Chapter-1

Evaluation of some selected natural products for Antioxidant Activity and their role in Reactive Oxygen Species mediated Oxidative Stress in Biological system

1.1 Status of Medicinal Plants in the Health care System

India is endowed with a rich wealth of medicinal plants. These plants have made a good contribution to development of *Materia medica*, one of the earliest treatises on Indian medicine. Charaka Samhita (1000 BC) records the use of over 340 drugs of vegetable origin. Thus, despite the rich heritage of knowledge on the use of plant drugs, little attention had been paid to grow them as field crops in the country [1].

During the past seven or eight decades, there has been a rapid extension of the Ayurvedic system of medical treatment in India [2]. The curative properties of drugs due to the presence of complex chemical substances of varied composition (present as secondary plant metabolites) in one or more parts of these plants. The alkaloids form the largest group, which includes morphine and codein (Poppy), strychnine and brucine (*Nux vomica*), quinine (*Cinchona*), ergotamine (*Claviceps*), hyocyamine (*Belladonna*), scolapomine (*Datura*), emetine (*Ipecac*), ephedrine (*Ephedra*), reserpine (*Rauwolfia*), caffeine (*Thea*) and a large number of others [3]. Glycosides form another important group represented by digoxin (*Foxglove*), strophanthin (*Strophanthus*),
glycyrrhizin (Liquorice), etc. Steroids have come into prominence recently and
diosgenin (Dioscorea), solasodin (Solanum) etc, now command large world
demand.

Apart from the above, taxol (Taxus baccata), used for the treatment of
ovarian cancer and Indian Himidesmus indicus is reported to be used to cure
34 types of diseases, Aegle marmelos 31 types and Phyllanthus embilica, 29
types of disease etc. Currently approximately 25% of drugs in market were
derived from plants, and many other synthetic analogues are built prototype
compounds isolated from plant species [4]. In India, nearly 17,000
species of higher plants, and 7500 are known for medicinal uses. As per the
literature, a list of 2,97,000 species were available on the world flora, out of
which only 10% are medicinally useful. About 171 species are potentially
useful for various ailments. Interestingly out of 675 species available in the
Himalyan regions as an edible and for curing various diseases [5]. In our
country, 75% of the population use herbal medicines for their health care.
Indian systems of medicine believe that complex diseases can be treated with
complex combination of herbs, unlike in the West with single drugs [5,6].

Decades of research in Medicinal Plants have led to the improvement of
health care but the search for newer drugs for other ailments still goes on and
has reached unprecedented levels that we never contemplated during the last
century. The Pharmaceuticals from Medicinal plants thus, serves as an
important source for varied range of pharmaceutically active drugs, which
forms the very basis and the remedy in the cure of diseases. Recent trend
witnessed by researchers world wide is the incorporation of these pharmaceutically significant natural products into formulation by the herbal industry for example, in the treatment of liver disorders, more than an estimated 40 different formulations are presently available in the Indian market, some of which have indeed become household names like Liv-52, Livol etc., which contains the chief principles namely silybin, andrographolide and the extracts of Picrorrhiza kurroea and Scipta alba [7]. The never ending inquisitive nature of mankind to constantly explore and reveal to the world of science remains inextinguishable and further paves the way for the search of newer and effective pharmaceuticals.

1.2 Promising lead molecules of Biological interest

Crude drugs subjected to the extraction process are isolated and further purified which are then incorporated as active ingredients in the system of Herbal Medicine. Some examples are the isolation of reserpine, an antihypertensive drug from the roots of Rauwolfia serpentina and the miraculous discovery of the two major alkaloids of vinca namely vinblastine and vincristine from Catharanthus roseus and also the Anthracene glycosides, the well known sennosides from the leaves and pods of Cassia species which are widely accepted laxatives.

Each class of natural products differs from the other, in possessing a potential to elicit its own therapeutic property. This concept had led scientist to open up a new chapter, now popularly known as drug design, which has paved
the way, or the design of several synthetic molecules that has blossomed out of their quantitative structure activity relationship. The alkloids quinine and quinidine which are used as antimalarialis were isolated from the bark of different Cinchona species, the cardac glycosides, digitoxin obtained from Digitalis purpurea and Digitalis lanata are all prodigies of the former discovery process [8].

Pharmaceutically active compounds derived from medicinal plants have been used for a wide spectrum of diseases, namely the traditional and continued use of the opium alkaloids, morphine and codeine as analgesic, in the relief of pain and cough suppressants [9]. The use of alkaloids ephedrine isolated from Ephedra spp. In treating bronchial asthma is also recorded in the literature. Pharmaceutically significant products have also found their way for treating inflammation, for eg: a flavonoids “Galangin” isolated from Alpinia officinarum is said to inhibit the enzyme cyclo-oxygenase and the amazing molecule diosgenin a corticosteroid from Discorea spp. being used to treat inflammation and also as a precursor for many sex hormones [10].

One of the first members in their race to fight cancer from a medicinal point of view is the discovery of an alkaloid monocrotaline from Crotalaria pectabilis, which received much popularity in the fight against cancer than the popular podophyllotoxin from P.hexandrum [11]. The potent drug namely Indicine–N-oxide from the well know source Heliotropium indicum has been effective in controlling the proliferation of cancer cells. The taxols obtained from the genus Taxus represents three potent diterpene members with
remarkable antineoplastic properties, which received much attention and recognition all throughout the world. **Taxol** has been approved and certified by the U.S. FDA for the treatment of ovarian and non-small cell lung cancer [12].

Medicinal plant constituents like “**silymarin**’ (*Silybum marianum*) which consists of three isomeric flavanolignans is the most important discovery in the treatment of liver disorders and jaundice [13]. Other molecules include the lactone, *andrographolide* which is obtained from *Andrographolide paniculata* which has become a household remedy in treating jaundice. Medicinal plants like *Eclipta alba* from which the molecules *wedelolactone* and **ecliptine** were derived and the iridoid glycosides such as **picroside I, II** and also **kukoside** from *Picrorhiza kurroa* have found their way as the fundamental ingredients in the manufacture of hepatic herbal formulations like livol, Liv-52 etc [14].

Medicinal plants have also been used through the ages for treating diabetes. Some of the reknowned molecules which have produced marked reduction in blood sugar levels and is the U.S patented diterpene **saudin** from *Cluytia richardiana* [15] and also **peganine** from *Peganum harmala*. A guanidine resembling molecule namely **galegine** from *Galega officinalis* and the constituent **allicin** isolated from the common onion also exhibited good degree of hypoglycemic property. A number of other plants include *Eugenia jambolona* and *Delonix elata* are being used widely in the formulations in the herbal industry for treating diabetes.

The most important contribution of medicinal plants witnessed in the field of medicine for treating viral diseases like HIV and AIDS include some of
the most promising drugs which are at present in their early stages of development, but a few have found their way into the clinical trials, some of these include the alkaloids castanospermine (*Castanospermum australe*) discovered by Bell and his colleagues at the University of London. Other compounds include hypoglycin A and B which are cyclopropanoid amino acids from the fruits of *Blighia saida* and also the talked about pentacyclic trierpene glycyrhrizin from *Glycyrrhiza* which has been said to delay the development of AIDS symptoms of the effected patients [16]. The anthraquinone of *Hypericum* species namely hypericin is in for clinical testing for its remarkable antiretroviral properties [17].

Other miscellaneous drugs include the ergot alkaloid ergotamine from *Claviceps purpurea* an uterine stimulant and also the bacoposide from *Centella asiatica* that has been used as memory enhancers and are included as ingredients in memory capsules. The antiamoebicide include the alkaloid emetine from the roots of *Ipecacuanha*. Resins like gugulipids are used as hypolipidemic and hypocholestermic drugs to treat heart diseases [18].

Flavouring agents are also contributions made by the plant kingdom, which include a number of essential and volatile oils like peppermint, eucalyptus, citronelia and clove oils. Although the most exotic of the volatile oils are those associated with the perfume industry, these are all important components in the formulation of perfumes. For example, Citral from lemon sandal wood oil from *Santalum* have been used in the prepration of soaps and perfumes. Colouring matter like bixin from the seeds of *Bixa* species are used
in the process of dyeing in the textile industry.

