CHAPTER 1

REVIEW OF LITERATURE

1.1 FREE RADICALS

1.1.1 Definition

Several definitions of the term free radical exist, but Halliwell and Gutteridge (1989) adopt a broad approach and define a free radical as any species (atom or molecule) that has one or more unpaired electrons. This definition embraces the atom of hydrogen (one unpaired electron), most transition metal ions and the oxygen molecule.

1.1.2 Formation of Free Radicals

The human body is composed of many different types of cells Cells are composed of many different types of molecules. Molecules are composed of atoms bonded together. This bonding process is accomplished by the sharing of electrons. When two atoms come together and their electrons pair up, a bond is created. It is a general principle of quantum chemistry that only two electrons can exist in one bond Specifically, each electron must have opposite spin from the other Paired electrons are quite stable; nearly 100% of all electrons in the human body exist in a paired state.

When a bond is broken (by radiation, for example), the electrons can stay together (i.e., both go to one of the atoms and the other atom gets none) or they can split up (one electron goes to each atom). If they stay together, the molecular fragments are called ions, and they are electrically charged (the atom with the electrons is negatively charged and the one without the electrons is positively charged). A good example of this is sodium chloride (salt), which splits up into a chloride anion (Cl^-) and a sodium cation (Na^+).

If the electrons split up, the atoms are free radicals (molecules with an unpaired electron). The unpaired electrons are highly energetic. This is a very hazardous, unnatural and unstable state, because electrons normally come in pairs. This odd, unpaired electron in a free radical causes it to collide with other molecules so that it can steal an electron from them, which changes the structure of these other molecules and causes them to also become free radicals. This can create a self-

perpetuating chain reaction in which the structures of millions of molecules are altered in a matter of nanoseconds reeking havoc with our DNA, protein molecules, enzymes and cells. Once the process is started, it can cascade, finally resulting in the disruption of a living cell This electron rip off is what makes free radicals both useful and dangerous.

1.2 REACTIVE OXYGEN SPECIES

Reactive oxygen species (ROS) is a collective term which is used by biologists to include not only oxygen radicals ($O_2^{\bullet-}$, $^{\bullet}OH$) but also some derivatives of oxygen that do not contain unpaired electrons such as H_2O_2, singlet oxygen ($^1O_2$), hypochlorous acid (HOCl) and peroxynitrite ($ONOO^-$) (Hemnani and Parihar, 1998).

The ground state diatomic oxygen molecule (O_2) is itself a radical, with two unpaired electrons each located in a π^* antibonding orbital The electronic structure causes O_2 to form water by a stepwise (univalent) reduction with four electrons (Fig. 1.1).

$$O_2 + 4H^+ + 4e^- \text{ --------> } 2H_2O$$

The two unpaired electrons have the same spin quantum number (parallel spin), and so if O_2 attempts to oxidise another atom or molecule by accepting a pair of electrons from it, both new electrons must be of parallel spin to fit into the vacant spaces in the π^* orbitals. Most biomolecules are covalently bonded nonradicals, and the two electrons forming a covalent bond have opposite spins and occupy the same molecular orbital.

Hence the reaction of oxygen with biomolecules is spin restricted. The spin restriction itself is of benefit for life in an oxygen-rich environment since it slows down the reaction of molecular oxygen with biological molecules. There is also an important orbital restriction Survival, however, ultimately depends on the constant repair of oxidative damage. This is supported by specific (enzymatic) antioxidant protection and less specific scavenger molecules, which protect key sites and limit overall damage. Transition metals are found at the active sites of most

oxidases and oxygenases because their ability to accept and donate single electrons can overcome the spin restriction of oxygen.

1.2.1 Singlet Oxygen

Another way of increasing the reactivity of O_2 is to move one of the unpaired electrons in a way that alleviates the spin restriction. This requires an input of energy and generates the singlet states of O_2. Singlet oxygen, the most important in biological systems, has no unpaired electrons and thus does not qualify as a radical. Singlet oxygen usually decays before it has time to react with anything. Excitation of O_2 to the singlet state can be achieved when several pigments are illuminated in the presence of O_2. The pigment absorbs light, enters higher electronic excitation state and transfers energy onto the O_2 molecule to make singlet O_2. Singlet O_2 formation is thus likely to occur in many pigmented systems exposed to light; the lens of the eye and the illuminated chloroplast are examples. It is often stated that singlet O_2 is formed by the dismutation of radicals and during the respiratory burst of neutrophils There are no specific scavengers of singlet O_2. All react with hydroxyl radical or with atleast one organic peroxyl radical and with hypochlorous acid formed by the action of myeloperoxidase in activated neutrophils (Halliwell and Gutteridge, 1984).

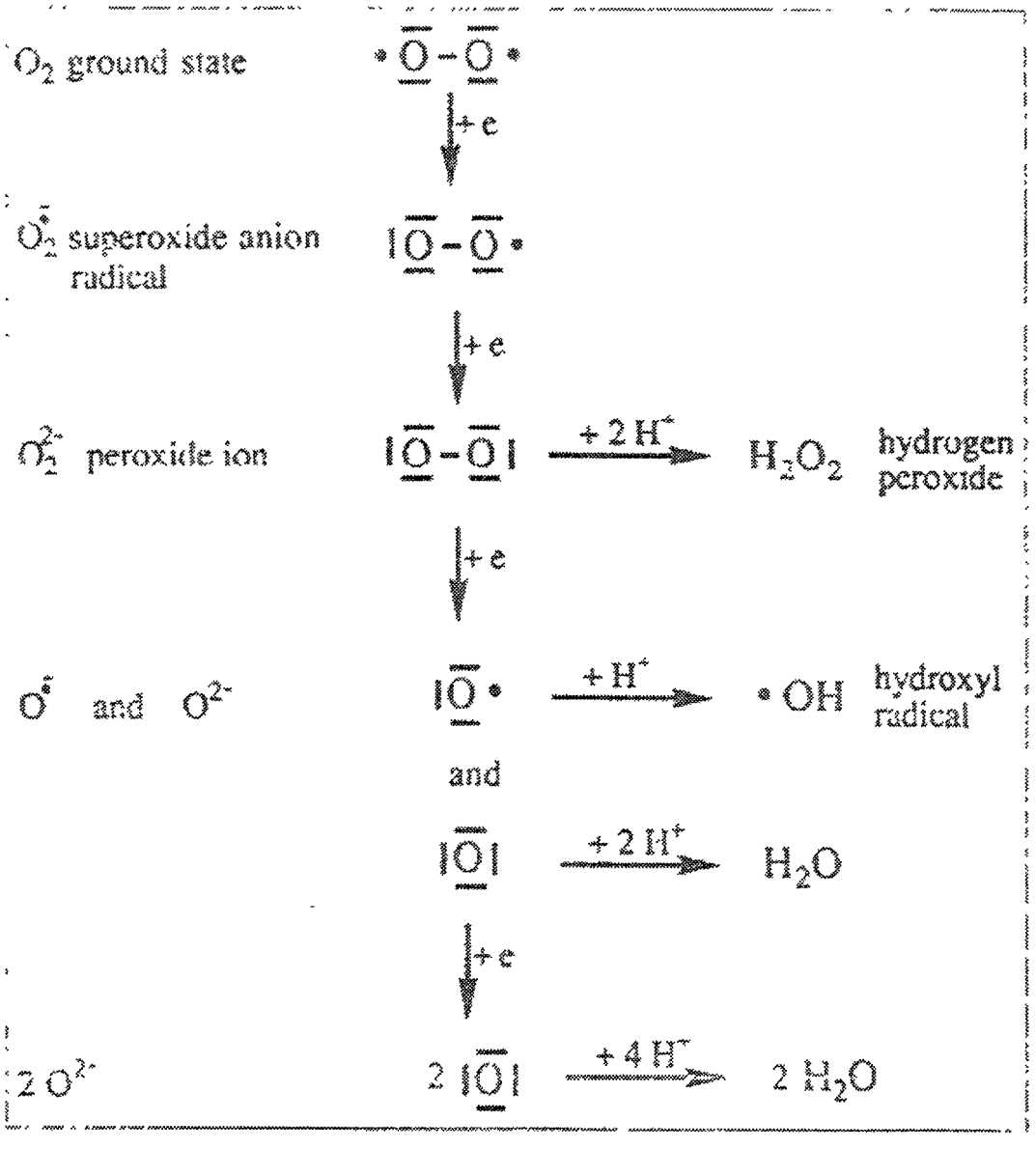


Fig. 1.1: The univalent reduction of oxygen. (Bast et al., 1991)

1.2.2 Superoxide Radical

One-electron reduction of oxygen produces superoxide radical O_2^-. This is frequently written as $O_2^{\bullet-}$. Superoxide is formed in almost all aerobic cells, a major source being leakage of electrons from various components of the cellular electron transport chains, such as those of mitochondria, chloroplasts and the endoplasmic reticulum. The amount of leakage, and hence the rate of $O_2^{\bullet-}$ production, increases as the O_2 concentration is raised. $O_2^{\bullet-}$ is produced during the respiratory burst of phagocytic cells (neutrophils, monocytes, macrophages and eosinophils). This plays a key role in the killing of several bacterial strains. Evidence is accumulating that superoxide is also produced *in vivo* by several cell types other than phagocytes, including lymphocytes and fibroblasts. Superoxide produced by such cells is often thought to be involved in intercellular signalling and growth regulation. The univalent reduction of O_2 forming $O_2^{\bullet-}$ also occurs from other normal biochemical oxidation-reductions, both enzymatic (e.g. xanthine oxidase) and non-enzymatic reactions (such as autoxidation of catecholamines). $O_2^{\bullet-}$ is metabolised by metalloenzymes, superoxide dismutases (SODs), to form H_2O_2 and O_2 (Halliwell and Gutteridge, 1984).

$$O_2 + e^- \xrightarrow{+1e,\ +2H} O_2^{\bullet-} \xrightarrow{SOD} H_2O_2 + O_2$$

1.2.3 Hydrogen Peroxide

A two-electron reduction of oxygen would yield hydrogen peroxide.

$$O_2 + 2e^- \longrightarrow H_2O_2$$

Superoxide can undergo a non-enzymatic or SOD catalyzed dismutation reaction to generate hydrogen peroxide.

$$2\ O_2^{\bullet-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Hydrogen peroxide is very diffusible within and between cells. Besides arising from superoxide, hydrogen peroxide is produced by the action of several oxidase enzymes (amino acid oxidases, xanthine oxidase) *in vivo*. Oxygen is simultaneously reduced both to superoxide and to hydrogen peroxide by these enzymes. Both superoxide and hydrogen peroxide can find some targets within cells at which they can do direct damage, although on the whole their reactivity is limited. Several metabolic roles of hydrogen peroxide are reported which include the formation of thyroid hormone, gene expression controlled by NKkB, induction of genetic expression of the provirus human immunodeficiency virus I and activation of human atherosclerotic lesions (Riley and Behrman, 1991).

H_2O_2 can be safely decomposed by glutathione peroxidase (GPx or GSH-Px) and catalase (CAT). At low concentrations, H_2O_2 is removed by reacting with reduced glutathione (GSH) to form oxidised glutathione (GSSG) and H_2O, catalysed by the selenium-dependent GPx.

$$2GSH + H_2O_2 \xrightarrow{GPx} GSSG + 2\,H_2O$$

Glutathione is a tripeptide of glutamate, cysteine and glycine. As an antioxidant, besides being a substrate for GSH peroxidase enzymes, it can also scavenge various free radicals directly though due to its hydrophilic nature it is a poor scavenger of radicals formed in the lipid membrane. GSH is regenerated by the action of glutathione reductase (GRD), a flavoprotein (FAD-containing) enzyme.

$$GSSG + NADPH + H^+ \xrightarrow{GRD} 2GSH + NADP^+$$

At high concentrations, H_2O_2 is removed by the heme-containing enzyme catalase.

$$2\,H_2O_2 \xrightarrow{CAT} O_2 + 2\,H_2O$$

Catalases are present in the peroxisomes of mammalian cells, and serve to destroy hydrogen peroxide generated by oxidase enzymes located within these subcellular organelles.

H_2O_2 can react non-enzymatically with Fe^{2+} and Cu^{1+} or chelates in Fenton type reactions, thereby being converted into reactive $OH^{\bullet}$radical.

$$Fe^{2+} + H_2O_2 \text{ ---------> } Fe^{3+} + OH^{\bullet} + OH^{-}$$

1.2.4 Hydroxyl Radical

Much of the damage done by superoxide and hydrogen peroxide *in vivo* is thought to be due to their conversion into highly-reactive oxidants, the major one being hydroxyl radical $OH^{\bullet}$.

$$O_2 \xrightarrow{-1e} O_2^{\bullet-} \xrightarrow[2H+]{+1e} H_2O_2 \xrightarrow[+1H+]{+1e} OH^{\bullet} \xrightarrow[+1H+]{+1e} H_2O$$

Hydrogen peroxide can react nonenzymatically with ferrous and cupric ions or chelates in Fenton-type reactions, thereby converted to reactive hydroxyl radical. The oxidised form of the metal and hydroxide ion are also products of the reaction.

$$Fe^{2+} + H_2O_2 \text{ ---------> } Fe^{3+} + OH^{\bullet} + OH^{-}$$

The Fenton reaction can be augmented by the reduction of ferric ion by superoxide anion radical, regenerating ferrous ion. The net result is the production of hydroxyl radicals as in the iron-catalysed Haber-Weiss type of reaction.

$$Fe^{3-} + O_2^{\bullet-} \text{ ---------> } Fe^{2+} + O_2$$

$$Fe^{3-} + H_2O_2 \text{ --------> } Fe^{2+} + O_2$$

$$O_2^{\bullet-} + H_2O_2 \text{ ---------> } O_2 + OH^{\bullet} + OH^{-}$$

$OH^{\bullet}$ is also formed by the decomposition of $ONOO^-$. $ONOO^-$ is formed in cell when $O_2^{\bullet-}$ reacts with nitric oxide radical ($NO^{\bullet}$) in a radical addition reaction.

$$O_2^{\bullet-} + NO^{\bullet} \text{ ------> } ONOO^-$$

$$ONOO^- + H^+ \text{ -----> } {}^{\bullet}OH, NO_2, NO_2^+$$

Formation of hydroxyl radical from superoxide requires traces of catalytic transition metal ions of which iron is the most important *in vivo*, although copper ions might also play a role.

