on identifying an array of promising antioxidants that may purge the body of cell damaging free radicals. Among the ones identified are bioflavonoids and carotenoids such as lycopene, lutein and zeaxanthin. Synthetic antioxidants such as butylated hydroxy toluene, propyl gallate and potassium sorbate have also been shown to have antioxidant properties.

In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show the immense potential of medicinal plants used in various traditional systems. The traditional systems of medicines may be using these plants in their therapeutic modalities unaware of the fact that these plants may be actually acting as antioxidants. For the present study, Crataegus oxyacantha extract was evaluated for its antioxidant properties.

To start with, the non-toxic nature of the drug was established. Haematological and biochemical parameters which were of toxicological interest were estimated in control and drug treated groups. Carbon tetrachloride was used as a toxicant to induce oxidative stress. Carbon tetrachloride continues to provide an important service to elucidate mechanisms of action of hepatotoxic effects such as fatty degeneration, fibrosis, hepatocellular death, and carcinogenicity. The toxicity of this molecule depends upon its ability to form free radicals, since the toxic effect is inversely related to the ease of dissociation of the carbon-chlorine bond. Carbon tetrachloride is microsomal reduced by mixed function oxidases to reactive trichloromethyl radical. Since the liver is the site of almost all types of detoxification reactions, the hepatoprotective nature of the drug was evaluated. Renal toxicity due to carbon tetrachloride is less dramatic than hepatotoxicity. Nevertheless, the
protective effects of the drug on the kidney was also evaluated. The red blood cell is almost always exposed to oxidative stress and therefore red blood cells were also taken for evaluation.

Once the antioxidant potential of the drug was established in the in vivo model, the potential was evaluated in vitro in the human model. Blood tissue from normal donors were obtained and both the peripheral lymphocytes and erythrocytes were used for analysis. Hydrogen peroxide was used to induce oxidative stress. The potential of the drug to prevent hydrogen peroxide induced apoptosis in lymphocyte was also studied.