Thus plants continue to be sources of new drugs as witnessed by the recent trends and approved of numerous new plant derived drugs and many synthetic drugs based on the secondary metabolites. Plant drugs of clinical significance also include the discovery of artemisinin, a rapidly acting antimalarial agent from the Chinese drug Artemisia annua and also Forskolin, a naturally occurring antihypertensive agent from Coleus forskohlii. The Indian Institute of Chemical Technology, Hyderabad has recently developed the indigenous production of etopside, a new semi-synthetic antineoplastic agent obtained from Podophyllum peltatum. Contributions in the area of pharmaceuticals from medicinal plants has also witnessed the isolation of a peruvoside from Thevetia neriiifium by Prof. E. Venkata Rao et al, (Department of Pharmaceutical Sciences, Andhra University, India) which is now used as a cardiotonic and has been patented [19].

Similarly, in the field of cancer research, two dimeric benzylisoquinoline alkaloids, thalicarpine and Tetrandrine from Cyclea peltata have quite promising and hence are selected for future anticancer drug development. Camptothecin an alkaloid isolated from Camtotheca acuminata, exhibited moderate anticancer activity, while the natural 10-hydroxyl Camptothecin was more potent [20]. The diterpenes triptolide and tripidiolide from Tripterygium wilfordii are seen as promising antineoplastics. Novel alkaloidal esters, Harringtonine and homoharringtonine from C.harringtonia have showed some interesting results and are seen as potent
anticancer agents [21].

**Natural compounds as neuroprotective agent**

Plants derived pharmaceuticals showed potential neuroprotective agents, some examples, **Resveratrol**, obtained from *Polygonum cuspidatum* and red wine contained polyphenols showed potent neuroprotective effects [22]. **Panaxadiol** (a group ginsenosides) from ginseng, **Withanolides** from ashwaganda (an ayurvedic herb), and **trigonelline** from coffee beans, all are have strong evidence and several studies indicated potent neuroprotective effects. **Theophylline**, a related compound that has A (2A) receptor blocking properties, has been shown in one small trial to improve motor function in patients with PD. **Curcumin**, is promising agent in the treatment and/or prevention of Alzheimer’s disease (AD), effective in lowering oxidative damage, cognitive deficits, synaptic marker loss and amyloid deposition. Curcumin administration also decreased lipid peroxidation, mitochondrial dysfunction, and the apoptotic indices [23]. **Wognin**, a flavonoid originated from *Scutellaria baicalensis*, inhibits ischemic brain injury and used in treatment of stroke. **Baicalin**, a flavonoid had a protective effect on ischemic like or excitotoxic injury, partially related to inhibition of PKC-α, **Salvianic acid A (SA)**, isolated from the Chinese herbal medicine *Salvia miltiorrhiza*, is capable of protecting diverse kinds of cells from damage caused by a variety of toxic stimuli and therapeutic strategy for the treatment of progressive neuroprotective diseases such as Parkinson’s disease. **Ginseng** is one of the most widely used of all
traditional Chinese herbs, ginsenosides isolated from this herb, improve performance in a passive avoidance learning paradigm and enhance cholinergic metabolism [24].

Intravenous injection of epicatechin or catechin to mice improved the memory impairment induced by cerebral ischemia [25]. The oral administration of EGCG restored the dopaminergic neurotransmission in rats injected with N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a drug used to reproduce a parkinsonian syndrome [26]. The chronic consumption of ferulic acid with the drinking water protected from the deleterious effects of an intra cerebral injection of β-amyloid peptide, a component of senile plaques postulated to be involved in the pathogenesis of Alzheimer’s disease [27]. Similar protective effects were observed with curcumin in an Alzheimer transgenic mouse model [28].

**Natural compounds as anti-inflammatory and anti-allergic**

Some evidence has already been indicated that a large number of flowering plants owe their anti-inflammatory properties containing flavonoids, which inhibits a broad spectrum of enzymes and scavenging free radicals. Eugenol and n-cis-feruloyl-trramines, inhibits the synthesis of prostaglandins and also Anthocyanins and hydrolysable tannins are anti-inflammatory because of their ability to scavenge free radicals. Triterpene as anti-inflammatory principle are to be found particularly in the Diospyros, Crateva species, and are known to elaborate a series of pentacyclic triterpenes.
including **betulin**, **betulinic acid** and **urosolic acid**. **Lupeol**, isolated from the stem bark of *Crataeva magna* (L) reduces the foot-pad thickness and complement activity in arthritic rats [29]. **Oleanolic acid** saponins isolated from the roots of *Momordica cochinensis* (L) is anti-pruritic in rodent [30]. Classical examples of herbs traditionally used to treat inflammation in western medicine are *Matricaria chamomilla*, *Salix alba* and *Glycyrrhiza glabra*. *Arnica montana* mainly they contains **Apignin**, **sesquiterpene lactones**, such as **helnalin** and **dihydrohelenalin**, which are thought to inhibit the activation of transcription factor nuclear factor NF-kB, which is responsible for the transcription of genes involved in encoding mediators for the inflammatory process [31]. *Curcuma longa* L is a yellow pigment, **curcumin**. This dye inhibits the enzymatic activity of both COX and nitric oxide synthetase (NOS) and showed clinical potentials for the treatment of inflammation.

**Natural compounds as hepatoprotective agents**

The management of liver disease is still a challenge to the modern medicine. The modern allopathic drugs have very little to offer alleviation of hepatic ailments and some of these drugs adversely affect the liver function. The traditional system of medicine a major role in the treatment of liver ailments. Presently plant based many herbal formulations **HD-03** [32], **Jigrine**, **Enliv**, **Trikatu churna** are available for treating liver disorders. Liv-52, an indigenous multi-herbal hepatotonic, has been widely used as a hepatoprotective agent during the last three decades, as it contains several
well-known plant principles [33]. It mainly contains powders of *Capparis spinosa*, *Cichorium intybus*, *Solanum nigrum*, *Terminalia arjuna*, *Cassia occidentalis*, *Achillea millefolium*, *Tamarix gallica* and Mandura bhasma. Recently, there are some extracted hepatoprotective compounds from medicinal plants *Achillea millefolium*, *Azadirachta indica* and *Cassia accidentalis* which have been used traditionally for liver disorders [34].

**Natural compounds as antidiabetics**

Many plants have been traditionally used in the treatment of diabetes. Polyphenols contained in these plants may explain some of their therapeutic activity. **Caffeic acid** and **isofurulic acid**, reduce the fasting glycemia and attenuate the increase of plasma glucose in an intravenous glucose tolerance test [35, 36]. Similar effects were observed with 4-hydroxybenzoic acid. **Catechin** improved the tolerance to glucose induced by starch or sucrose ingestion. Quercetin inhibited glucose transport by GLUT2 in a transected oocyte model and also inhibited glucose absorption [37]. Black and green tea extracts and **EGCG** also increased glucose uptake by epididymal adipocytes, both in the presence or absence of insulin [38]. Polyphenolic exhibits thier action, inhibition of gluconeogenesis [39], adrenergic stimulation of glucose uptake [40], or the stimulation of insulin release by pancreatic β-cells [41]. **p-Hydroxybenzoic acid**, which shows hypoglycemic effects in diabetic rats when submitted to a glucose tolerance test, had no effect on insulinemia and hepatic glycogen [42]. *Gymnema sylvestris*, contains **GS4**, **Gymnemic acid** and *Ficus*
*bengalenis*, Leucocyanidine-3-0-beta cellabioside showed regeneration and stimulation activity of Beta cells of pancreas. *Mamordica charinta* contains **polypeptide-P**, is effective hypoglycemic agent, which acts like insulin [43, 44].

**Natural compounds as anti-osteoporosis agents**

The plant-derived pytoestrogens are metabolized to **genistein** and **daidzein**, ligands for both estrogen α and β receptors, with a greater affinity for β receptors (45) showed potential anti-osteoporosis activity in estrogens deficiency caused osteoporosis.