The hydroxyl radical is the most reactive of the oxygen radicals. It combines with almost all molecules found in living cells. It is so reactive that no enzyme systems involving it as a substrate exist. The cell's efforts are directed at preventing its formation by removing hydrogen peroxide and moving transition metals to inactive sites. Because of its reactivity, the hydroxyl radical does not travel far and has a half-life of a few microseconds. However, hydrogen peroxide can cross cell membranes and lead to hydroxyl radical formation at more distant sites (Halliwell, 1994; Cheesman and Slater, 1993).

Because it is a radical, however, its reactions leave behind a legacy in the cell in the form of propagating chain reactions. Thus if $^{\bullet}OH$ attacks DNA, free radical chain reactions occur and cause strand breakage, deoxyribose fragmentation and extensive chemical alteration of the purine and pyrimidine bases (Von Sonntag, 1987). Imperfect repair of DNA damage caused by $^{\bullet}OH$ can result in proto-oncogene activation and carcinogenesis. Hence, high-energy radiation can lead to cancer, which implies that $^{\bullet}OH$ is a complete carcinogen (Breimer, 1988).

Perhaps the best characterised biological damage caused by $^{\bullet}OH$ is its ability to initiate the free radical chain reaction known as lipid peroxidation; leading to disruption of membrane function and can cause it to collapse (Halliwell, 1987).

1.2.5 Hypochlorous acid

Although not a free radical, hypochlorous acid is a potent chlorinating and oxidising agent. HOCl attacks primary amines and sulfhydryl groups in proteins and may chlorinate purine bases in DNA. One of the most important targets attacked by HOCl is alpha antiproteinase, which is the major inhibitor in body fluids (Arouma, 1994).

1.2.6 Nitric Oxide

It is only relatively recently that the biological importance of nitric oxide (NO) has been appreciated. This radical has a structure similar to that of superoxide, except that it has $2e^-$ less. It is widely thought that the endothelium-derived relaxing factor (EDRF) produced by vascular endothelium, which is an important mediator of vascular responses induced by several pharmacological agents is nitric oxide.

The free radical nitric oxide is synthesized from the amino acid L-arginine in the presence of the enzyme nitric oxide synthase, by vascular endothelial cells, phagocytes, certain cells in the brain and many other cell types. Nitric oxide is a very unstable species; under aerobic conditions it reacts with oxygen to produce, through intermediates such as NO_2, N_2O_4, N_3O_4, the stable products nitrate and nitrite. Nitric oxide acts as a neurotransmitter, prevents platelet aggregation and is a defense molecule of immune system against tumor cells, parasites and bacteria. Nitric oxide can react with superoxide anion to generate peroxynitrite. Peroxynitrite itself a damaging species, can get protonated and undergo decomposition to hydroxyl radical, causing additional damage (Moncada and Higgs, 1993).

1.3 SOURCES OF FREE RADICALS

Oxidative reactions continuously take place during normal cellular metabolism. A secondary effect of these reactions is free radicals. Free radicals are a fact of life. Life as we know it could not exist, without free radicals. When the cells and body carry on with their daily functions, oxygen is used in the process and oxidation takes place - and although

these are normal functions, they do cause free radicals, the waste material of these processes that can have an influence on the forming of cancer, arterial damage, inflammation and accelerated aging through oxidative damage. They are also caused by a diet high in fried and barbequed foods, pollution, radiation, etc.

Free radicals are produced during normal body processes, as well as from tissue injury, and as a result of exposure to tobacco smoke, sunlight, x-rays, and other environmental factors.

In aerobic cells, incomplete reduction of oxygen in the mitochondrial electron transport chain releases superoxide anion radicals into the cytosol. The superoxide radical is relatively unreactive but may interact with transition metal ions such as iron or copper to produce the highly reactive and damaging hydroxyl radical. Powerful metal binding mechanisms exist to prevent its participation in redox reactions The enzyme xanthine oxidase may also be a source of superoxide radicals during reperfusion of ischaemic tissues. Inflammatory cells such as macrophages and neutrophils produce hydrogen peroxide and hypochlorous acid as a means of bacterial killing. These oxidants may nevertheless damage innocent bystander cells and be responsible for much of the damage associated with the inflammatory process. Drugs may exert toxic effects by promoting free radical formation during their metabolism, e.g. the hepatotoxicity associated with paracetamol (acetaminophen) Cigarette smoke represents a major threat to health and many of its damaging effects can be attributed to its free radical content and subsequent oxidative damage (Maxwell, 1995).

1.4 REACTIVE OXYGEN SPECIES AND ITS BIOLOGICAL CONSEQUENCES

Free radicals disrupt the equilibrium of biological systems by damaging their major constituent molecules, leading eventually to cell death. Poly-unsaturated fatty acids within cell membranes and lipoproteins are particularly susceptible to oxidative attack (lipid peroxidation), often as a result of metal-ion dependent hydroxyl radical formation (Aust et al., 1985). Following initiation by a single radical, if

oxygen is present, long chains of lipid peroxides may be formed by a rapid free radical chain reaction causing serious disruption of cell membrane function (Sevenian and Hochstein, 1985).

Proteins exposed to free radical attack may fragment, cross-link or aggregate. The consequences include interference with ion channels, failure of cell receptors and failure of oxidative phosphorylation. Free radical-induced damage to DNA may cause destruction of bases and deoxyribose sugars or single and double strand breaks (Aruoma et al , 1989). These events have been implicated as a cause of mutagenesis, carcinogenesis and cell death (Scholes, 1983)

Reactive oxygen species can thus attack vital cell components like polyunsaturated fatty acids, nucleic acids, proteins and carbohydrates. These reactions can alter intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein cross linking, inhibition of protein synthesis, DNA damage: ultimately resulting in cell death. Some of the well-known consequences of generation of the free radicals *in vivo* are: DNA strand scission, nucleic acid base modification, protein oxidation and lipid peroxidation.

1.4.1 Lipid Damage (Lipid Peroxidation)

Oxygen radicals catalyse the oxidative modification of lipids Lipids are byfar the most susceptible target for free radical attack. Hydrogen peroxide may be reduced by free iron (or certain types of iron chelates) to the hydroxyl radical and this is the species, which is the initiator of lipid peroxidation. Hydroxyl radical can initiate the oxidation of polyunsaturated fatty acids by abstraction of a hydrogen atom from a methylene group of the fatty acid. This abstraction of hydrogen can also be brought about by transition metal ions. The result is the formation of the lipid radical. The lipid radical formed, tends to stabilise by molecular rearrangement to form a conjugate diene. The conjugate diene readily combines with oxygen to give peroxyl radical. The peroxyl radicals are capable of abstraction of hydrogen from another lipid molecule (from an adjacent fatty acid chain) propagating the chain reaction. The peroxyl radical combines with hydrogen atom to give lipid hydroperoxide (or lipid

peroxide). The peroxyl radicals are the carriers of the chain reaction. They can oxidise further PUFA molecules and initiate new chains producing lipid hydroperoxides that can break down (in the presence of transition metal ions) to yield more radical species and to a wide range of compounds, notably aldehydes, many of them being cytotoxic These aldehydes can diffuse from the original site of attack and spread the damage to other parts of the cell. Lipid peroxidation has been implicated in a wide range of tissue injuries and diseases (Halliwell and Gutteridge, 1990a).

$LH + R^{\bullet} \text{----------} > L^{\bullet} + RH$

Lipid radical

$L^{\bullet} + O_2 \text{---------} > LOO^{\bullet}$

Lipid peroxyl radical

$LOO^{\bullet} + LH \text{-------} > LOOH + L^{\bullet}$

Lipid hydroperoxide

LOOH ----------> LO, LOO, aldehydes

Process of Lipid Peroxidation

1.4.2 DNA Damage

DNA is readily attacked by oxidizing radicals if they are formed in its vicinity. Free radical damage of the DNA of living organisms may be due to breakage of the main strand, degradation of the bases or cleavage of hydrogen bonds. All components of nucleic acids may be exposed to free radical damage, which may become permanent or may be repaired by special mechanisms. Unrepaired damage in bases may lead to mutation, while damage in the pentose part, to chain breakage. When cells are subjected to oxidant stress, for example, by generation of superoxide and hydrogen peroxide, by treatment with high external concentrations of hydrogen peroxide or by exposure to cigarette smoke, DNA strand

breakage usually occurs. This could occur because the oxidant stress leads to activation of some specific DNA-cleaving mechanism, such as calcium dependent endonuclease. Hydrogen peroxide can also react with intracellular metal ions to give hydroxyl radical which fragments the DNA by site-specific hydroxyl radical attack. Oxidant stress can also liberate iron ions from their sites of sequestration within the cell, so that they can bind to DNA. Recent studies show that $^1O_2$ is capable of inducing DNA damage. Its reaction with DNA results in single strand breaks besides formation of the altered bases. The biological consequences associated with these modifications in DNA are the loss of biological activity as assessed by transforming ability as well as mutagenicity and genotoxicity (Halliwell and Aruoma, 1993).

1.4.3 Damage to Enzymes and Proteins

Metal catalyzed oxidation has been identified as a post translational covalent modification of proteins which may be important in several physiological and pathological processes which include the ageing process, intracellular protein turnover, arthritis and pulmonary diseases. Proteins are also membrane constituents; therefore, their damage explains the membrane-damaging effect of free radicals, while the loss of specific activity of enzymes may also have severe consequences. Introduction of carbonyl groups into amino acid residues of proteins is a hallmark of oxidative modification. Oxygen free radical attack on proteins also results in loss of catalytic activity, loss of histidine residue and changes in surface hydrophobicity. Oxidative damage results in the oxidation of -SH groups of proteins, cross-linking of proteins and peptide fragmentation. Hydroxyl radicals are capable of attacking many amino acid residues. Proteins often bind transition metal ions, making them a target for attack by site-specific hydroxyl radicals (Halliwell, 1994).

1.4.4 Damage to Carbohydrates

Sugars including glucose, mannitol and deoxysugars react readily with hydroxyl radical. Hyaluronic acid, which forms the central axis of proteoglycans and maintains the viscosity of synovial fluid, is fragmented

following exposure to free radicals resulting in destabilization of connective tissue and loss of synovial fluid viscosity (Ryan and Aust, 1992).

1.4.5 Lipofuscin Pigments

Lipofuscin pigments accumulate in human and animal tissues as a result of ageing. Recent studies have shown that they are lipid-protein complexes resulting from the peroxidation of polyunsaturated fatty acids of subcellular membranes and correspond to the so-called residual body, the end product of lysosomal digestion. The pigments have characteristic fluorescent spectra. The Schiff base product of malondialdehyde (one of the terminal products of lipid peroxidation) cross linking with primary amino groups of proteins, and with the amino groups of nucleic acids and nucleic acid bases, phospholipids has similar fluorescent characteristics (Halliwell and Aruoma, 1993).

1.5 DEFENSES AGAINST FREE RADICALS: ANTIOXIDANTS

The term "antioxidant" is frequently used in the biomedical literature, but it is rarely defined. Often the term is implicitly restricted to chain-breaking antioxidant inhibitors of lipid peroxidation, such as alpha-tocopherol. However, Halliwell and Gutteridge (1990b) take a much broader view and define an antioxidant as "any substance that when present at low concentrations compared to those of an oxidisable substrate significantly delays or inhibits oxidation of that substrate". The term 'oxidisable substrate' includes almost everything found in living cells, including proteins, lipids, carbohydrates and DNA.

Antioxidants (also known as free radical scavengers) function by offering easy electron targets for free radicals. In absorbing a free radical, antioxidants "trap" (de-energize or stabilize) the lone free-radical electron and make it stable enough to be transported to an enzyme, which combines two stabilized free radicals together to neutralize both. Antioxidants can act at different levels in an oxidative sequence. This may be illustrated by considering one of the many mechanisms by which oxidant stress can cause damage: stimulating the free radical chain

reaction of lipid peroxidation. Antioxidants could act against lipid peroxidation by

1. Decreasing localized O_2 concentrations (e.g. by combining with O_2 or displacing it).
2. Preventing initiation of peroxidation by scavenging species capable of abstracting hydrogen atoms, such as hydroxyl radical, $^{\bullet}OH$.
3. Quenching or scavenging singlet O_2 that can react directly with membrane lipids to produce peroxides. For example, lycopene has been reported to be the best lipid soluble quencher of singlet O_2 in human plasma.
4. Binding metal ions in forms that will not generate reactive species (such as $^{\bullet}OH$, ferryl, or $Fe^{2+}/Fe^{3+}/O_2$ complexes) and/or will not decompose lipid peroxides to peroxyl and alkoxyl radicals
5. Removing peroxides by converting them into non-radical products, such as alcohols. For example, glutathione peroxidases act as peroxide-removing antioxidants
6. Chain breaking, i.e., reacting with chain-propagating radicals (peroxyl and possibly alkoxyl), so preventing continued hydrogen abstraction from fatty acid side chains. Chain-breaking antioxidants are often phenols or aromatic amines and α-tocopherol is the major although probably not the only, lipid soluble chain-breaking antioxidant in human tissues.

Antioxidants inhibiting lipid peroxidation by mechanisms 1, 2, 3, or 4 can be called preventative antioxidants. Those acting by mechanism 4 would not be expected to be consumed during the course of the reactions. Antioxidants of the fifth type are also preventative antioxidants, but they may or may not be consumed during the reaction, depending on their chemical behaviour (e.g. glutathione peroxidase acts by this mechanism and being an enzyme, is a catalyst and is not consumed). Chain breaking antioxidants, acting by combining with chain-propagating radicals will be consumed, as will antioxidants acting by scavenging singlet O_2 (Halliwell and Gutteridge, 1990b).

1.5.1 Endogenous Antioxidants

1.5.1.1 Antioxidant Defense *In Vivo*: Intracellular

The major intracellular antioxidants in the human body are probably the enzymes superoxide dismutase, catalase and glutathione peroxidase. Two isoenzymes of SOD exist in human cells: an enzyme containing copper and zinc ions (largely located in the cytosol) and mitochondrial SOD containing manganese ions. Both catalyze the same reaction. In addition, membranes contain the chain-breaking antioxidant α-tocopherol, a lipid soluble molecule that is located in the interior of biological membranes. α-tocopherol is the most important chain-breaking antioxidant present in human membranes but not the only one (Halliwell, 1991).

1.5.1.2 Antioxidant Defence *In Vivo*: Extracellular

Antioxidant defence in the extracellular compartments of the human body relies largely on different strategies than does intracellular defence, α-tocopherol is still important. Thus, the content of α-tocopherol in plasma lipoproteins helps to determine their resistance to lipid peroxidation, but it is not the only antioxidant present.

By contrast with the intracellular environment, enzymic antioxidant defence enzymes are much less prominent in extracellular fluids. Blood plasma, tissue fluid, cerebrospinal fluid, synovial fluid and seminal plasma contain little or no catalase activity, and only low activities of superoxide dismutase and selenium-containing glutathione peroxidase can be measured. There is also very little reduced glutathione (GSH) in most extracellular fluids (Halliwell, 1991). Glutathione is vital to maintaining a variety of intracellular functions, including detoxification, antioxidation, tertiary protein configuration, and redox balance (Kidd, 1997)

'Extracellular' superoxide dismutase (EC-SOD) enzymes have also been described. Although EC-SODs contain copper and zinc, they are very different from intracellular CuZnSOD in that EC-SODs have a much higher relative molecular mass and possess attached carbohydrate. The biological role of EC-SODs is unclear; such low activities are present in

plasma and in other extracellular fluids that bulk scavenging of $O_2^{\bullet-}$ in extracellular fluids has always seemed to be an unlikely physiological function for them. Marklund (1990) has shown that some EC-SODs bind to heparin and has proposed that, *in vivo*, they may be associated with endothelial cell surfaces as a protective antioxidant 'layer' over the cells Trace amounts of EC-SODs are found attached to cells in various tissues, so the term 'extracellular' does not really mean a location within extracellular fluids.

Thus, enzymic removal of $O_2^{\bullet-}$ and H_2O_2 contributes little to the antioxidant activity of extracellular fluids. Indeed, $O_2^{\bullet-}$ and/or H_2O_2 generated extracellularly in small amounts by endothelium, lymphocytes, platelets, fibroblasts and other cells may play important physiological roles and thus should not be scavenged with 100% efficiency. Halliwell and Gutteridge (1990a,b) have argued that a major antioxidant defence of human plasma is to prevent $O_2^{\bullet-}$ and H_2O_2 from dangerous species such as $^{\bullet}OH$, by binding transition metal ions in forms that will not stimulate free radical reactions, or by otherwise preventing the metal ions from participating in such reactions. Thus, safe sequestration of iron and copper ions into forms that will not catalyze free radical reactions is an important antioxidant strategy in the human body.

The iron-transport protein transferrin in plasma is only 20 to 30% loaded with iron, so that the content of free ionic iron in plasma is effectively nil. Iron, bound to transferrins will not participate in $^{\bullet}OH$ radical formation or lipid peroxidation (Aruoma and Halliwell, 1987; Gutteridge et al., 1981).

Haemoglobin can be liberated into plasma from disrupted erythrocytes and myoglobin can be released from muscles after injury (e.g. myocardial infarction, or skeletal muscle damage during strenous exercise). Both of these haem-containing proteins are potentially dangerous in that they can accelerate peroxidation of lipids, including plasma lipoproteins in the presence of H_2O_2. Plasma contains haemoglobin-binding proteins known as haptoglobins, as well as a haem-binding protein (haemopexin). Binding of haemoglobin to haptoglobins or of haem to haemopexin diminishes their effectiveness in stimulating lipid

peroxidation. The haemoglobin-haptoglobins or haem-haemopexin complexes are rapidly cleared from the circulation.

The plasma copper containing protein caeruloplasmin is thought to play an essential role in iron metabolism, but it also has antioxidant properties. First, caeruloplasmin has a ferroxidase activity – it oxidises Fe^{2+} to Fe^{3+} whilst reducing oxygen to water. However, unlike the non-enzymatic oxidation of Fe^{2+} ions by O_2, this caeruloplasmin-catalysed oxidation of Fe^{2+} does not release any damaging oxygen radicals: they are kept on the active site of the protein. The ferroxidase activity of caeruloplasmin allows it to inhibit iron ion-dependent lipid peroxidation and $^{\bullet}OH$ formation from H_2O_2 under most conditions and this is probably the major antioxidant activity of the protein (Halliwell, 1991).

Albumin can also bind copper ions and it usually inhibits copper ion-dependent lipid peroxidation and $^{\bullet}OH$ radical formation In fact, the reactions often continue at the sites of metal ion binding and damage the protein, but the high concentration of albumin in plasma and the rapid turnover of this protein mean that such damage is probably biologically insignificant. Thus, binding to albumin of any copper ions released into plasma may lead to damage to the protein if $O_2^{\bullet-}$ and H_2O_2 are generated in plasma. However, albumin may well prevent the copper ions from attaching to more important targets, such as –SH groups essential to membrane function, e.g. on the membrane proteins of endothelial cells of erythrocytes, where binding of Cu^{2+} ions could lead to oxidative damage. Binding to albumin might also help to stop copper ions from accelerating the peroxidation of low-density lipoproteins and promoting atherosclerosis. The copper ion-albumin complex might be a safe 'transit form' of copper that can be removed from the circulation by the liver.

Albumin transports fatty acids in the blood and the bile pigment bilirubin is bound to it. It has been claimed that bilirubin acts as an antioxidant inhibitor of lipid peroxidation *in vitro*. Perhaps bilirubin protects albumin-bound fatty acids against peroxidation *in vivo*. Induction of the enzyme haem oxygenase (occurs in cells exposed to oxidative stress) serves not only to remove a pro-oxidant (haem) but also to increase

antioxidants (e.g. bilirubin). Albumin is also a powerful scavenger of HOCl in plasma (Halliwell, 1991).

Ascorbic acid, present in plasma has multiple antioxidant properties, probably including the ability to regenerate α-tocopherol by reducing α-tocopheryl radicals at the surface of lipoproteins and membranes. High concentrations of ascorbic acid are also present intracellularly, especially in brain and lung cells. Mixtures of ascorbic acid and H_2O_2 with iron or copper ions have powerful pro-oxidant properties, however, because they can form $^{\bullet}OH$ and other reactive species. This emphasizes the need for careful sequestration of 'free' transition metal ions in the human body in order for ascorbic acid to exert its antioxidant action. The fact that large amounts of ascorbic acid can safely be given to healthy humans illustrates how effective this sequestration of iron and copper ions is, and means that ascorbic acid is probably an important antioxidant in healthy humans (Halliwell, 1991).

Uric acid can act as an antioxidant, both by binding iron and copper ions in forms that do not accelerate free radical reactions, and by directly scavenging oxidizing species such as singlet O_2, HOCl and peroxyl radicals Uric acid does react with some oxidants *in vivo*; however, reaction of uric acid with certain oxidizing species, such as $^{\bullet}OH$ or peroxyl radicals, can generate uric acid radicals that are themselves capable of doing biological damage, e.g. by inactivating certain enzymes. Fortunately, these uric acid-derived radicals can be reduced by ascorbic acid, one aspect of the many antioxidant actions of this molecule (Halliwell and Gutteridge, 1990b).

1.5.2 Exogenous Antioxidants

Endogenous antioxidant defence systems, though scavenge and minimize the formation of oxygen free radicals, are not 100% effective, especially so, in pathological conditions demanding the use of exogenous antioxidants. Hence large number of drugs and natural compounds have been studied and characterized as potent antioxidants. A few of them are discussed below.

The antioxidant property of glutathione has lead to the study of several sulfhydryl-containing compounds for their antioxidant activity. Lipoic acid and N-acetylcysteine have received most attention in this regard. Both these compounds act by replenishing the reduced glutathione (Moldeus and Cotgreave, 1994). Lipoic acid also acts as a singlet oxygen scavenger. Dihydrolipoic acid inhibits hepatic microsomal lipid peroxidation. N-acetylcysteine protects against free radical mediated cellular damage (due to its ability to act as a precursor for the cysteine portion of the tripeptide glutathione) and is a powerful scavenger of hypochlorous acid. N-acetylcysteine has been reported to be of use in several conditions including AIDS. Sulfhydryl containing drugs diethyldithio carbamic acid (a disulfuram metabolite), captopril, 6-mercaptopurine and 2-mercaptopropionylglycine have been studied for their antioxidant properties (Egan et al , 1988).

A large series of anti-inflammatory drugs have been recently tested with regard to their iron binding and hydroxyl radical scavenging actions. and were found to be protective against site-specific damage by the hydroxyl radical (Aruoma and Halliwell, 1988; Sorenson, 1984) There is an antioxidant component in the effect of the known anti-inflammatory agents. Nonsteroidal anti-inflammatory agents act not only by inhibiting cyclooxygenase, but also by the superoxide dismutating effect of their copper complex, which is comparable in strength to that of SOD. Such an effect has been demonstrated for penicillamine, indomethacin, piroxicam and acetylsalicylic acid.

Other examples include iron porphyrins, a complex of manganese ions with the chelating agent desferrioxamine or copper ion chelated to amino acids. The superoxide dismutating effect of the copper complexes is probably catalytic, as opposed to the stoichoimetric effect of scavengers. Therefore the complexes are effective in lower doses and have a more lasting effect. Compared with SOD, they also have the advantage of a low molecular weight, thus being able to enter the cells more easily than the high molecular weight SOD. Superoxide scavengers that do not involve metal ions such as nitroxides have also been described. Several

nonsteroidal anti-inflammatory agents have also been shown to inhibit the respiratory burst of phagocytes (Sorenson, 1984).

Carotenoid pigments, such as β-carotene, are powerful quenchers of singlet oxygen and have found therapeutic use in protecting porphyria patients against photosensitization damage to the skin and in protecting animals against photosensitizing drugs.

Flavonoids have a prominent antioxidant activity. They are reported to chelate metal ions preventing free radical damage. Many of the flavonoids are reported to be scavengers of superoxide anion, hydroxyl radicals, singlet oxygen and inhibitors of iron stimulated lipid peroxidation (Halliwell and Gutteridge, 1990a) The antioxidant activity of other polyphenolic natural pigments like polyhydroxynaphytoquinones, gossypol and ellagic acid is also reported.

Ebselen is a newly designed anti-inflammatory agent, which has peroxidase activity with the endogenous thiol glutathione as a co-factor. Studies have shown that ebselen inhibits the oxidative burst of alveolar macrophages.

Curcumin, the major colouring component of turmeric, which is credited with several pharmacological properties including anti-cancer and anti-inflammatory activities is a scavenger of oxygen free radical and a potent inhibitor of lipid peroxidation.

Many β-adrenoreceptor blocking drugs, calcium antagonists and anti-arrhythmic agents have an antioxidant action. Several antibiotics have been tested for their radical scavenging effect. Some could inhibit the inactivation of alpha 1-protease by hypochlorous acid. Allopurinol, oxypurinol, folic acid and pterin aldehydes are reported to inhibit superoxide generation by xanthine oxidase by inhibiting the enzyme (Wasil et al., 1988). They have a potent hydroxyl radical scavenging activity as well.

Butylated hydroxy toluene derivatives are being developed as lipoygenase inhibitors and radical scavengers. Recently, several vitamin E analogues have been synthesized which have shown chain-breaking antioxidant activity. Also vitamin C analogues such as 2-O-alkyl ascorbic acids have been synthesized and studied extensively.

Pycnogenol, also known as OPCs (Oligomeric proanthocyanidin complexes) are obtained from the bark of French maritime pine trees. This antioxidant protects cells from free radical activity, helps strengthen capillaries and possesses anti-inflammatory properties.

Potentiation of the protection against free radicals has also been attempted by administration of the endogenous enzymes superoxide dismutase and catalase. Chemically modified SOD or catalase is being developed in order to extend their half-lives, target them and reduce their antigenecity (Ogino et al., 1988).

Desferrioxamine is a powerful chelator of ferric and inhibits iron ion-dependent lipid peroxidation and the generation of highly reactive oxidising species such as hydroxyl radical from superoxide and hydrogen peroxide in the presence of iron ions. It can also react directly with several reactive oxygen species including the lipid peroxyl radical, superoxide radical, hydrogen peroxide and peroxynitrite (Ursini et al , 1989).

1.6 FREE RADICALS AND HUMAN DISEASES

"Oxidative stress" is associated with a disturbance in the pro-oxidant- antioxidant balance in favour of the pro-oxidant The occurrence of reactive oxygen species (ROS), known as pro-oxidants is an attribute of normal aerobic life. Aerobic life is characterized by a steady formation of pro-oxidants balanced by a similar rate of their consumption by antioxidants. To maintain homeostasis, there is a requirement for the continuous regeneration of antioxidant capacity, and if this is not met, oxidative damage accumulates, resulting in pathophysiological events (Sies, 1986).

The theory is that a process called oxidation damages important molecules in the body and harms vital structures in our cells - in the same way that rust damages the metalwork of a car. This oxidation contributes to many of the degenerative diseases that are common as we age - from heart disease to dementia, from cancer to cataracts. Antioxidants are chemicals that may be able to block this process.