[Chemical structures of xenoestrogens, genistein, daidzein, zearalenone, and coumestrol are shown.]

Estrogenic compounds and chemical structure

For example, the phenolic isoflavone phytoestrogen **genistein**, which is
abundant in soy products, exhibits high affinity and selectivity for ER-β. Genistein also exhibits high binding affinity for GPR30, and its binding to GPR30 results in the activation of mitogen-activated protein kinases (MAPKs) through this mechanism act as a anti-osteoporosis [46]. The involvement of coumestrol, another common phenolic phytoestrogen, act as antiosteoestrogenic activity through estrogen-receptor-mediated signaling pathways. The supplementation of the diet with genistein [47], daidzein, or their glycosides during several weeks prevents the loss of bone mineral density and trabecular volume caused by the ovariectomy [48]. Rutin, added to the diet of ovariectomized rats restored the loss of bone mineral density induced by the ovariectomy and was even more efficient than isoflavones. Catechins abundant in tea could possibly counteract the effects of tea caffeine, known for its adverse effects on bone metabolism.

Natural compounds as anticancer agents

A number of important commercial drugs have been obtained from nature or by structural modification of a natural product as a anticancer drugs [49], best know example Paclitaxel and related taxanes exert their anticancer effects by promoting tubulin polymerization and stabilizing microtubules, leading to mitotic G2/M arrest and apoptosis [50]. Docetaxel, a second–generation taxane, is one of the most powerful drugs against breast cancer [51]. Vinca alkaloids (vincristine, vinblastine) and their derivatives (e.g vinorelbine) block mitosis through metaphase arrest by binding specifically to tubulin and leading to its depolymerization [52]. Camptothecin and its
derivatives (Irinotecan and topotecan) inhibit topoisomerase I, which is involved in the cleavage and reassembly of DNA [53]. Podophyllotoxin is the most abundant lignan isolated from podophyllin, a resin produced by Podophyllum species, but it was found to be too toxic for clinical use. Etoposide and teniposide, two semi-synthetic derivatives of podophyllotoxin, inhibit topoisomerase II and have shown good clinical efficacy against several types of neoplasms including testicular and small cell lung cancers, lymphoma and leukemia. Plant-derived compounds currently under investigation are flavopiridol, homoharringtonine, β-lapachone, combretastatin A4. Flavopiridol is a synthetic flavone derived from the plant alkaloid rohitukine, which was isolated from leaves and stems of Amoora rohituka and later from Dysoxylum binectariferum. Flavopiridol is a cyclin–dependent kinase inhibitor [54]. Homoharringtonine is an alkaloid isolated from the Chinese tree Cephalotaxus harringtonia acts mainly efficacy against various leukaemias [55-57].

Dieary polyphenolic compounds have been shown to have anticancer effects such as ability to induce apoptosis and inhibit cell growth and kinase activity [58-60]. Flavonoids, rich in soybeans, tea, fruits and leafy vegetables, are the most abundant polyphenols in our daily diets, including flavones quercetin, myricetin and kaempferol, and the flavones luteolin, epigallocatechin-3-gallate (EGCG), apigenin, genistein and fisetin inhibit cultured tumor [61,62]. Epigallocatechin-gallate (EGCG) and resveratrol are such example for selectively inhibit endothelial cell growth at lower
concentrations as compared with tumor cells [63, 64]. **Apigenin** and **luteolin** are considered as the most potent anti-tumor proliferative flavonoids. **Fisetin** and **myricetin** is widely distributed in fruits and vegetables. Both these compounds, acts through by inhibit endothelial cell proliferation and migration, further potent angiogenesis inhibitor.

### 1.3 Oxidative stress—An introduction

Molecular oxygen (dioxygen: O$_2$) is essential for the survival of all aerobic organisms. In mitochondria, energy is generated by the consumption of oxygen and the reduction of oxygen to water by the transfer of four electrons without formation of intermediates. In this process of oxidative phosphorylation, the oxido-reduction energy of mitochondrial electron transport is further converted to the high-energy phosphate bond of ATP via a multi component NADH dehydrogenase enzymatic complex. Partially reduced and highly reactive metabolites of O$_2$ are formed during these electron transfer reactions. These O$_2$ metabolites include superoxide anion (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$), formed by one- and two-electron reductions of O$_2$, respectively. In the presence of transition metal ions, the even more reactive hydroxyl radical (OH$^-$) can be formed. These partially reduced metabolites of O$_2$ are often referred to as "reactive oxygen species" (ROS) due to their higher reactives relative to molecular O$_2$. ROS from mitochondria and other cellular sources have been traditionally regarded as toxic by-products of the cellular metabolism with the potential to cause damage to lipids, proteins, and DNA [65]. To protect the host against the damaging effects of ROS, many antioxidative defense mechanisms
have evolved. In addition to several antioxidant enzymes such as superoxide dismutase (which reduces O$_2^·$ to H$_2$O$_2$), catalase, and glutathione peroxidase (which reduces H$_2$O$_2$ to H$_2$O), a broad range of non-enzymatic scavengers exists. “Oxidative stress” may be broadly defined as an imbalance between oxidant production and the antioxidant capacity of the cell to prevent oxidative injury, and is thought to contribute to the pathogenesis of a number of human diseases including rheumatoid arthritis, ischemia / reperfusion injury in brain and heart, diabetes mellitus, stroke, atherosclerosis, pulmonary fibrosis, allergy, cancer, neurodegenerative diseases, osteoporosis, and ageing [66, 67]. These diseases fall into two groups (i) the first group involves diseases characterized by pro-oxidants shifting the thiol/disulphide redox state and impairing glucose tolerance-so called “mitochondrial oxidative stress” conditions (Cancer and diabetes mellitus); (ii) the second group involves diseases characterized by “inflammatory oxidative stress” conditions and enhanced activity of either NAD(P)H oxidase (leading to atherosclerosis and chronic inflammation) or xanthine oxidase –induced formation of ROS (implicated in ischemia and reperfusion injury). The process of aging is to a large extent due to the damaging consequence of free radical action (lipid peroxidation, DNA damage, protein oxidation) [68]. During the past decade however, reduction-oxidation (redox) reactions that generate ROS (including H$_2$O$_2$, O$_2^·$ and OH·) have been identified as important chemical mediators in the regulation of signal transduction processes involved in cell growth and differentiation [69]. ROS may interact with and modify cellular proteins, lipid
and DNA, which results in altered target cell function, consequences to biological systems exposed to uncontrolled oxygen radicals flux can produce functional damage, structural damage and ultimately cell death [70]. Oxidative stress is associated with increases formation of ROS that modifies phospholipids and proteins leading to peroxidation and oxidation of thiols groups [71, 72]. The ROS lead to changes in membrane permeability, membrane lipid bilayer disruption and functional modification of various cellular proteins. The sources of ROS and antioxidant defense system in endogenous and exogenous were presented in table 1.1.

Table 1.1. The sources of ROS and antioxidant defence system

<table>
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<tr>
<th>SOURCES OF ROS</th>
<th>ENDIGENOUS</th>
<th>EXOGENOUS</th>
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<tr>
<td>NADPH oxidase</td>
<td>Environmental toxins</td>
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<tr>
<td>Mitochondria</td>
<td>Ionising radiation</td>
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<tr>
<td>Peroxisomes</td>
<td>Ultraviolet light</td>
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<tr>
<td>Cytochrome P450</td>
<td>Electrical field</td>
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<tr>
<td>Xanthine oxidase</td>
<td>Chemotherapeutics</td>
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<tr>
<td>Cyclooxygenase</td>
<td>Inflammatory cytokines</td>
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<td>Lipooxygenase</td>
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<tr>
<td>Γ-Glutamyl transpeptidase</td>
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<table>
<thead>
<tr>
<th>ANTIOXIDANT DEFENCE</th>
<th>ENDIGENOUS</th>
<th>EXOGENOUS</th>
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<tbody>
<tr>
<td>Superoxide dismutase</td>
<td>Glutathione</td>
<td></td>
</tr>
<tr>
<td>Catalase</td>
<td>Thioredoxin</td>
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<tr>
<td>Glutathion peroxidase</td>
<td>Glutaredoxin</td>
<td></td>
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<tr>
<td>Prion protein</td>
<td>Vitamin C,A,E</td>
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<td></td>
<td>Lipoate</td>
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<td></td>
<td>Urate</td>
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<tr>
<td></td>
<td>Ubiquinone</td>
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<td></td>
<td>Pyruvate</td>
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A free radical is any species capable of independent existence that contains one or more unpaired electrons. Free radicals are constantly produced
in the body during the normal cellular metabolism and oxidative phosphorylation. There are two important pathways for the production of radicals in living systems

1. Enzymatically controlled one electron reduction of oxygen eg. formation of superoxide anion radicals by xanthine oxidase, aldehyde oxidase, dihydro orotate dehydrogenase and peroxidases.