Antioxidant defenses act in concert in cell differentiation and growth, immune responses, cell membrane integrity and normal DNA

repair. Despite the existence of endogenous defense mechanisms against ROS, it has been observed that whenever either the level of the cellular antioxidant systems goes down or when the ROS reach abnormally high levels, oxidative damage to the cells occurs, finally leading to several pathological conditions. Oxidative stress occurs when there are more free radicals than can be dealt with due to environmental insult, disease or malnutrition. An improper balance between formation and destruction of free radicals may play a role in degenerative disease and aging. Antioxidants are really a class of vitamins and nutritional ingredients that help fight and rid the body of free radicals that can cause untold damage to our body. The importance of antioxidants is nowadays accepted by even the most conservative medical fields and people find great benefits from these nutritional ingredients in achieving optimum health.

About 100 disorders, like rheumatoid arthritis, hemorrhagic shock, cardiovascular diseases, cystic fibrosis, metabolic disorders, neurodegenerative diseases, gastrointestinal ulcerogenesis and AIDS have been reported as the ROS-mediated disorders. Some specific examples of the ROS-mediated diseases are Alzheimer's disease, Parkinson's disease, oxidative modification of low-density lipoprotein in atherosclerosis, cancer, Down's syndrome and ischemic reperfusion injury in different tissues including heart, brain, kidney, liver and gastrointestinal tract (Bandyopadhyay et al., 1999).

1.6.1 Role of free radicals in Ulcers

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side effects (arrythmias, impotence, gynaecomastia and haematopoeitic changes) of modern medicine, indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer (Akhtar et al., 1992).

Although in most of the cases the etiology of ulcer is unknown, it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism (Piper and Stiel, 1986). To regain the balance, different therapeutic agents including herbal preparations are

used to inhibit the gastric acid secretion or to boost the mucosal defense mechanism by increasing mucus production.

There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammation and gastric ulcer (Repetto and Llesuy, 2002). Recently oxygen-derived free radicals have been postulated to play an important role in the pathogenesis of acute gastric mucosal injuries such as ischemia-reperfusion (Pery et al., 1986)-, stress (Cochran et al., 1982)-, ethanol (Pihan et al., 1987)-, anti-inflammatory drug (Del Soldato et al., 1985)- and pylorus-ligation (Rastogi et al., 1998)- induced gastric mucosal injuries in rats. Recent studies in the rat showed that oxygen-derived radicals are directly implicated in the mechanism of acute and chronic gastroduodenal ulceration and that scavenging them, stimulates the healing of ulcers (Salim, 1992). It has been demonstrated that many drugs or formulations possess potent antioxidant actions and are effective in healing experimentally induced gastric ulcers.

The gastrointestinal epithelium is continuously exposed to reactive oxygen metabolites that are generated within the lumen. Inspite of this exposure, the healthy epithelium appears unaffected, suggesting efficient mechanisms for protection against these potentially cytotoxic oxidants.

There is growing body of experimental data that suggests that reactive oxygen metabolites such as superoxide radical, hydrogen peroxide and hydroxyl radical mediate some of the cellular injury associated with enteritis, ischemic bowel disease, gastric ulceration and perhaps inflammatory bowel disease (Grisham et. al., 1986).

Although it is recognized that the epithelial, endothelial and phagocytic cells of the mucosa are important sources of these oxidants, there is evidence that the gastrointestinal mucosa is exposed to oxidants produced within the lumen. Potential sources of luminal oxidants include ingested food, cast-off mucosal cells, cigarette smoke and tar.

Oxygen derived free radicals damage cellular membranes and crosslink lipids, proteins and nucleic acids. In addition they cause

degradation of hyaluronic acid, the principal component of the epithelial basement membrane and thus promote mucosal injury. Reactive oxygen species, particularly hydrogen peroxide or subsequent oxidizing species such as $OH^{\bullet}$, cause injury to rat gastric mucosal cells *in vitro* (Hiraishi et al , 1987). Intracellular production of $OH^{\bullet}$ from H_2O_2, catalyzed by iron (Fenton reaction) seems to be mainly responsible for inducing cultured gastric cell injury exposed to reactive oxygen species (Hiraishi et al., 1987)

Free radicals in the process of aspirin and other NSAIDs and ethanol-induced injury may come from more than one source. Both the agents depress the gastric mucosal blood flow, aspirin via the inhibition of prostaglandin synthesis and ethanol by a direct action. The resulting tissue hypoxia and ischemia induce transformation of the native xanthine dehydrogenase into a predominantly oxidase form (Itoh and Guth, 1985) During ischemia, xanthine oxidase will use any available oxygen as an electron acceptor and convert xanthine or hypoxanthine into uric acid with the production of superoxide radical. The production of this radical then greatly increases when the oxygen supply is restored and extensive free radical-mediated tissue damage results (McCord, 1985).

Subsequently, superoxide and H_2O_2 react in the presence of transition metals or their chelates to form the highly reactive and cytotoxic hydroxyl radical.

On the other hand, depression of gastric mucosal blood flow reduces bicarbonate secretion and mucus production, thus allowing back-diffusion of H^{+}. This diffusion may lead to several adverse effects:

- Disruption of the mucosal vasculature, an action, which results in intramural hemorrhage, in addition to enhancing the degree of tissue ischemia thereby ensuring, continued presence of xanthine oxidase and subsequent production of superoxide radicals.
- Change in the mucosal pH that can convert the enzyme xanthine dehydrogenase to an oxidase form, thereby generating oxyradicals.
- Tissue irritation and microscopic injury, which may result in increased free radical production.

The potential source of release of free radicals is perhaps the neutrophils which release free radicals on activation and have been

implicated in the mechanism underlying oxidative damage following ischemia-reperfusion in the gastric mucosa (Smith et al., 1987). Indeed depletion of circulating neutrophils has recently been shown to reduce gastric mucosal damage/injury induced by indomethacin or naproxen.

The polymorphonuclear leucocytes infiltrating the vicinity of the aspirin- and ethanol-induced injury may be another source of free radicals. These cells undergo oxidative bursts that generate highly reactive metabolites of oxygen such as H_2O_2 and hypochlorite, as well as superoxide and hydroxyl radicals. Although these products of oxygen are involved in bacterial killing, their formation outside the cell may also cause tissue damage. The hydroxyl radical is the most reactive of the oxygen radicals and combines with almost all molecules found in the living cells.

Bandyopadhyay et al. (1999) have reported that during oxidative stress $OH^{\bullet}$ causes oxidative damage of the gastric mucosa and that the radical is formed by the metal catalyzed Haber-Weiss reaction between $O_2^{\bullet}$ and H_2O_2.

The free radical oxidation of PUFA to form hydroperoxides in biological systems, known as lipid peroxidation decreases the defence potentialities of gastroduodenal mucosa. Lipid peroxidation plays a crucial role in the pathogenesis of gastric ulceration. Oner et al. (1994) have reported that some environmental factors involved in lipid peroxidation disrupt the gastric mucosal protection and may impair the mucosal barrier facilitating the occurrence of gastric ulcers.

Neutrophil adherence to the vascular endothelium and the subsequent release of oxygen-derived free radicals and proteolytic enzymes has also been implicated as a critical event in the pathogenesis of various forms of gastric ulceration.

Local infusion of a superoxide generating system into the rat celiac artery has been shown to cause gastric mucosal bleeding as assessed by the leakage of radiolabelled erythrocytes (Wadhwa and Perry, 1987). Intraarterial infusion of hydrogen peroxide, which can form hydroxyl radicals or the superoxide generating system, was found to induce hemorrhagic damage in the rat gastric mucosa and this damage was attenuated by concurrent administration of catalase or SOD. Attempts

were made therefore to prevent gastric injury using chelators and the antioxidant enzymes, catalase or SOD.

Experimentally, injury in the stomach following hypovolemic shock can be reduced by agents that interfere with the free radical generation such as xanthine oxidase inhibitor, allopurinol or by free radical scavengers (Itoh and Guth, 1985).

Reports suggest an association between psychological stress and gastric ulcers. Stress ulceration of the stomach is associated with clinical conditions like trauma, head injury, burns, shock, sepsis and neurological diseases. Stress causes ischemic conditions in the gastric mucosa by reducing blood flow following activation of parasympathetic and sympathetic nervous system resulting in the constriction of the smooth muscles of the blood vessels and gastric tissue. This causes $O_2^{\bullet-}$, which is dismutated by SOD to form H_2O_2. Stress produces loss of gastroprotection and acidity, increased pepsin and histamine aggravate the situation (Das and Banerjee, 1993).

Pylorus-ligation induced ulcers

It is well known that pylorus-ligation causes gastric hypersecretion (Shay et al., 1945; Brodie et al., 1962; Ishii, 1969) due to poorly understood mechanisms. The activation of the vagus-vagal reflex by stimulation of pressure receptors in the antral gastric mucosa in the hypersecretion model of pylorus ligature is believed to increase gastric tonus and secretion. The stomach digestive effect of accumulated gastric juice in the induction of gastric ulcers is well documented in the pylorus-ligation model (Brodie, 1966). The gastric distension produced by accumulated secretion seems to influence the secretion of gastric acid in this model, possibly by increasing the release of gastrin hormone, and consequently further increasing acid secretion (Nagy et al., 1968).

Studies have shown alterations in the antioxidant status following ulceration, indicating that free radicals seem to be associated with the pylorus ligation-induced (Rastogi et al., 1998) ulceration in rats. They observed a time-dependent increase in the normal lesion index, acid content and pepsin activity in the gastric juice after pyloric ligation. They

also observed a time-dependent increase in the TBA reactive material, MDA indicating lipid peroxidation in the gastric mucosa after pyloric ligation. An increase in the myeloperoxidase (MPO) activity, a neutrophil marker enzyme following ulceration had also been observed. Neutrophil migration in the mucosa following pyloric ligation suggested that these cells might be involved in gastric mucosal injury. It might be possible that free radicals released from neutrophils cause lipid peroxidation and damage the cell membrane. The activity of SOD and glutathione peroxidase and content of GSH were found to decrease with time after pylorus ligation. In order to ascertain the role of free radicals in pylorus-ligation induced ulcers they also studied ulcer-induced changes 4h after ligation in rats pretreated with GSH depletor, N-ethyl maleamide (NEM) or catalase inhibitor (aminotriazole). Depletion of gastric glutathione content by NEM or inhibition of catalase activity by aminotriazole augmented gastric ulcer index, acid contents and peptic activity which was in agreement with the existing reports in other models of ulcers Thus the results obtained in their above investigation indicated that generation of free radicals is augmented following ulceration. Fasting, which predisposes the animals for ulceration was also found to reduce the antioxidant levels in the gastric tissue. Antioxidant depletion in the animals was also found to elevate acidity and peptic activity in the gastric juice. Thus free radicals seem to be associated with pyloric ligation-induced ulcers

Ethanol-induced ulcers

Ethanol is a necrotizing agent that produces gastric ulceration by causing direct damage to the mucosa independent of gastric acid secretion (Szabo, 1987; Cho and Ogle, 1992). Intragastric instillation of excessive ethanol results in gastric mucosal injury characterized by mucosal edema, subepithelial hemorrhages, cellular exfoliation and inflammatory cell infiltration (Eastwood and Kirchner, 1974; Laine and Weinstein, 1988). Studies focusing on the pathogenesis of ethanol-induced injury have suggested that several factors are implicated in such processes: products of arachidonate metabolism (e.g. leukotriene) (Peskar et al., 1986), mast cell secretory products (Oates and Hakkinen, 1988) and reactive oxygen species (Mizui et al., 1987; Pihan et al., 1987; Mutoh et al., 1990; Szelehji

and Brune, 1988). The mechanism by which ethanol induces gastric damage involves the disruption of the defensive factors such as the gastric mucosal barrier, gastric mucus and mucosal circulation (Takase et al., 1994). The involvement of ethanol-generated toxic oxygen radicals causing ethanol-induced gastric lesions has also been suggested (Mutoh et al , 1990; Szelehji and Brune, 1988), leading to increased lipid peroxidation, which causes damage to cell and cell membranes. Accumulation of activated neutrophils in the gastric mucosa may be a source of free radicals (Tepperman and Soper, 1990).

Several studies have demonstrated that many agents, including several antioxidants can prevent ethanol-induced gastric mucosal injury. Pretreatment of DMSO and allopurinol completely protected the rats from ethanol-induced mucosal injury (Salim, 1992) The beneficial effect of allopurinol suggests that xanthine oxidase, which has been considered a major source of free radicals in ischemia/reperfusion, may be one source of free radicals in ethanol-induced lesions Gastric mucosa of the rat and human are shown to possess alcohol dehydrogenase activity (Seitz et al , 1989), which can convert ethanol to acetaldehyde, acetaldehyde can be a substrate of xanthine oxidase to produce ROS including O_2^- (Steinbeck et al , 1993). The intracellular glutathione protected the rats from ethanol-induced gastric cell injury (Mutoh et al., 1990) and also prevents ethanol-induced gastric mucosal damage and depletion of sulphydryl compounds in humans (Loquerico et al., 1993). The protective effect of SOD against gastric injury was also established (Terano et al., 1986).

With regard to maintaining the integrity of the gastric mucosa, several agents, such as prostaglandins (Guth et al., 1984; Lacy and Ito, 1984); sulphydryl compounds (Boyd et al., 1981; Szabo et al , 1981); BHT, a potent free radical scavenger; quercetin, a bioflavonoid having antiperoxidative activity and quinacrine, which inhibits lipid peroxidation via interaction with membrane phospholipid have been shown to protect the stomach from ethanol injury (Mizui et al., 1987).