2. Reaction initiated by xenobiotics eg: CCl₄ and environmental contamination eg: fluoride, lead etc., These substances can lead to the production of radicals by several distinctive mechanisms. (a) Substances can trigger the production of H₂O₂ and O₂⁻ from cells. (b) Xenobiotics can be metabolized by radical mediated paths, for eg CCl₄ (reduced by cytochrome p450 to Cl⁻ and CCl₃); Fluoride (F) combines with aluminum in drinking water, it becomes aluminum fluoride (AlF₄⁻), it reduced to form reactive (AlF₃⁻). (c) A few toxins are themselves radicals eg are NO, NO₂ and organic combustion products such as tobacco smoke and automobile exhaust. These materials can react with bimolecular to reproduce radicals without intervention of enzymes. (d) A group of toxins, while not radicals themselves, can react to form radicals or radical precursors compounds by non-enzymatic pathway like ozone and single oxygen. When a radical reacts with a new compound, more free radicals are generated, this chain reaction leads to thousands of events. Reactive oxygen species (ROS) are likely to be involved in the pathophysiology of many human diseases [73, 74]. Almost all biological macro molecules are damaged by free radicals, eg. peroxidation of PUFA in plasma membranes, oxidative
inactivation of sulfhydryl contains enzymes [75], polysacaride depolymerisation and DNA breaks. These results in the inhibition of loss of membrane functions such as absorption and secretion, inhibition of protein and enzyme synthesis and indirectly cause cell death or mutation and carcinogenesis [76]. The schem of oxidative stress induced by ROS/RNS was presented in scheme 1.1.

**Scheme 1.1.** Oxidative stress induced by ROS/RNS

![Scheme 1.1. Oxidative stress induced by ROS/RNS](image)

ROS can also act as signaling molecules in the regulation of genetic expression, cell growth and cell death. Extracellular ROS can initiate cellular signaling (eg: activation of receptor kinase by UV radiation) and intracellular ROS are generated as second messengers (eg: Tumor Necrosis factor-α (TNF-α) activate transcription factor NF-κB through H$_2$O$_2$). There is strong evidence that signaling pathways are involved in the enhanced expression of oxidative stress inducible genes such as C-fos, C-jun and transcription factors AP-1 and NF-κB.
Initiation of these signaling events at the plasma membrane involve kinase cascades and redox regulation [77, 78]. Therefore, there need not be cascade of events initiated by oxidative stress, rather a cycle of events of which oxidative stress is a major component. Inhibition of oxidative stress might break the cycle of cell death of neurons, thus much efforts is devoted to developing “rational drug” or “genetic therapy” targeted at the “oxidative stress components” of the cycle.

It is also clear from a whole range of chronic modern medical treatment and the difficulty/failure to treat diseases such as cancer, cardiovascular diseases, diabetes, rheumatism and newer diseases like AIDS demand effective drugs. Moreover, problems with drug resistance microorganisms, side effects of modern drug and emerging new diseases for no effective drugs are available, have encouraged new interest once again to re-examine plant remedies described in ancient and medieval texts as a significant sources of new drugs. The molecules from medicinal plants thus serve as important sources for varied range of pharmaceutically active drugs that forms the very basis and the remedy in the cure of diseases. A detailed pharmacological investigation of these plants used traditionally can lead to the development of invaluable plant drugs for many dreaded diseases of neurodegenerative, respirator, cardiovascular, endocrine and renal systems. Human body has inherent mechanism to reduce the free radical induced injury by endogenous enzymes such as superoxide dismutase, gluthathione peroxidase, catalase and others such as vitamins E, exogenously administered ascorbic acid etc., Some times
these protective mechanisms were found to be not sufficient, when compared to the insult produced to the body. Hence the search for exogenous antioxidants is continued. With this consideration, the plant derived antioxidants and their role in reactive oxygen species mediated oxidative stress in biological system must be evaluated for their potential activity.

Chemistry of ROS

Free radicals can be defined as molecules or fragments of molecules containing one or more unpaired electrons in atomic or molecular orbitals [79]. Molecular oxygen (dioxygen) has a unique electronic configuration and is itself a radical. The addition of one electron to dioxygen forms the superoxide anion radical (O$_2^•$) [80]. Superoxide anion, arising either through metabolic processes or following oxygen “activation” by physical irradiation, is considered the “primary” ROS, and can further interact with other molecules to generate “secondary” ROS, either directly or prevalently through enzyme-or metal-catalyzed processes.

Reactive oxygen species can be classified into oxygen-centered radicals and oxygen-centered non radicals. Oxygen-centered radicals are superoxide anion (·O2−), hydroxyl radical (·OH), alkoxy radical (RO·), and peroxyl radical (ROO•). Oxygen-centered non-radicals are hydrogen peroxide (H$_2$O$_2$) and singlet oxygen (·O$_2$). Other reactive species are nitrogen species such as nitric oxide (NO•), nitric dioxide (NO$_2$•), and peroxynitrite (OONO−)[81]. Reactive oxygen species in biological systems are related to free radicals, even though there are non radical compounds in reactive oxygen species such as singlet oxygen and
hydrogen peroxide. A free radical exists with one or more unpaired electron in atomic or molecular orbital.

Free radicals are generally unstable, highly reactive, and energized molecules. Reactive oxygen species or free radicals in biological systems can be formed by prooxidative enzyme systems, lipid oxidation, irradiation, inflammation, smoking, air pollutants, and glycoxidation. Clinical studies reported that the reactive oxygen species are associated with many age related degenerative diseases, including atherosclerosis, vasospasms, cancers, trauma, stroke, asthma, hyperoxia, arthritis, heart attack, age pigments, dermatitis, cataractogenesis, retinal damage, hepatitis, liver injury, and periodontis [82.83]. Singlet oxygen can attack various pathogens and induce physiological inflammatory response [84]. The reactive oxygen species are associated with many age related degenerative diseases are presented in scheme 1.2.

**Schem 1.2.** Reactive oxygen species and age related degenerative diseases

Superoxide anion (·O2−)

Superoxide anion is a reduced form of molecular oxygen created by
receiving one electron. Superoxide anion is an initial free radical formed from mitochondrial electron transport systems. Mitochondria generate energy using 4 electron chain reactions, reducing oxygen to water. Some of the electrons escaping from the chain reaction of mitochondria directly react with oxygen and form superoxide anions. The superoxide anion plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical, or singlet oxygen ($2\text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$) in living systems [85]. The superoxide anion can react with nitric oxide (NO) and form peroxynitrite (ONOO$^-$), which can generate toxic compounds such as hydroxyl radical and nitric dioxide ($\text{ONOO}^- + \text{H}^+ \rightarrow \cdot\text{OH} + \cdot\text{NO}_2$).

Hydroxyl radical (OH)

Hydroxyl radical is the most reactive free radical and can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions such as copper or iron.

$\cdot\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \cdot\text{OH} + \cdot\text{OH}^- + \text{O}_2$

Hydroxyl radicals have the highest 1-electron reduction potential (2310 mV) and can react with everything in living organisms at the 2nd-order rate constants of 109 to 1010/M/s [84]. The resulting radicals can react with oxygen and generate other free radicals. Hydroxyl radicals react with lipid, polypeptides, proteins, and DNA, especially thiamine and guanosine [86]. Hydroxyl radicals also add readily to double bonds. The barrier to the addition
of hydroxyl radicals to double bonds is less than that of hydrogen abstraction, so that in competition addition is often favored. When a hydroxyl radical reacts with aromatic compounds, it can add on across a double bond, resulting in hydroxyl cyclohexadienyl radical. The resulting radical can undergo further reactions, such as reaction with oxygen, to give peroxyl radical, or decompose to phenoxy type radicals by water elimination [86].

**Hydrogen peroxide (H₂O₂)**

Hydrogen peroxide can be generated through a dismutation reaction from superoxide anion by superoxide dismutase. Enzymes such as amino acid oxidase and xanthine oxidase also produce hydrogen peroxide from superoxide anion. Hydrogen peroxide is highly diffusible and crosses the plasma membrane easily. Hydrogen peroxide is the least reactive molecule among reactive oxygen species and is stable under physiological pH and temperature in the absence of metal ions. Hydrogen peroxide is a weak oxidizing and reducing agent and is thus regarded as being poorly reactive. Hydrogen peroxide can generate the hydroxyl radical in the presence of metal ions and superoxide anion [87].

\[
\cdot O_2^- + H_2O_2 \rightarrow \cdot OH + O_2^- + O_2
\]

Hydrogen peroxide can produce singlet oxygen through reaction with superoxide anion or with HOCl or chloroamines in living systems. Hydrogen peroxide can degrade certain heme proteins, such as hemoglobin, to release iron ions.

**Nitric oxide and nitric dioxide**
Nitric oxide (NO\(^\cdot\)) is a free radical with a single unpaired electron. Nitric oxide is formed from L-arginine by NO synthase. Nitric oxide itself is not a very reactive free radical, but the overproduction of NO is involved in ischemia reperfusion, and neurodegenerative and chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease. Nitric oxide, exposed in human blood plasma, can deplete the concentration of ascorbic acid and uric acid, and initiate lipid peroxidation [88]. Nitric dioxide adds to double bonds and abstract labile hydrogen atoms initiating lipid peroxidation and production of free radicals. It also oxidizes ascorbic acid [89].

**Lipid peroxidation:**

ROS mediated impairment of membrane function can occur directly through the oxidation of the polyunsaturated (lipid peroxidation), or indirectly through the inhibition of lipid synthesis, fatty acid desaturation, or activation of lipases [90].

| Initiation | LH + \cdotOH \rightarrow H_2O + L\cdot |
| Propagation | L\cdot + O_2 \rightarrow LOO\cdot |
| | LOO\cdot + LH \rightarrow LOOH + L\cdot |
| Termination | LOO\cdot + LOO\cdot \rightarrow \text{Inert Product} |
| | L\cdot + L\cdot \rightarrow \text{Inert Product} |
| | LOO\cdot + L\cdot \rightarrow \text{Inert Product} |

The lipids initially attacked by free radicals become oxidized to lipid peroxides. Lipid peroxides are potentially toxic and possess the capacity to damage most cells.
Malonaldehyde (MDA) is the major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acid (PUFA). MDA is a secondary product of LPO, which is used as an indicator of tissue damages by a series of chain reactions. MDA reacts with thiobarbituric acid and produce red coloured products. MDA can modify xanthine oxidoreductase activity through interaction with XO and/or xanthine dehydrogenase (XDH). MDA is a mutagenic and genotoxic agent that may contribute to the development of human cancer. Lipid hydroperoxides may directly induce DNA chain breaking. Lipid peroxyl and alkyl radicals may cause base oxidation in DNA [91].

**Fluoride induced oxidative stress**

Compelling evidence indicates that fluoride produces injury to the central nervous system (CNS) by several mechanisms. Particular interest is the ability of fluoride to induce free radical generation and lipid peroxidation in the brain, especially in the hippocampus. In addition, fluoride enhances aluminum absorption from the gastrointestinal mucosa and across the blood-brain barrier. Particular concern is the recent demonstration that fluoride readily forms a chemical complex with aluminum, similar to the phosphate ion, which is toxic to neurons at low concentrations and can act as an activator of G-proteins, a membrane link to second messenger activation.

While it appears that the toxicity of fluoride is secondary to many widely divergent and unrelated processes, there is compelling evidence that a central mechanism may be involved called excitotoxicity [92]. The excitotoxicity effect of fluoride/aluminium is given in table 1.2.
Table 1.2. Comparison of the effects of fluoride/Aluminium and excitotoxicity

<table>
<thead>
<tr>
<th></th>
<th>Fluoride/Aluminium</th>
<th>Excitotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased brain ROS and RNS</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Increased lipid peroxidation (LPO)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Decreased glutathione</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Decreased SOD</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Elevated brain ascorbate</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hippocampal apoptosis necrosis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>G-protein activation</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Synaptic injury</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Impaired glutamate uptake</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Microglial activation</td>
<td>? for fluoride</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Yes for aluminium</td>
<td></td>
</tr>
<tr>
<td>ROS in other tissues</td>
<td>? for fluoride</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Yes for aluminium</td>
<td></td>
</tr>
<tr>
<td>DNA injury</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Excitotoxicity is a common mechanism seen in many neurological disorders, including strokes, brain trauma, CNS infections, autoimmune disorders, multiple sclerosis, heavy metal toxicity, brain tumors, and the majority of neurodegenerative diseases, such as Alzheimer’s dementia, Parkinson’s disease [93].

The ionotrophic receptors control the passage of sodium, potassium, and calcium through membrane channels, which in turn initiates neuronal depolarization (excitation). Most important to the excitotoxic process is calcium accumulation within the cytosol following glutamate receptor activation. Intracellular calcium triggers numerous cellular reactions including the activation of nitric oxide synthase and protein kinase C [94]. These in turn can activate free radical generation and lipid peroxidation as well as eicosanoid
activation, should glutamate persist too long in its receptor [95]. These processes play a major role in excitotoxic injury and neuronal death. When these receptors are stimulated by glutamate, the G-protein within the cell membrane is activated, which in turn activates several second messengers within the neuron, including IP₃ (inositol 1,4,5-trisphosphate), cAMP (cyclic adenine monophosphate), or cGMP (cyclic guanine monophosphate). There is also evidence that they regulate intracellular calcium. Another mechanism by which fluoride might increase brain free radical generation and lipid peroxidation would be through activation of protein kinase C by a fluoroaluminum complex. It is known that a major mechanism by which glutamate induces excitotoxicity is activation of protein kinase C. Blocking this enzyme affords significant protection against excitotoxicity. Lead dramatically increases protein kinase C activity in a manner similar to glutamate, thereby triggering excitotoxicity [96].

**Carbon tetrachloride (CCl₄) induced oxidative stress**

The liver is the largest organ of the human body, this organ highly exposed for the metabolism of substances and exposed to toxic compounds for metabolism in liver leads to hepatotoxicity. The exact mechanism of hepatotoxicity is unclear, but most probably resulting from toxic intermediates that binds covalently to hepatocytes and causes a centrilobular hepatic necrosis. Alternate explanations of necrosis are depletion of antioxidant enzymes and induce lipid peroxidation with oxidation of thiol groups due to production of ROS in liver [97]. Human beings are exposed to many foreign
compounds through environmental exposure, consumption of contaminated food or during exposure to chemical substances in the occupational environment and being consume a lot of synthetic drugs during diseased conditions, which may produce a variety of toxic manifestations.