1.6.2 Role of free radicals in Myocardial infarction

Reactive oxygen species (ROS) are implicated in many pathogenic processes including the cardiovascular system. Detoxification of ROS by antioxidants (AO) therefore affords protection against such diseases. There is a growing body of evidence suggesting that antioxidants contribute to cardioprotection.

The proper use of the non-medical term "heart attack" is "Myocardial infarction". Either term is scary. "Myocardial infarction" (abbreviated as "MI") means there is death of some of the muscle cells of the heart as a result of a lack of supply of oxygen and other nutrients. This lack of supply is caused by closure of the artery ("coronary artery") that supplies that particular part of the heart muscle with blood. This occurs 98% of the time from the process of arteriosclerosis ("hardening of the arteries") in coronary vessels.

Although it once was felt that most heart attacks were caused from the slow closure of an artery, say from 90 or 95% to 100%, it is now clear that this process can occur in even minor blockages where there is rupture of the cholesterol plaque. This in turn causes blood clotting within the artery, blocking the flow of blood. The heart muscle, which is injured in this way, can cause irregular rhythms, which can be fatal, even when there is enough muscle left to pump plenty of blood. When the injured area heals, it will leave a scar. While the heart won't be able to pump quite as much as before, there is often plenty of good muscle left to take care of the job and recovery can be quite complete.

Cholesterol deposition in the artery wall leaves less room for the blood to flow through the channel in the middle. Furthermore, it leads to inflammation. which may eat away at the cells lining the artery. Thus, the contents of the plaque may be exposed to the bloodstream. When a substance other than what is supposed to is sensed within the bloodstream, the body's natural reaction is to build a clot around it to keep it from doing damage. If the clot grows too large, the artery is occluded and a heart attack results.

Factors other than cholesterol are important in the development of Coronary Artery Disease (CAD). Risk factors include smoking, which

damages the lining to the arteries, promotes the clotting of blood, lowers the level of "good" cholesterol in the blood and promotes spasm of the vessels, tending to keep it closed. Other factors, which promote coronary artery disease are high blood pressure, diabetes, male sex, sedentary lifestyle, overweight and family history of the problem.

Until recently there has been a reluctance to accept that oxidative stress can be important in the pathogenesis of cardiac disease however, recent investigation suggests that oxidative stress may be a very important contributor to the deterioration of the hypertrophied or failing myocardium. For example, reactive oxygen species have been shown to be critical components of the apoptosis pathway; myocyte loss by apoptosis is now thought to be a significant contributor to the inexorable deterioration of the failing myocardium. The importance of oxidative stress in heart failure is not surprising because a number of factors associated with heart failure, such as increased plasma catecholamines and cardiac sympathetic tone, microvascular reperfusion injury, cytokine stimulation and mitochondrial DNA mutations (particularly complex I) are known stimuli for free radical production and oxidative stress.

Peroxidative damage has been demonstrated in the hearts of dogs, guinea pigs and rats with heart failure due to pressure or volume overload. Coenzyme Q10, taurine (and its precursor cysteine) and vitamin E are important endogenous antioxidants or antioxidant precursors and their administration benefited both myocardial structure and function. Decrease in the levels of glutathione peroxidase and α-tocopherol and a concomitant increase in protein oxidation in the myocardium of cardiomyopathic hamsters during the late stages of hamster cardiomyopathy, an elevation of myocardial free radicals and lipid peroxides have been demonstrated in this model. The administration of vitamin E was found to completely normalize these findings.

Recently, it has also been demonstrated that a significant increase in the plasma level of lipid peroxides and malondialdehyde, markers of oxidative stress occurs in patients suffering from congestive heart failure. The increase in oxidative stress was related to the clinical severity of heart failure with the highest levels of lipid peroxidation and malondialdehyde

being observed in class 3 and class 4 patients. Increased free radical activity is also seen in patients on life support or in intensive care unit settings. These observations suggest that antioxidant supplements should be important additions to the therapy of heart failure and severely ill patients. The ability to withstand peroxidative injury is partially dependent on diet. A good dietary intake of the antioxidant vitamins C and E, and trace nutrient minerals such as selenium together with adequate cysteine as a precursor for glutathione synthesis are important for protection against free radical injury. Dietary antioxidants may reduce the risk of ischemic heart disease and the extent of myocardial infarction

Oxidative stress plays a major role in the biochemical and pathological changes associated with myocardial ischemic-reperfusion injury (IRI). The need to identify agents with a potential for preventing such damage has assumed great importance. Oxidative stress is a well-established etiopathogenic factor of ischemic heart disease (IHD) and its consequences. Reperfusion of the ischemic myocardium is the only logical approach for the successful management of patients with acute obstruction of coronary arteries. Morphologic observations of the ischemic myocardial tissue undergoing reperfusion suggest that reperfusion injury is a true pathologic phenomenon and a distinct entity from the preceding ischemic injury. Generation of reactive oxygen species immediately upon reperfusion has been documented in experimental conditions, as well as in patients with acute myocardial infarction undergoing thrombolysis, coronary angioplasty or open-heart surgery (Bolli, 1998). Upon reperfusion, molecular oxygen undergoes sequential reduction to form reactive oxygen species, including superoxide anion and hydroxyl radical, in addition to hydrogen peroxide. The interaction of oxygen-derived free radicals with cell membrane lipids and essential proteins contribute to myocardial cell damage, leading to depressed cardiac function and irreversible tissue injury with concomitant depletion of certain key endogenous antioxidant compounds, e.g., superoxide dismutase (SOD), catalase, reduced glutathione (GSH) and glutathione peroxidase (GPx) (Ferrari et al., 1991).

Although antioxidant administration during reperfusion injury has been shown to reduce the severity of the ischemic reperfusion injury, yet some properties of antioxidants such as cytotoxicity (Visweswaran et al., 1997), pro-oxidant activity (Edge and Truscott, 1997) or high molecular weight in case of SOD, limited their therapeutic application. Use of plant extracts (Bhattacharya et al., 1999), food supplement (Seneviratne et al., 1999) and even drugs (Cavanagh et al., 1997) which augment major cellular endogenous antioxidants following chronic administration have been identified as a promising therapeutic approach to combat oxidative stress associated with ischemic heart disease. The property of augmenting cellular endogenous antioxidants has been defined as a major constituent of myocardial adaptation against oxidative stress (Das et al., 1995).

Myocardial adaptation against oxidative stress is mediated through augmentation of a number of cellular antioxidants, such as SOD, catalase, glutathione peroxidase, glutathione (Das et al., 1995; Schaefer et al , 1998) As IRI is a common sequel of ischemic heart disease (IHD) and oxidative stress plays a central role in its etiopathogenesis, protection against oxidative stress through a novel mechanism like myocardial adaptation holds promise as an effective therapeutic approach. Myocardial adaptation occurs in response to various kinds of obnoxious stimuli, like ischemia (Lawson et al., 1993; Maulik et al., 1995a), certain endotoxins (Sun et al., 1996), reactive oxygen species (Asimakis et al., 1992), etc. and protects heart from subsequent exposure to injuries of similar or more severe nature (Li et al., 1990; Tosaki et al., 1997). It is interesting to note that different plants and plant extracts can also stimulate the synthesis of cellular antioxidants (Banerjee et al., 2001; Gauthaman et al., 2001; Geng and Lau, 1997; Bhattacharya et al., 1999).

Isoproterenol-induced myocardial infarction

Isoproterenol-induced myocardial infarction serves as a well-standardized model to study the beneficial effects of many drugs. Isoproterenol, a non-selective ß- adrenergic agonist, has been reported to cause oxidative stress in the myocardium resulting in infarct like necrosis of the heart muscle (Wexler and Greenberg, 1978). Animals develop

'infarct-like' lesions when injected with isoproterenol (ISP), a potent synthetic catecholamine. These lesions are morphologically similar to those of 'coagulative myocytolysis' (COAM) or myofibrillar degeneration, one of the findings described in acute myocardial infarction and sudden death in man. Myocardial necrosis induced by ISP is probably due to a primary act on the sarcolemmal membrane, followed by stimulation of adenylate cyclase, activation of Ca^{2+} and Na^{+} channels, exaggreated Ca^{2+} inflow, excess of excitation-contraction coupling mechanism, energy consumption and cellular death. The close resemblance of human COAM to ISP-induced lesions suggests that similar mechanisms may be involved (Milei et al., 1978).

The mechanism for catecholamine-induced myocardial scarring is not limited to ischaemia. Along with several other mechanisms, formation of high concentrations of free fatty acids by catecholamine-induced lipolysis can be very injurious. Furthermore, in the presence of an endothelial injury, an ischaemic insult to the myocardium may occur at lower (i.e., more plausible) catecholamine concentrations than those possibly required for a direct myocardial injury. One factor would be pathological ventricular hypertrophy since this process may itself cause myocardial necrosis (Laks, 1991).

During isoproterenol-induced myocardial infarction enhanced free radical formation and lipid peroxide accumulation have been proposed as one of the possible biochemical mechanism for myocardial damage (Sushmakumari et al., 1989).

Myocardial infarction is accompanied by the disintegration of membrane polyunsaturated fatty acids expressed by increase of thiobarbituric acid reactive substance (TBARS), a measure of lipid peroxides and by the impairment of natural scavenging, characterized by the decrease in the levels of superoxide dismutase, catalase and reduced glutathione (Nirmala and Puvanakrishnan, 1996).

Antioxidants like carnitine (Sushmakumari et al., 1989), vitamin E (Ithayarasi and Shyamala Devi, 1997), curcumin (Nirmala and Puvanakrishnan, 1996) and AO-8, a herbal formulation (Mitra et al., 1999)

were found to help the myocardium to survive from the oxidative stress induced by isoproterenol.

1.6.3 Role of free radicals in Kidney Damage

Nephrotoxicity is an inherent adverse effect of certain anticancer drugs. Renal dysfunction can be categorised as prerenal uraemia, intrinsic damage or postrenal uraemia according to the underlying pathophysiological process. Renal hypoperfusion promulgates prerenal uraemia. Intrinsic renal damage results from prolonged hypoperfusion. exposure to exogenous or endogenous nephrotoxins, renotubular precipitation of xenobiotics or endogenous compounds, renovascular obstruction, glomerular disease, renal microvascular damage or disease, and tubulointerstitial damage or disease. Postrenal uraemia is a consequence of clinically significant urinary tract obstruction (Kintzel 2001).

Mechanisms of chemotherapy-induced renal dysfunction generally include damage to vasculature or structures of the kidneys, haemolytic uraemic syndrome and prerenal perfusion deficits. Patients with cancer are frequently at risk of renal impairment secondary to disease-related and iatrogenic causes. Dose-related nephrotoxicity subsequent to administration of certain chloroethylnitrosourea compounds (carmustine, semustine and streptozocin) is commonly heralded by increased serum creatinine levels, uraemia and proteinuria. Cisplatin and carboplatin cause dose-related renal dysfunction. In addition to increased serum creatinine levels and uraemia, electrolyte abnormalities, such as hypomagnesaemia and hypokalaemia, are commonly reported adverse effects. Rarely, cisplatin has been implicated as the underlying cause of haemolytic uraemic syndrome. Pharmaceutical antidotes to cisplatin-induced nephrotoxicity include amifostine, sodium thiosulfate and diethyldithiocarbamate. Dose- and age-related proximal tubular damage is an adverse effect of ifosfamide. Acute renal failure is reported following administration of high doses of methotrexate. Acute renal failure is a rare adverse effect of treatment with interferon-alpha.

Renal failure in cancer patients is a common problem in oncology; this complication is frequently multifactorial in origin. Several antineoplastic agents are potentially nephrotoxic; previous renal impairment as well as combinations with other nephrotoxic drugs may increase the risk of nephrotoxicity during administration of chemotherapy (Ries and Klastersky, 1986).

Platinum-based drugs represent an important class of antitumor agents used to treat various malignancies. Several pathways have been shown to contribute to the toxicity including formation of reactive oxygen species, direct damage to renal tubule cells and activation of systemic inflammatory cells, which infiltrate and damage the kidney.

Cell injury by oxidative stress has been implicated in renal epithelial cell destruction during the progression of kidney diseases.

Cisplatin-induced nephrotoxicity

Cisplatin [cis-diamminedichloroplatinum (II): CDDP] is a widely used cancer chemotherapeutic agent whose clinical use is limited by its renal toxicity, an effect which has been well documented in all species studied to date including mice, rats, dogs and humans (Goldstein and Mayor, 1983). Cisplatin was discovered to have cytotoxic properties in the 1960s, and by the end of the 1970s it had earned a place as the key ingredient in the systemic treatment of germ cell cancers. Since the early seminal work in the preclinical and clinical development of this drug, several thousand analogues have been synthesized and tested for properties that would enhance the therapeutic index of cisplatin. Although renal toxicity is the dose-limiting factor for the use of cisplatin, other associated toxicities include emesis, nausea, diarrhea, anorexia, hair epilation and myelosuppression (Daly, 1996).

Studies point to the likelihood that cisplatin nephrotoxicity is not related to the toxicity of platinum per se as has been commonly presumed. Rather it seems that a metabolite of cisplatin, possibly an electrophile such as the aquated and/or hydroxylated form of cisplatin, mediates its nephrotoxicity. This reactive metabolite of cisplatin may bind covalently to

essential macromolecules of the kidney, resulting in nephrotoxicity (Goldstein and Mayor, 1983).

Mechanisms for renal toxicity range from definitive histologic changes found in the proximal convoluted tubules to physiologic and biochemical alterations involving a decrease in mitochondrial respiratory function, enzymic activity in the respiratory chain and glutathione peroxidase, and effects on cellular calcium homeostasis. Important factors related to nephrotoxicity include age, renal irradiation, and concurrent alcohol intake. A potential mechanism influencing the sensitivity of cells to cisplatin may result from the interaction of specific proteins with cisplatin-damaged DNA. Other molecules, including RNA and proteins, also react with cisplatin. These reactions occur either by a direct pathway in which the co-ordinated chlorides are displaced by nucleophilic entering groups or by an indirect pathway in which the entering nucleophiles react with cisplatin molecules that have already exchanged chloride groups for solvent (H_2O). Cisplatin protein interactions are important in determining the therapeutic efficacy of the antitumour agent Binding of cisplatin to plasma proteins significantly alters the rate of clearance of the drug from circulation. Reactions of native proteins with cisplatin may be responsible for the observed toxicity to the kidneys and gastrointestinal tract (Daly, 1996).