A typical and most frequently used experimental model to study liver damage involves the use of carbon tetrachloride (CCl₄). It is an established potent toxin that is metabolized by a microsomal drug oxidizing system to a more toxic metabolite, the CCl₃ radical, which initiates peroxidative changes in polyunsaturated fatty acid constituents of various biomembranes. The mechanism of CCl₄-induced liver damage is considered to be due to the enzymatic activation (cytochrome P450) of CCl₄ into the trichloromethylfree radical (CCl₃•) within the membrane of the endoplasmic reticulum.

\[
\text{CCl}_4 \rightarrow \text{CCl}_3^\cdot + \text{Cl}^\cdot
\]

This is followed by chloromethylation, saturation, peroxidation and progressive destruction of the unsaturated fatty acid of the endoplasmic reticulum membrane phospholipids. These processes are known as lipid peroxidation, leading to its functional and structural disruption. The carbon tetrachloride induced liver damage was shown in scheme1.3.
Scheme 1.3. Carbon tetrachloride induced liver damage
Reactive oxygen metabolites may play an important role in the
inflammation process after intoxication by CCl₄. It has also been found that metabolism CCl₄ involves the production of free radicals through its activation by drug metabolizing enzymes located in the endoplasmic reticulum. Cellular sources of ROS production include plasma membrane NADPH oxidase and intracellular cytosolic xanthine oxidase, peroxisomal oxidases, endoplasmic reticular oxidases, and mitochondrial electron transport components. Finally, CCl₃ reacts with sulfhydral groups, which mediate the function of the many cell proteins, including a number of important enzymes and this reaction leads to their alkalyation and subsequent loss of function. It consists of a rapid decrease in synthesis of the protein albumin as well as inactivation of cytochrome P-450 system [98].

**Ischemia /reperfusion injury**

Cerebrovascular disease is a group of brain dysfunctions related to disease of blood vessels supplying the brain. Some of the most common devastating disorders such as ischemic stroke, hemorrhagic stroke, cerebrovascular anomalies etc. Cerebrovascular diseases ranked as the second leading cause of death after ischemic heart disease.

The World Health Organization (WHO) definition of stroke is- “rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin” [99].

The pathological background for stroke may either be ischemic or hemorrhagic disturbances of the cerebral blood circulation. Thrombotic
cerebral infarction results from the atherosclerotic obstruction of large cervical and cerebral arteries, with ischemia in all or part of the occluded artery. This can be due to occlusion at the site of the main atherosclerotic lesion or to embolism from this site to more distal cerebral arteries. A thrombosis, an embolism or systemic hypoperfusion, all of which result in a restriction of blood flow to the brain, can cause an ischemic stroke, which results in insufficient oxygen and glucose delivery to support cellular homoeostasis. This elicits multiple processes that lead to cell death- excitotoxicity, acidotoxicity and ionic imbalance, peri-infarct depolarization, oxidative and nitrative stress, inflammation and apoptosis. Within the core of the ischemic area, where blood flow is most severely restricted, excitotoxic and necrotic cell death occurs within minutes. In the periphery of ischemic area, collateral blood flow buffers the full effects of the stroke and degree of ischemia is less. In this area cell death occurs less rapidly via mechanisms such as apoptosis and inflammation.

**Cerebral ischemia/reperfusion injury**

The term cerebral ischemia (Gr. *ischein* to suppress + *haima* blood) means lack of blood supply to the brain. Ischemic injury to the brain can occur in two, principally different modes.

**i. Global cerebral ischemia**

A complete cessation of blood flow to the brain is termed *global ischemia* and is most commonly the result of a cardiac arrest and the subsequent circulatory failure. It involves a reduction or absence of cerebral blood flow
(CBF) to the entire brain.

**ii. Focal cerebral ischemia**

Focal cerebral ischemia entails reduction in CBF to a specific vascular territory, usually encountered clinically due to thrombotic, embolic or hemorrhagic strokes [100]. The tissue in the centre of the ischemic area with severe CBF reduction is termed as *ischemic core*. The majority of strokes are a result of focal ischemia and one of the major blood vessels affected is the middle cerebral artery (MCA) [101].

**Excitotoxicity**

Excitotoxicity is a common mechanism seen in many neurological disorders, including strokes, brain trauma, CNS infections, autoimmune disorders, multiple sclerosis, heavy metal toxicity, brain tumors, and the majority of neurodegenerative diseases, such as Alzheimer’s dementia, Parkinson’s disease and Lou Gehrig’s disease (amyotrophic lateral sclerosis, ALS). Glutamate is the major excitatory neurotransmitter in the brain, plays an important role in excitotoxicity. An increased glutamatergic transmission has been implied in the pathogenesis of several neurological disorders including cerebral ischemia [102]. This “excitotoxic hypothesis” was initiated by the discovery of a neurotoxic effect of glutamate in developing animals and the *in vitro* findings that synaptic release of glutamate mediated hypoxia induced injury.

The sequence of excitotoxicity starts with the excessive extracellular accumulation of glutamate. Extracellular levels of glutamate in experimental
models of stroke have been reported to increase after ischemic insult [103]. But in pathological conditions like ischemia/reperfusion, the over-stimulation of the NMDA receptor causes increased entry of $\text{Ca}^{2+}$ into the cells thereby mediating the excitotoxicity and neuronal damage. The excess of $[\text{Ca}^{2+}]_c$ is highly toxic for the cells, promoting cerebral edema formation and activation of intracellular self-destruction cascade. $\text{Ca}^{2+}$, a cellular messenger that controls important aspects of cell and tissue physiology, can be turned into death signals when delivered at a wrong time and at a wrong place [104].

During ischemia, ATP is degraded to adenosine, ionsine and hypoxanthine. The enzyme xanthine dehydrogenase is converted to xanthine oxidase. However, it is important that, at the moment of reperfusion of blood, oxygen combines with hypoxanthine in the presence of xanthine oxidase and produces uric acid and the superoxide radical. ATP- adenosine triphosphate AMP- adenosine monophosphate [105]. The free radical production by the xanthine oxidase pathway was presented in scheme 1.4 and 1.5.

**Scheme 1.4.** Free radical production by the xanthine oxidase pathway

![Scheme 1.4. Free radical production by the xanthine oxidase pathway](image-url)
Scheme 1.5. The major adverse effects of a nonphysiological rise in (Ca^{2+})

Energy failure /depolarization

Transmitter release and Receptor activation

Ca^{2+}

Lipolysis

DAG↑ PKC↑ Protein phosphorylation

FFAs↑ LPLs↑

Dysfunction of receptor and ion channels

Enzyme conversion

Damage to membrane structure and function

Free Radical formation

Inflammatory and allergy reactions

Allergy is not a single disease it includes allergic rhinitis, atopic rhinitis,
atopic dermatitis, anaphylaxis, allergic pruritis, asthma are the most common cause of chronic ill health [106]. Antigens had induced changes in reactivity. Now this condition frequently known as IgE mediated allergic disease. The term atrophy is often used to describe IgE mediated diseases. Persons with atrophy have a hereditary predisposition to produce IgE antibodies against common environmental allergens and have one or more atrophic diseases (Allergic rhinitis, asthma, and atopic eczema). Some allergic diseases such as contact dermatitis and hypersensitivity pneumonitis develop through IgE independent mechanisms and in this sense can be considered nonatopic allergic conditions [107-111].

**Reactive oxygen species and allergic reaction**

Mast cells play a key role in allergic and inflammatory reactions. Mast cells and some tumor cell lines express the highy-affinity age receptor (FcεR1) on their cell surface. The activation these receptor release of Ca\(^{2+}\) leads to the depletion of intracellular Ca\(^{2+}\) stores and then triggers store operated or capacitative Ca\(^{2+}\) entry through the plasma membrane. Stimulation of age-bound FcεR1 by antigen initiates cascades of intracellular signaling events that lead to the secretion of different types of inflammatory mediators such as performed granular substances (histamine, serotonin, proteases, and some cytokines, referred as to degranulation ) and newly synthesized archidonate metabolites, cytokines and chemokines. Recent studies have demonstrated that stimulation of mast cells / basophils through FcεR1 induces the production of intracellular reactive oxygen species (ROS) such as the superoxide anion (O\(_{2}^{-}\))
and hydrogen peroxide (H$_2$O$_2$), which appears to act as a second messenger in the signal transduction pathways leading to degranulation and release of LTC4[112] and cytokines such as IL-4 and IL-6. It has been shown that agonist that interact with different primary cell targets, including epidermal growth factor (EGF), bradykinin, thapsigargin (TG), and the Ca$^{2+}$ ionophore A23187 and compound 48/80 stimulate the production of ROS in human systems [113]. Rat peritoneal mast cells (RPMC) generate intracellular reactive oxygen species (ROS) following incubation with gold, compound 48/80 and ionophore. Reactive oxygen species have also been shown to enhance mast cell histamine release. The secretagogues used in the present study also caused a concentration–dependent release of histamine, confirming published data [114].