The effect of cisplatin on liver and kidney functions has been reported in several studies. Treskes et al. (1992), Hanigan et al (1994) and Bogin et al. (1994) showed that an injection of cisplatin changed liver and kidney enzyme activities. Also, Nair et al. (1991) reported that body weight; haemoglobin levels and leucocyte counts were decreased after cisplatin injection in mice. Renal failure in rats treated with cisplatin could be a result of the lower enzymatic activities in the kidney, as well as the less efficient oxidative phosphorylation and adenosine triphosphate production of the mitochondria. Administration of cysteine and vitamin E together with cisplatin partially reversed the uraemia and many of the biochemical changes induced by cisplatin.

Biochemical studies with heavy metals show that they react with free sulphydryl groups. It is postulated that the nephrotoxicity caused by

several heavy metals, e.g. mercury, is related to the intracellular decrease of reduced glutathione, enzymes are then inactivated because their SH-groups are not maintained in a reduced form. Levi *et al.* (1980) showed that following the administration of cisplatin to rats, there was a significant decrease of SH groups per gram wet weight. This change was seen prior to the elevation of blood urea nitrogen (BUN) and creatinine in the blood, suggesting that SH group depletion may be a primary event leading to the renal failure. The decrease of SH groups was due to a decrease of protein-bound SH, with the greatest decline in the cytosolic and mitochondrial fractions. Ways of protecting SH groups and reducing the toxic effects caused by cisplatin have been reported (Daly, 1996).

Administration of substances containing sulphydryl groups, or with antioxidant properties, before cisplatin, significantly reversed the inhibitory effects caused by cisplatin alone, supporting the hypothesis that the cisplatin toxicity is associated with the reduction of free sulphydryl groups (Daly, 1996).

Studies also suggest that apoptotic cell death may play an important role in the development of cisplatin-induced acute renal failure (Zhou et al., 1999). Findings also suggest that the nephrotoxic effects of cisplatin may, in part, be related to protein kinase C (PKC) activation in the renal tubules (Ikeda et al., 2000).

Cytokines are synthesized primarily by immune cells and have pleotrophic effects on various cells. It has been shown that the expression of various cytokines and chemokines including TNF-α are upregulated in cisplatin-induced renal failure in mice. TNF-α is known to induce apoptosis in cultured renal proximal tubule cells. Using pharmacological agents, which antagonize the secretion and/or action of TNF-α, helped to alleviate the severity of cisplatin toxicity as measured by blood urea nitrogen and changes in gene expression of cytokines suggesting the direct role of TNF-α in cisplatin-induced nephrotoxicity. The mechanism of cisplatin-induced upregulation of TNF-α expression and the role of TNF targets in producing nephrotoxicity is being studied.

Previous reports suggest that cisplatin-induced nephrotoxicity is by increase in lipid peroxidation (Hanneman and Baumann, 1991) and

depletion of cellular thiols (Levi et al., 1980) in kidney tissues following cisplatin treatment. Cisplatin inhibits activities of antioxidant enzymes (SOD and catalase) in rat kidneys suggesting that cisplatin nephrotoxicity results from generation of reactive oxygen species (Sdzuka et al., 1992). Enhancement in lipid peroxidation and decrease in reduced glutathione and antioxidant enzymes (SOD and catalase) contributes to cisplatin-induced nephrotoxicity.

Effects of several antioxidants like butylated hydroxyl anisole (BHA) and glutathione (GSH) on cisplatin-induced renal injury have been studied and these compounds prevent cisplatin-induced lipid peroxidation and depletion of glutathione (Kim et al., 1997) and antioxidant enzymes

1.6.4 Role of free radicals in Liver Damage

In order to efficiently metabolize drugs, during the process of evolution, the liver has developed "drug metabolizing nzymes" which are different from the enzymes of intermediate metabolism (Rao, 1973). Most of these enzymes are largely located in the hepatic microsomes. Biotransformation of a drug or xenobiotic compound following its exposure can alter its distribution and action leading to its detoxification and excretion or enhance its toxicity due to the activation of the compound or due to the biochemical disruption caused by reactive metabolites arising from biotransformation (Athar et al., 1997; Plaa, 1991). Biotransformation of xenobiotics usually occurs in two phases. Phase I metabolism (detoxification) involves oxidative, reductive and/or hydrolytic reactions that cleave substrate molecules to producea more polar moiety. Phase II reactions (synthetic reactions) involve conjugation of certain endogenous molecules to the products of phase I reaction (Remmer, 1970). Cytochrome P_{450} (Cyt. P_{450}) enzymes are responsible for the metabolic conversion of many drugs to the polar metabolites via Phase I and Phase II reactions to earlier excretion.

Acute liver failure (ALF) is a dramatic and challenging syndrome in clinical medicine. Although an uncommon disorder, it is usually fatal (Hoofnagle et al., 1995; Lee, 1994). There is little information on the epidemiology of ALF in various regions of the world. Crude estimates

suggest that there are approximately 2000 deaths per year from ALF related to viral hepatitis in the United States. 1 In contrast, ALF caused by hepatitis E is a national health problem in the developing countries. Hepatitis E causes large-scale epidemics of hepatitis in the Indian subcontinent, involving hundreds of thousands of cases with high mortality (Khuroo and Khuroo, 1997).

ALF can result from diverse etiological agents. Of these, hepatitis viruses, acetaminophen overdose and idiosyncratic drug reactions constitute the bulk of cases. Metabolic and vascular liver diseases, liver diseases unique in pregnancy and a number of miscellaneous liver diseases cause a small number of the remaining cases (O'Grady, 1993).

Lipid peroxidation in tissues and in tissue fractions represents a degradative process, which is the consequence of the production and the propagation of free radical reactions primarily involving membrane polyunsaturated fatty acids (PUFA), especially arachidonic acid (Slater, 1972). The peroxidative breakdown of PUFA has been implicated in the pathogenesis of many types of liver injury and especially in the hepatic damage induced by several toxic substances. Among these are the haloalkanes, carbon tetrachloride, trichlorobromo-methane, chloroform, 1,2-dibromomethane and halothane; in addition, paracetamol, bromobenzene, iron, bipyridyl compounds, allyl alcohol and in some instances, ethanol have been shown to stimulate lipid peroxidation.

Lipid peroxidation can be stimulated in the membranes of the endoplasmic reticulum by iron-chelated complexes like ADP-iron, by cumene hydroperoxide, by ascorbate and also by the metabolic activation of toxic agents such as carbon tetrachloride (Poli et al., 1987).

Subcellular aspects of oxidative liver injury:

The peroxidation of PUFA within biological membranes results in a complex series of biochemical and biophysical events, which leads to inactivation of enzymatic functions in several subcellular organelles. These alterations include changes in the physical properties of the lipid bilayer, reactions between acylperoxyl radical and membrane proteins and

formation of reactive products originating from the degradation of peroxidised fatty acids.

The stimulation of lipid peroxidation in either artificial membrane of liposomes or in subcellular organelles has been shown to increase membrane rigidity. Such a loss of fluidity seems not to be dependent upon an increase in the ratio between cholesterol and phospholipids, but is rather an effect on the formation of cross-linking between acyl chains and of the depletion of long chain polyenoic fatty acids. In addition to the changes in fluidity, lipid peroxidation causes an increase in the ionic permeability and affects the surface potentials of the membranes.

In the liver, the membranes of mitochondria and endoplasmic reticulum vesicles contain unsaturated fatty acids in high proportion and therefore are vulnerable to peroxidative attack. At the same time they contain enzymes of the electron transport systems, which make them capable of producing free radical species (Poli et al , 1987).

Effect of lipid peroxidation in liver mitochondria

The addition of iron chelated with ADP, citrate or gluconate has been shown to stimulate lipid peroxidation in isolated mitochondria. It has been postulated that Fe^{3+} is taken up by the mitochondria and subsequently reduced by the respiratory chain to Fe^{2+} in order to take part in a Fenton-type reaction with endogenously formed hydrogen peroxide. It is also possible that $O_2^{\bullet-}$ leaking out from the respiratory chain may contribute to the iron reduction. Mitochondrial swelling and uncoupling of oxidative phosphorylation follow the addition of iron and both these phenomena are prevented by EDTA, which also inhibits iron-mediated MDA formation. Interestingly, the same alterations of liver mitochondria are also produced by CCl_4 intoxication, suggesting that they could similarly be induced by a peroxidative damage.

The mechanism involved in causing the peroxidative damage of mitochondria is still not completely understood. Stimulation of lipid peroxidation brings about a progressive fall in the mitochondrial membrane potential, which is associated with a release of K^+ and Ca^{2+}.

These effects are blocked by the addition of antioxidants such as butylated hydroxytoluene (BHT) or Trolox C, a synthetic analog of vitamin E.

Peroxidation of mitochondrial membranes causes alterations of mitochondrial integrity by inducing the oxidation of pyridine nucleotides and an uncontrolled calcium recycling (Poli et al., 1987).

Effect of lipid peroxidation in liver microsomes

The biochemical alterations induced by lipid peroxidation in the endoplasmic reticulum (ER) of the liver have been extensively studied. Early reports showed that isolated microsomal vesicles undergo swelling and rough endoplasmic membranes lose bound ribosomes when exposed to peroxidising agents. Membrane breakages and loss of intramembranous particles have been revealed in microsomes treated with CCl_4.

The pro-oxidant effects of CCl_4 in liver microsomes were also found to be associated with the inactivation of glucose-6-phosphatase and of cytochrome P-450-dependent monoxygenase system. Peroxidative events were the main cause of enzyme damage.

Among the enzymatic functions of ER calcium sequestration activity is considered to play an important role in the regulation of cellular Ca^{2+} homeostasis. Recent studies have demonstrated that calcium uptake by liver microsomes is rapidly inhibited following stimulation of lipid peroxidation by either CCl_4 or iron (Poli et al., 1987).

Effect of lipid peroxidation in lysosomes and plasma membranes:

The damage of lysosomes as a consequence of peroxidative events has been observed in irradiated tissues where the release of hydrolytic enzymes correlates with the content of MDA in the tissue itself. The addition of antioxidants prevents the enzyme leakage supporting a cause-effect relationship between the two events.

The alterations of lysosomal membranes and the consequent release of hydrolytic enzymes are known to cause severe damage to cellular structures and therefore they could contribute to bringing about cell death. Among the effects of lipid peroxidation on plasma membranes, CCl_4 intoxication of rats *in vivo* was found to increase the calcium permeability

of plasma membranes. Such an effect is ascribed to the ionophoretic properties of certain peroxidised unsaturated fatty acids since the Ca^{2+}-ATPase activity is not significantly affected.

The stimulation of lipid peroxidation by several hepatotoxic agents causes a severe impairment of plasma membrane structure resulting in the leakage of intracellular enzymes. It is possible that the alterations of plasma membrane fluidity, as a consequence of the changes in the lipid composition along with the increased permeability to ions, might be relevant factors in causing irreversible membrane lesions (Poli et al , 1987).

CCl_4 - induced hepatotoxicity

CCl_4-induced hepatotoxicity in rats represents an adequate experimental model of cirrhosis in man and it is used for the screening of hepatoprotective drugs (Al-Shabanah et al., 2000; Pérez-Tamayo, 1983; López-Novoa et al., 1977). The liver represents the principal site of toxicity, although it induces sublethal proximal tubular injury in the kidney and focal alterations in granular pneumatocytes (Striker et al., 1968).

CCl_4 - induced hepatotoxicity is also a commonly used model for investigating lipid peroxidation-related tissue injury. The biotransformation of CCl_4 occurs in the ER and is mediated by Cyt. P_{450} (Castro et al., 1968). Cyt. P_{450} is inhibited suicidally by the reactive metabolites of CCl_4 (Athar et al., 1997). $CCl_3^{\bullet}$ radical initially formed being relatively unreactive reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy radical ($CCl_3OO^{\bullet}$), which is the probable initiator of lipid peroxidation (Bhat and Madyastha, 2000). The involvement of free radical-mediated reactions in the development of CCl_4 - induced hepatic injury has been implicated in various *in vitro* and *in vivo* studies. Using electron spin resonance measurements, the formation of trichloromethyl free radicals from CCl_4 metabolism has been demonstrated in isolated liver microsomes and in intact liver tissues. These trichloromethyl free radicals can react with macromolecules and initiate lipid peroxidative reactions, resulting in the disruption of cellular and subcellular structures and subsequent generalized tissue damage.

Pretreatment with antioxidants such as α-tocopherol and BHT has been shown to protect against CCl_4 - induced hepatic damage (Ko et al., 1993)

Ko et al. (1993) have also provided evidence for impairment in hepatic antioxidant status during the development of CCl_4 - induced hepatotoxicity. They also demonstrated that the impairment in the hepatic antioxidant status and the associated hepatocellular damage were attenuated by a loading dose of vitamin E, a lipid-soluble antioxidant, or multiple doses of *Polygonum* or *Cassia* extracts, preparations reported to possess antioxidant properties, supports the involvement of uncontrolled free radical-mediated processes in the pathogenesis of CCl_4 hepatotoxicity (Ko et al., 1993).