Compound 48/80 has been shown to penetrate cellular membranes and directly activates G-proteins by interaction of an aromatic ring within compound 48/80 with the COOH-terminal domain of the alpha sunuit of a G-protein involved in mast cell exocytosis [115]. Compound 48/80 administration for four day once daily induces, mast cell degranulation and extensive gastric mucosal lesions, authors have suggested that oxygen free radicals and lipid peroxidation may play an important role in the pathogenesis of gastric mucosal lesions in rats treated repeatedly with compound 48/80 and that both the xanthine–XOD system or neutrophiles may be important for the sources of oxygen free radicals. In addition, gastric mucosal XOD activity was found to increases with a decrease in gastric mucosal XDH activity, without change in
the sum of both activities during the compound 48/80 treated rats. This conversion of XDH to XOD seems to result in the excessive production of oxygen free radicals such as superoxide anion (O$_2^-$) and H$_2$O$_2$ in the gastric mucosal tissue [116].

**Osteoporosis**

Osteoporosis, which comes from Greek meaning porous bone, is a condition or disease process that is marked by structural deterioration of bone tissue and low bone mass. Low bone mass can lead to bone fragility and increase a women’s risk of fractures. Osteoporosis is the most frequent metabolic condition experienced by elderly individuals. It is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture [117].

Estrogen replacement therapy is approved for the prevention of bone loss in postmenopausal women and is efficacious in reducing the incidence of skeletal fractures [118]. However, estrogen use and compliance are limited due to its numerous undesirable side effects such as uterine and breast cancer [119].

The cytokine responsible for communication between the osteoblasts, other bone marrow cells, and osteoclasts has been identified as RANK ligand (receptor activator of NF-$\kappa$B, RANKL). RANKL, a member of the TNF family, is secreted by osteoblasts and certain cells of the immune system. The osteoclast receptor for this protein was referred to as RANK. Activation of RANK by RANK-
L is a final common pathway in osteoclast development and activation. A humoral decoy for RANKL, also secreted by osteoblasts, is referred to as osteoprotegerin. Modulation of osteoclast recruitment and activity appears to be related to the interplay among these three factors. Additional influences include nutrition (particularly calcium intake) and physical activity level [120].

**Reactive oxygen species and osteoporosis**

Postmenopausal osteoporosis is a disease of high prevalence and a cause of substantial morbidity and mortality. We hypothesized that estrogen might similarly augment oxidant defenses in bone. Such an augmentation of oxidant defenses would be expected to lower the concentration of reactive oxygen species (ROS) within cells. Although at high concentrations ROS damage many cell constituents, they also affect many signaling proteins at levels considerably lower than those that cause oxidative injury. Signaling cascades can be modulated by ROS following oxidant stress-or cytokine-regulated generation of ROS or through modulation of oxidant defenses [121]. Therefore, if estrogen does increases oxidant defences in bone, this might modulate signal transduction in bone cells with signaling pathways that are sensitive to ROS.

ROS are also potent inducers in many cells of TNF-α and other cytokines strongly implicated in the bone loss of estrogen deficiency. Thus, if estrogen increases oxidant defences in bone, estrogen deficiency might directly or indirectly stimulate osteoclastic bone resorption.

Osteoporotic women had increased oxidative stress with respect to non-
porotic controls recognised by increased MDA levels, an endproduct of lipid peroxidation, in the plasma and erythrocytes and significantly reduced erythrocytes CAT enzyme activity. Erythrocyte NO levels were also found significantly increased in osteoporotic women. Results of previous animal studies have revealed that osteoclastic differentiation and functions were stimulated by ROS, particularly hydrogen peroxide (H$_2$O$_2$) and superoxide anion [122]. Osteoclasts express NADPH oxidase and generate large quantities of ROS during bone resorption. Muthusami et al showed that levels of lipids peroxidation and H$_2$O$_2$ were increased and enzymatic antioxidants like SOD, GSH-Px, Glutathione-S-transferase were decreased in overiectamized rats compared to control rats and assumed that hydrogen peroxide was essential for osteoclast differentiation [123].

**Diabetes and oxidative stress**

Diabetes mellitus is a metabolic disease prevailing throughout the world irrespective of age, sex and race. Diabetes is syndrome characterized by hypoglycaemia, altered metabolism of lipids, carbohydrates, and proteins, and an increased risk of complications from vascular diseases. The etiology of the disease is complicated and treatment is still complicated because of the dramatic change that takes place in the metabolism of body. The lack of exact etiology, diagnostic procedures, specific drugs for correcting all abnormalities of the disease and the effects of synthetic drugs are contributing factors for complications in the treatment. There is a need to search for new drugs with minimum side effects and good therapeutics activity. The therapy today aims
only to temporarily control the metabolic aberrations of glucose. Other metabolic changes such as proteins, lipid and fatty acid metabolisms are not taken into consideration.

Numerous studies have been demonstrated that oxidative stress, mediated mainly by hyperglycaemia-induced generation of free radicals, contributes to the development and progression of diabetes and its complications. Abnormally high levels of free radicals which cause membrane damage due to peroxidation of membrane lipids and protein glycation and the simultaneous decline of antioxidant defense mechanisms leads to cell and tissue damage [124]. Pancreatic-β cells are particularly susceptible to the deleterious effects of reactive oxygen species (ROS), because of their low expression of the anti-oxidant enzymes genes as compared to other tissues. Thus, the increase of ROS leads to damage of β cells through the induction of apoptosis and suppression of insulin biosynthesis [125]. As a new strategy for alleviating the oxidative damage in diabetes, interest has grown in the usage of natural antioxidants. Increased oxidative stress has been postulated in the diabetic state. Oxidative stress in diabetes coexists with a reduction in the antioxidant status. It has been know that preclinical model, alloxan or streptozotocin induces its diabetogenic activity mainly by inducing oxygen free radicals and thereby damaging the pancreas [126]. Alloxan / streptozotocin have several effects on β-cells of the pancreas and it is likely that a combination of these effects results in the destruction of β-cells. Alloxan a higly reactive molecule is reduced to dialuric acid, which is then auto-oxidation back
to alloxan resulting in the production of \( \text{H}_2\text{O}_2, \cdot\text{O}_2 \) and \( \text{OH}^- \). The \( \text{H}_2\text{O}_2 \) causes DNA fragmentation, which in turn activates nuclear poly ADP-ribose synthetase resulting in deletion of cellular DNA levels. Two factors appear to make islet especially sensitive to the effect of alloxan [127]. The hyperglycemia induced oxidative stress is shown in scheme 1.6.

**Scheme 1.6.** Hyperglycemia induced oxidative stress

\begin{center}
\begin{tikzpicture}[node distance=2cm]
    \node (hyperglycemia) {Hyperglycemia \atop \rightarrow FFA \atop / Inflammation \rightarrow \cdot\text{OH}};
    \node (xanthine_oxidase) [below of=hyperglycemia] {Xanthine Oxidase \rightarrow \text{O}_2^-};
    \node (superoxide_dismutase) [below of=xanthine_oxidase] {Superoxide dismutase \rightarrow \text{H}_2\text{O}_2};
    \node (catase) [right of=superoxide_dismutase] {Catase \rightarrow \text{H}_2\text{O}};
    \node (stress_sensitive_gene_expression) [below of=superoxide_dismutase] {Stress-sensitive Gene Expression};
    \draw[->] (hyperglycemia) -- (xanthine_oxidase);
    \draw[->] (hyperglycemia) -- (superoxide_dismutase);
    \draw[->] (hyperglycemia) -- (catase);
    \draw[->] (xanthine_oxidase) -- (stress_sensitive_gene_expression);
    \draw[->] (superoxide_dismutase) -- (stress_sensitive_gene_expression);
    \draw[->] (catase) -- (stress_sensitive_gene_expression);
\end{tikzpicture}
\end{center}

ROS play a significant role in activating stress sensitive signaling pathways that regulate gene expression resulting in cellular damage. One major intracellular target of hyperglycemia and oxidative stress is the transcription factor NF-kB. NF-kB can be activated by a wide array of exogenous and endogenous stimuli including hyperglycemia, elevated FFA, ROS, TNF-\( \alpha \), IL-1\( \beta \), p38, MAPA, DNA damage and UV irradiation [128].
Reactive oxygen species in cancer

Reactve oxygen species (ROS) such as the superoxide radical ($O_2^-$), hydrogen peroxide ($H_2O_2$), the hydroxyl radical (OH) and singlet oxygen are produced by normal metabolism, irradiation, or some chemicals including tumor promoters. There is evidence that reactive oxygen plays an important role in multistep carcinogenesis, diseases, and aging [129].

Oxidative stress induces cellular redox imbalances, which has been found to be present in various cancer cells compared with normal cells. The redox imbalances has may be related to oncogenic stimulation. Permanent modification of genetic materials resulting from “oxidative damage” incidents represents the first step involved in mutagenesis, carcinogenesis, and aging. DNA mutation a critical step in carcinogenesis and elevated levels of oxidative DNA lesions have been noted in various tumors. DNA damage can result in either arrest or induction of transcription, induction of signal transduction pathways, replication errors, and genomic instability, all of which associated with carcinogenesis [130]. Apart from DNA damage, the lipid peroxidation products also implicated in the mechanism of carcinogenesis. DNA damage, mutations, and altered gene expression are thus main key players in the process of carcinogenesis.

Many of the biological effects of antioxidants appear to be related to their
ability not to scavenge deleterious free radicals, but also modulate cell signalling pathways [131]. Thus, the modulation of cell signaling pathways by antioxidants could help prevent cancer by i. Preserving normal cell cycle regulation, ii. Inhibiting proliferation and inducing apoptosis, iii. Inhibiting tumor invasion and angiogenesis, iv. Suppressing inflammation and v. Stimulating phase II detoxification enzymactivity and other effects.

**Antioxidant defense mechanism against free radical damage**

The term antioxidant has been defined as “any substance that inhibits or delays oxidative damage to a target molecule” [132]. To counter the harmful effects of ROS and RNS, antioxidant defense mechanism operates to detoxify or scavenge these reactive oxygen species. Antioxidants are effective because they are willing to give up their own electrons to free radicals. When free radicals gain the electron from an antioxidant, it no longer needs to attack the cell and the chain reaction of oxidation is broken. After donating an electron, an antioxidant because free radical by definition. Antioxidant this state is not harmful because they have the ability to accommodate the change in electrons without becoming reactive. The human body has an elaborate antioxidant defense system. The antioxidant system comprises different types of functional components classified as first line, second line, third line defenses.

i. **Enzymatic antioxidants**

The first line of defenses $O_2^\cdot$, $H_2O_2$ mediated injury are superoxide
dismutase, glutathione peroxidase and catalase. These enzymes are present inside the cell and called as water-soluble antioxidants.

(a) **Superoxide dismutase (SOD)**

Aerobic life forms have evolved an elaborate system for scavenging the free radicals, SOD removes $O_2^-$, by convert to $H_2O_2$ following reactions

$$2H^+ + O_2^- + O_2^- \rightarrow H_2O_2 + O_2$$

SOD is the most important enzyme found in both prokaryote and eukaryote cells. Human SOD is the Cu-Zn-SOD enzyme. This ubiquitous enzyme requires Cu and Zn for its activity and has great physiological significance and therapeutic potential [133].

Two possible mechanisms have been proposed for the action of SOD that inhibiting transformation. 1) It is likely to act at the level of the cell membrane and remove or prevent radical formation that would otherwise cause membrane lipid peroxidation and leads to a chain of extra–nuclear and nuclear events, ultimately expressed as transformation. 2) It has been suggested that oxygen radicals could be formed in growth medium and that these radicals may act as promoters in the transformation process, would be removed by SOD.

(b) **Catalase (CAT)**

CAT is a heme-containing protein present in almost all type of cells extensively present in blood cells. Catalase activity in mammalian tissues varies greatly. It is highest in liver, kidney and low in connective tissue. It catalyses the decomposition of hydrogen peroxide to water and oxygen.

(1) A catalytic role in the decomposition of $H_2O_2$
\[
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\]

(2) A peroxidic role in which the peroxide is utilized to oxidize a range of \( \text{H} \) doners (\( \text{AH}_2 \))

\[
\text{AH}_2 + \text{H}_2\text{O}_2 \rightarrow \text{A} + 2\text{H}_2\text{O}
\]

CAT is found to act \( 10^4 \) times faster than peroxidase. CAT is present in peroxisome (80%) and cytosol (20%). It has 240,000 molecules weight and consists of four protein subunits, each containing a heme Fe (III)-protoporphyrin group bound to its active site [134]

(c) Glutathione system

Glutathione, a major non-protein thiol in living organisms, plays a central role in coordinating the body’s antioxidant defense processes. Perturbation of glutathione status of a biological system has been reported to lead serious consequences. Reduced thiols have long been reported to be essential for recycling of other antioxidants like vitamin E and vitamin C. Liver plays a major role in glutathione homeostasis and is the main organ for glutathione synthesis. GSH is a substrate for GPX, which removes \( \text{H}_2\text{O}_2 \) and also acts as substrates for dehydro ascorbate reductase. In addition GSH is a scavenger of \( \text{OH}^- \) and singlet oxygen. GSH can reactivate some enzymes that have been inhibited by exposure to high \( \text{O}_2 \) concentrations.

(d) Glutathione Peroxidase (GPX)

Glutathione peroxidase enzyme is a well-known first line of defense against oxidative stress, which in turn requires glutathione as co-factor. Glutathione peroxidase catalyses the oxidation of GSH to GSSG at the expense of \( \text{H}_2\text{O}_2 \). It is a selenium-containing enzyme which catalyses the reduction of
H₂O₂ and lipid hydroperoxide (LO₂ H), generated during lipid peroxidation to water using reduced glutathione as a substrate [135].

\[
\text{ROOH + 2GSH} \rightarrow \text{ROH + H₂O + GSSG}
\]

\[
\text{(Reduced)} \quad \text{(Oxidized)}
\]

or

\[
\text{H₂O₂ + 2GSH} \rightarrow 2\text{H₂O + GSSG}
\]

**iii. Antioxidant vitamins**

These are the second line defences, which cannot be synthesized by most membranes including human beings and therefore are required from diet. These vitamins (Vitamine-E and C) can prevent gentic damages by inhibiting DNA damage includes by the ROS in mitochondria [136].

In the light of the foregoing the author has selected 7 natural products isolated from *Hibiscus vitifolius* (gossypin), *Zizyphus glabrata* (gallic acid), *Diospyros sylvatica* (microphyllone), *Tephrosia pulcherrima* (candinone), *Diospyros sylvatica* (lupeol). Quercetin, (-)-epicatechine gallate and esculetin were procured from Sigma-Aldrich Co (St. Louis, MO, USA). The plants *Hibiscus vitifolius*, *Zizyphus glabrata*, *Diospyros sylvatica*, *Tephrosia pulcherrima* and their extracts were reported to posess antioxidant activity. Hence, the author has selected some plants, analysed by chemically, and characterized by conventional extraction and chromatographic methods. Isolated compounds were tested for various activities claimed in literature. Gossypin, quercetin, (-)-epicatchine gallate, gallic acid, microphyllone, candinone, esculetin and lupeol were tested for the following biological activities.
i. *In vitro* antioxidant activity

ii. *In vivo* antioxidant activity against CCl₄, Aluminium, Sodium fluoride and Streptozotacin induced oxidative stress.

iii. Neuroprotective activity of in global ischemia model.

iv. Anti-allergic activity in mast cell mediated allergy model.

v. Antiosteoporosis activity in overriectamized rats.

vi. *In vitro* anticancer activity in EAC and human cancer cell lines

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