Lipids of cellular membranes, e.g. lipids of the membranous components of the ER, are rich in polyenoic fatty acids, which are prone to undergo peroxidative decomposition Living cells are equipped with antioxidants and other means to prevent the process from taking place *in vivo* In living cells, as a result of the action of foreign agents, which initiate lipid peroxidation, the protective mechanisms may be overwhelmed. When this occurs, the results are catastrophic for the particular cells or subcellular components involved Thus, lipid peroxidative decomposition leads to disintegration of red cells and isolated liver mitochondria. Rapid loss of a variety of microsomal enzymic activities accompanies peroxidative decomposition of lipids of liver cell ER. Disintegration of lysosomes has been correlated with peroxidative decomposition of lysosomal lipids. Peroxidative decomposition of hepatocellular membrane lipids results in breakdown of the delicate and complex mechanisms responsible for formation of VLDL in the liver cell, their movement within the cell and their eventual extrusion into the plasma. This supposition is central to the lipoperoxidation theory of CCl_4 liver damage. The theory holds that the classical indices of CCl_4 liver damage, such as increased fat, loss of soluble enzymes into the plasma, shifts in electrolyte and water distribution and eventual necrosis, emerge at the pathophysiological level as resultants of structural and functional alteration of liver cell membranes, initiated by carbon-chloride bond cleavage and lipid peroxidation.

According to the lipoperoxidation theory for CCl_4 liver injury, the initial event (which precedes onset of lipid peroxidation) is cleavage of the carbon-chlorine bond in the hepatocellular mixed-function oxidase system (cytochrome P-450). This cleavage is believed to be homolytic. The physiological electron donor is NADPH and the electrons are transferred via cytochrome P-450 reductase to cytochrome P-450. CCl_4 first interacts with the active site of cytochrome P-450. Following one electron reduction and elimination of a chloride ion, a trichloromethyl radical ferric cytochrome P-450 complex is formed. From this complex the radical is either released to yield the trichloromethyl radical (1) or undergoes further reductive metabolism to yield CO. Thus, its initial products would be trichloromethyl and monoatomic chlorine free radicals. CCl_4 related lipid peroxidation may be initiated via two pathways. The first pathway requires the presence of O_2 to $CCl_3^{\bullet}$ to yield the trichloromethylperoxyl radical ($CCl_3O_2^{\bullet}$) (2). $CCl_3O_2^{\bullet}$ rapidly reacts with polyenoic fatty acids of membrane phospholipids (LH) yielding CCl_3O_2H and $L^{\bullet}$ (3a). CCl_3O_2H is assumed to decompose, probably with catalysis by metal ions (Me^{+}), to the respective trichloromethylalkoxyl radical ($CCl_3O^{\bullet}$) (4). The latter again may attack another LH leading to the formation of a second $L^{\bullet}$ (3b). In the second pathway, $L^{\bullet}$ is directly formed by $CCl_3^{\bullet}$ either by hydrogen atom abstraction, as evidenced by the formation of $CHCl_3$ (3c), or by radical addition to one of the double bonds of a PUFA (3d). While, in the first pathway requiring O_2, two $L^{\bullet}$ may be formed for every $CCl_3^{\bullet}$ reacting, in the second pathway $L^{\bullet}$ is formed by $CCl_3^{\bullet}$ in equimolar amounts and thus less efficiently. Reactions (5) and (6) leads to termination of the free radical chain reaction. Propagation and termination of CCl_4-induced lipid peroxidation essentially proceeds by the reactions 7-13.

$CCl_4 + e^- \text{-------} > CCl_3^\bullet + Cl^-$ (1)

$CCl_3^\bullet + O_2 \text{-----} > CCl_3O_2^\bullet$ (2)

$CCl_3O_2^\bullet + LH \text{---} > CCl_3O_2H + L^\bullet$ (3a)

$CCl_3O^\bullet + LH \text{-----} > COCl_2 + HCl + L^\bullet$ (3b)

$CCl_3^\bullet + LH \text{-------} > CHCl_3 + L^\bullet$ (3c)

$CCl_3^\bullet + LH \text{-------} > LHCCl_3^\bullet$ (3d)

$CCl_3O_2H + Me^+ \text{---} > CCl_3O^\bullet + OH^-$ (4)

$L^\bullet + CCl_3^\bullet \text{------} > LCCl_3$ (5)

$CCl_3^\bullet + CCl_3^\bullet \text{-----} > CCl_3CCl_3$ (6)

The molecular biological perturbations represented by lipid peroxidation, appearance of abnormal branched-chain fatty acids and rich incorporation of carbon from CCl_4 into microsomal lipids, take place extremely rapidly and occur just before or coincidently with major pathological disturbances of CCl_4 liver injury, such as accumulation of triglycerides and depression of protein synthesis (Recknagel et al., 1974)

It was noted that repeated doses of antioxidants are more effective than single dose in decreasing the activity of drug-metabolizing enzymes. It is concluded that repeated doses of antioxidants or garlic could reduce the toxic effects exerted by CCl_4 upon the liver, and probably other organs, through inhibition of cytochrome P450 system that activates CCl_4 into its active metabolite, trichloromethyl radical. Moreover, inhibition of cytochrome P450 system could also reduce the toxic and carcinogenic effects of chemical carcinogens such as benzo(a)pyrene and dimethylnitrosamine (Sheweita et al., 2001).

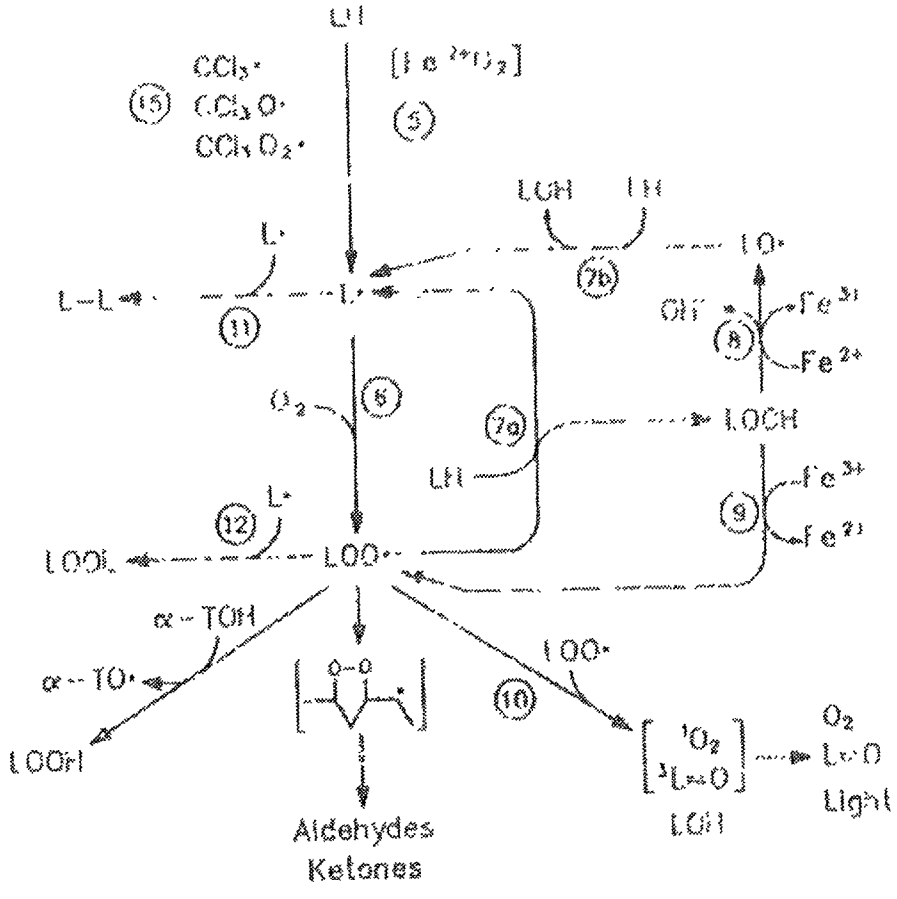


Fig. 1.2. Relation among initiation, propagation and termination reactions in CCl_4-induced lipid peroxidation (Recknagel et al., 1974).

1.6.5 Role of free radicals in Atherosclerosis

The clinical sequelae of atherosclerosis such as myocardial infarction and stroke account for the majority of premature deaths in developed countries. Reactive oxygen species seem also to play a role in the formation of foam cells in atherosclerotic plaques. Many studies have suggested that oxidation of low density lipoprotein (LDL) particles is a critical event in the development of the atherosclerotic plaque (Steinberg et al., 1989).

The cellular uptake of LDL is largely mediated by a high affinity cell surface receptor, a process first recognised by Brown and Goldstein (1974). This LDL receptor present on many cells recognises apo B100 and apo E in the LDL and cellular uptake of LDL occurs followed by digestion by lysosomes. Cholesterol and triacylglycerols are thus made available to cells. When LDL becomes oxidised, the structure of apolipoprotein B becomes modified and no longer binds to the LDL receptor, but instead it binds to the receptor present on the surface of macrophages, which act as scavengers. There may be more than one type of scavenger receptor (Gordon, 1996). LDL bound to these receptors is taken up with enhanced efficiency, so that cholesterol rapidly accumulates within the macrophage and may convert it into a foam cell (Mitchinson and Ball, 1987) Arterial endothelial cells, smooth muscle cells and macrophages have been shown to be capable of oxidising LDL *in vitro* so that macrophages will internalise it faster. The modification process that allows recognition by the scavenger receptors involves the derivatisation of lysine residues of the apoprotein B moiety of LDL by lipid peroxidation products, such as the cytotoxic aldehyde 4-hydroxynonenal.

Peroxidation of LDL within the vessel wall could have a number of deleterious effects. It has been suggested that products formed in peroxidised LDL, such as lysophosphatidyl choline, might act as chemotactic factors for blood monocytes, encouraging their recruitment into an atherosclerotic lesion and development of macrophages (Steinberg et al., 1989). In addition, low concentrations of lipid hydroperoxides might accelerate cyclooxygenase- and lipoxygenase-catalysed reactions in endothelium and in any platelets present, leading to enhanced formation

of prostaglandins and leukotrienes (Warso and Lands, 1983). Oxidised LDL might also stimulate the production of cytokines and growth factors by macrophages, which are complex multifunctional cells (Yokode et al., 1988). There have been several speculations that oxidation products of cholesterol might also be involved in atherogenesis, cholesterol is oxidised into a wide variety of products in peroxidising lipid systems (Smith, 1987) and oxidised cholesterol has been reported to be toxic to many cell types, including arterial smooth muscle cells and endothelial cells. It seems likely that cell breakdown in the advanced atherosclerotic plaque can lead not only to activation of lipoxygenases, but also to release of transition metal ions that can stimulate free radical reactions such as lipid peroxidation (Halliwell, 1989). Oxidised LDL inhibits the relaxation of smooth muscle cells by nitric oxide, which acts as an endothelial derived relaxing factor, and promotes formation of autoantibodies.

The clinical consequences of severe atherosclerosis (stroke and myocardial infarction) may also involve reactive oxygen species (Halliwell, 1991).

There has been much interest in recent years in the possible effect of antioxidants in retarding oxidative modification of low-density lipoproteins (LDL). The 'oxidative-modification hypothesis' of atherosclerosis stimulated interest in the natural antioxidant defence of LDL particles. This includes tocopherol, β-carotene and ubiquinol-10, which reduce the susceptibility of core fatty acids to oxidation (Esterbauer et al., 1992). LDL supplemented with tocopherol is more resistant to oxidation *in vitro* (Reaven et al., 1993). The fact that the oxidative resistance of LDL from patients with coronary artery disease in vitro is reduced suggested that antioxidant therapy might slow the progression of the disease (Regnstrom et al., 1992).

Initial trials of antioxidant vitamins and drugs such as probucol in animals suggested that the atherogenic process could indeed be retarded. These observations were strengthened by epidemiological surveys in humans linking low plasma levels or dietary intake of antioxidant vitamins (ascorbic acid, tocopherol and β-carotene) with ischaemic heart disease (Kardinaal et al., 1993; Manson et al., 1991). In this preventive role,

antioxidant therapy would necessarily last for many years and for this reason supplementation of the diet with natural antioxidants seems to be the safest approach.

1.7 HERBAL DRUGS AS ANTIOXIDANTS

A large section of the world's population relies on traditional remedies to treat a plethora of diseases. Medicinal herbs are an indispensible part of the traditional medicine practised all over the world due to low costs, easy access and ancesteral experience (Marini-Bettolo, 1980).

The use of natural products with therapeutic properties is as ancient as human civilisation and, for a long time, mineral, plant and animal products were the main sources of drugs. The industrial revolution and the development of organic chemistry resulted in a preference for synthetic products for pharmacological treatment The reasons for this were that pure compounds were easily obtained, structural modifications to produce potentially more active and safer drugs could be easily performed and the economic power of the pharmaceutical companies was increasing. Furthermore, throughout the development of human culture, the use of natural products has had magical-religious significance and different points of view regarding the concepts of health and disease existed within each culture. Obviously, this approach was against the new modus vivendi of the industrialised western societies, in which drugs from natural resources were considered either an option for poorly educated or low income people or simply as religious superstition of no pharmacological value.

However, even if we only consider the impact of the discovery of the penicillin, obtained from microorganisms, on the development of anti-infection therapy, the importance of natural products is clearly enormous. About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use. Of the 252 drugs considered as basic and essential by the World Health Organization (WHO), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors. Examples of important drugs obtained

from plants are digoxin from *Digitalis* spp., quinine and quinidine from *Cinchona* spp., vincristine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladona*, and morphine and codeine from *Papaver somniferum*. It is estimated that 60% of anti-tumor and anti-infectious drugs already on the market or under clinical trial are of natural origin. The vast majority of these cannot yet be synthesised economically and are still obtained from wild or cultivated plants. Natural compounds can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds. In addition, compounds such as muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicine and phorbol esters, all obtained from plants, are important tools used in pharmacological, physiological and biochemical studies

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants This interest in drugs of plant origin is due to several reasons, namely, conventional medicine can be inefficient (e g. side effects and ineffective therapy), abusive and/or incorrect use of synthetic drugs results in side effects and other problems, a large percentage of the world's population does not have access to conventional pharmacological treatment, and folk medicine and ecological awareness suggest that "natural" products are harmless (Rates, 2001).

However, the potential use of higher plants as a source of new drugs is still poorly explored. Of the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and even a smaller percentage has been properly studied in terms of their pharmacological properties.

The modern social context and economic view of health services, the needs of the pharmaceutical market and the recognition that research on medicinal plants used in folk medicine represents a suitable approach for the development of new drugs have led to an increase in the number of publications in this field, and private and governmental institutions are now financially supporting research programmes worldwide.

Today, the market is flooded with herbal medicines. A number of companies are entering into the arena of herbal medicines. These medicines are available for each and every disorder ranging from diabetes to rejuvenators.

Crude drugs or natural diet food which possess anti-oxidant or free radical scavenging activity has become a central focus for research designed to prevent or ameliorate tissue injury and may have a significant role in maintaining health (Jer-Min et al., 1995).

Antioxidant compounds must be constantly replenished since they are used up in the process of neutralizing free radicals. Therefore these have to be continuously ingested in diet or by supplementation. Antioxidant supplements were once thought to be harmless but increasingly we are becoming aware of potential interactions and potential toxicity. As an example, in normal concentrations found in humans, vitamin C and beta-carotene are antioxidants; but at higher concentrations they are pro-oxidants and can be harmful. It is also possible that unforeseen metabolic disturbances may occur after prolonged use of highly bioavailable pure compounds; such effects may not be apparent when antioxidants are obtained from natural foods. Thus, supplementation of antioxidants using natural sources seems to be a safe approach.

As plants produce a lot of antioxidants to control the oxidative stress, they can represent a source of new compounds with antioxidant activity. Ayurveda, the Indian traditional health care system is the oldest medical system in the world, which exploits the potential of various herbs generally in polyherbal formulations as drugs (Dash and Kashyap, 1980). A number of plants and plant isolates have been reported to protect free-radical induced damage in various experimental models.

Red/orange/yellow vegetables and fruits, nuts, peas, berries, broad beans and watermelon are some of the food sources of antioxidants. Oligomeric proanthocyanidin complexes (OPCs) from grape seed and pine bark, turmeric, resveratrol from grapes, soy isoflavones and garlic are also commonly mentioned. Lycopene (from tomatoes) is one of the latest entrants in the antioxidant group.

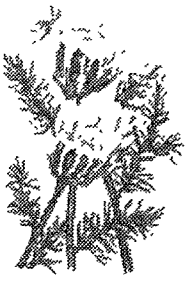
One of the clinical specialities of Ayurveda is Rasayana. Rasayana is not only a drug therapy but is a specialised procedure practised in the form of rejuvenating recipes, dietary regimen promoting good habit. The purpose of rasayana is two-fold: prevention of disease and counteraction of aging processes, which result from optimization of homeostasis. The meaning of the word Rasayana (rasa=essence, water; ayana=going) essentially refers to nutrition and its acquisition, movement, circulation and perfusion in the body tissues. With regard to the rasayana drug therapy the strong antioxidant activity of any rasayana has been reported: these compounds were found to be 1000 times more potent than ascorbic acid, α-tocopherol and probucol (Scartezzini and Speroni, 2000). *Emblica officinalis*, *Tinospora cordifolia*, *Asparagus racemosus*, *Tribulus terristris*, *Withania somnifera*, *Mangifera indica* are some examples of rasayana drugs.

Rasayanas are reputed to promote physical and mental health, improve defence mechanisms of the body and enhance longevity These attributes are similar to the modern concept of adaptogenic agents, which are, known to afford protection of the human physiological system against diverse stressors (Bhattacharya et al , 2000)

Bhasma is that which is burnt to powder. This name is generally applied to all that are subjected to the process of burning and reduction to ash. This term in ayurveda applies to the metals and minerals, marine and animal products, which are, by special processes (purification), calcinated in closed crucibles in pits with cowdung cakes (Anonymous, 1978).

Kwath are the preparations containing drugs or combination of drugs made into coarse powder and kept for the preparation of Kasaya (Anonymous, 1978).

1.7.1 *ANACYCLUS PYRETHRUM*



Latin Names: *Anacyclus pyrethrum* Link, *Pyrethrum radix*, *Anacyclus depressus* Maire, *Anacyclus freynu* Porta and Rigo (Asteraceae)

English Name: Pellitory

Sanskrit / Indian Name: Akarakarabha, Akarakara

Habitat

It is found in Himalayas, North India and Arabian countries.

Morphological Description

It is a perennial, procumbent herb, the roots of which are used in medicine.

Principal Constituents

They contain anacyclin, pellitorine, enetriyne alcohol, hydrocarolin, inulin, traces of volatile oil and (+)-sesamin.

Pharmacology

The roots possess stimulant and rubefacient properties.

Indications

Ayurveda prescribes this herb for nerve disorders, bowel conditions, seminal debility, gargle for tooth problems (e.g. toothache), sore throat and tonsils, paralysis, hemiplegia, epilepsy, rheumatism, promotes talking in retarded children, with honey for epilepsy (internal and as snuff), diabetes and promotes saliva.

Product Range

Used in Tentex Forte (Vigor Care for Men), Muscle and Joint Rub, Speman Forte Vet, Tentex Forte Vet.

1.7.2 *ARGYREIA SPECIOSA*

Latin Names: *Argyreia nervosa* (Burm.f.) Bojer.
(Convolvulaceae)/ *A.speciosa* Sweet.
English Names: Elephant Creeper, Woolly Morning Glory
Sanskrit Name: Vriddadaru
Hindi Name: Vidhara

History

The root of this large climber is used as a substitute for the drug described under the name of 'Vriddhadaraka'. In Ayurveda, the root is regarded as alterative, tonic and useful in rheumatic affections and diseases of the nervous system.

Habitat

Throughout India, up to an altitude of 300 meters high.

Morphological Description

It is a very large woody climber. The stem is stout, white and tomentose. The leaves are large, ovate-cordate, and glabrous above; persistently white tomentose beneath, base cordate and petiole is long. The flowers are in sub-capitate cymes. The calyx is white tomentose outside. The corolla is long, tubular-infundibuliform, silky pubescent outside and glabrous inner. The fruits are globose and apiculate.

Principal Constituents

It contains many ergoline alkaloids.

Pharmacology

'Speman', consisting of several ingredients of plant material including this species, is reported to exhibit anabolic-cum-androgen-like activity in mice. It possesses significant spasmolytic and hypotensive activities. It is a local stimulant and rubefacient. It is also reported to possess immunomodulatory activity (Gokhale et al., 2003).

Clinical Studies

A preparation made from this plant along with several other ingredients is used for curing sexual disorders in males.

Toxicology

A few of the ergoline alkaloids reported in this plant are hallucinogenic.

Indications

The root is bitter, aphrodisiac, diuretic and used in gonorrhoea, rheumatism and diseases of the nervous system. It is also used as a tonic.

Product Range

Confido (Speman forte), Geriforte (GeriCare / StressCare), Speman (ProstaCare), Tentex forte (VigorCare for Men), Geriforte Vet, Speman forte Vet, Speman Vet, Tentex forte Vet.

1.7.3 *ASPARAGUS RACEMOSUS*

Latin Name: *Asparagus racemosus* Willd (Liliaceae)
English Name: Asparagus
Sanskrit Name: Shatavari
Hindi Names: Satavar, Satmuli

History

Asparagus racemosus and *A. sarmentosus* are the 'Satavari' and 'Maha-satavari' of the 'Nighantas'. The tubers are candied and eaten as a sweetmeat. The fresh juice of the root is given with honey as a demulcent in bilious dyspepsia or diarrhea. It is a constituent in the preparation of medicated oils for external application in nervous and rheumatic affections and urinary troubles.

Habitat

It is found through out tropical Africa, Java, Australia, India, Sri Lanka and southern parts of China. In India it is found in plains to 4,000 ft high, in tropical, sub-tropical dry and deciduous forests and in the Himalayas.

Morphology Description

It is an under-shrub, climbs up to 1-3 m high, with stout and creeping rootstock. The root occurs in clusters or fascicle at the base of the stem with succulent and tuberous rootlets. The stem is scandent, woody, triquetrous, striate, terete and climbing. The young stem is delicate, brittle and smooth. The spines are long, sub-recurved or straight. Cladodes are in tufts of 2-6 in a node, slender, finely acuminate, and falcate divaricate. The flowers are solitary or fascicles, simple or branched racemes of 3 cm long. The pedicel is slender and joined in the middle. Perianth lobes are white, fragrant and 3 mm in length. The anthers are minute and purple. The berries are globular or obscurely 3 lobbed, purple-reddish, seeds hard with brittle testa.

Principal Constituents

Apart from saponins, the material contains alkaloids, proteins, starch, tannin, mucilage and diosgenin. The type of saponin varies with the geographical distribution of the species. Plants found in south India have saponin-A4 fraction but not in north Indian samples. Steroid saponin, shatavarin - is the major glycoside with 3 glucose and rhamnose moieties attached to sarsasapogenin, whereas shatavarin-IV has 2 glucose and one rhamnose moieties with sarsasapogenin. Vanillin, coniferin and sarsasaponin were also identified from the roots. The plant contains triterpene saponins - Shatavarin I - IV, which are phytoestrogen compounds.

Pharmacology

Root extracts were found to increase the weight of mammary glands in post-partum and estrogens-primed rats and uterine weight in

estrogens-primed group (Sabnis et al., 1968). It also has galactogogue action in buffaloes (Patel and Kamtkar, 1969). It increased the force and rate of contraction in isolated frog's heart, but in higher doses it caused cardiac arrest. Aerial parts have anticancer activity in human epidermal carcinoma of the nasopharynx. Rege et al. (1999) have reported the immunomodulating activity of *A. racemosus*.

The ulcer protective effect of methanolic extract of fresh roots of *A. racemosus* (ARM), 25-100 mg/kg given orally, twice daily for 5 days, was studied on different gastroduodenal ulcer models. ARM 50 mg/kg, twice daily, orally (total saponins 0.9%) showed significant protection against acute gastric ulcers induced by cold restraint stress (CRS), pyloric ligation, aspirin plus pyloric ligation, and duodenal ulcers induced by cysteamine. ARM in the above dose also significantly healed chronic gastric ulcers induced by acetic acid after 10 days treatment. However, ARM was ineffective against aspirin- and ethanol-induced gastric ulcers. Further, gastric juice and mucosal studies showed that ARM significantly increased the mucosal defensive factors like mucus secretion, cellular mucus, life span of cells and also possessed significant anti-oxidant effect, but had little or no effect on offensive factors like acid and pepsin (Sairam, 2003).

A.racemosus has potent antioxidant properties against damage induced by gamma radiation in mitochondrial membranes of rat liver (Kamat et al., 2000).

Clinical Studies

It is proved that it increases milk production in lactating women.

Toxicology

There is no report of toxic and adverse effects on use of this plant but *Asparagus officinalis* is an allergenic plant.

Indications

The roots have oleaginous, cooling, antispasmodic, indigestible, appetizer, alliterative, stomach, tonic, aphrodisiac, galactagogue, astringent, antidiarrhoeatic, antidysenteric, laxative properties and is

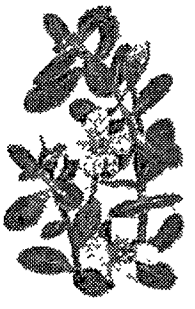
useful in tumors, inflamations, diseases of blood and eye, throat complaints, tuberculosis, leprosy, epilepsy, night blindness and kidney troubles.

Product Range

Abana (HeartCare), Diabecon (GlucoCare), Evecare (MenstriCare), Geriforte (GeriCare / StressCare), Himplasia, Lukol, Menosan, Renalka, Galactin Vet, Geriforte Vet, Nefrotec Vet, Shatavari.

1.7.4 *BACOPA MONNIERI*

Latin Names: *Bacopa monnieri* (Linn.) Wettst. /
Herpestis monnieria (Linn.) H.B. & K.
Scrophulariaceae
English Name: Thyme-Leaved Gratiola,
Smooth water hyssop, Moneywort
Sanskrit Names: Brahmi, Nira-brahmi
Hindi Name: Brahmi



Habitat

It commonly grows in wet, damp and marshy places throughout India, ascending to an altitude of 1,320 m.

Morphology Description

It is a small, perennial creeping herb; its stems are obtuse-angular; the leaves are short-petioled, cuneate to obovate and the capsules are ovoid. It can be easily grown in damp areas and can be propagated using seeds or vegetatively.

Principal Constituents

The herb contains the alkaloids brahmine, herpestine, and a mixture of three bases. It also contains the saponins, monnierin; hersaponin, bacoside A and bacoside B. Other constituents present in the

plant are D-mannitol, betulic acid, β- sitosterol, stigmasterol and its esters. Aspartic acid, glutamic acid and serine are also present

Pharmacology

The saponin, hersaponin, is reported to possess cardiotonic, sedative and spasmodic properties. It produced a mild inhibitory effect on the respiration of rat brain tissue, which was partially reduced by LSD 25 and potentiated by 5-HT. It was also found, as in the case of reserpine, to deplete nor-adrenaline and 5-HT content of the rat brain. An alcoholic extract of the plant, in a dose of 50mg/kg, produced a tranquilizing effect on albino rats and dogs but the action was weaker than that produced by chlorpromazine. The administration of an aqueous suspension of an alcoholic extract (40mg/kg, p.o.) for three or more days is reported to improve the performance of rats in various learning situations (Singh and Dhawan, 1982; Malhotra et. al., 1961).

The anti-ulcerogenic effect of fresh juice from the whole plant of *Bacopa monniera* Wettst. (BMJ) was examined using gastric ulcer models induced by ethanol, aspirin, 2 h cold restraint stress and 4 h pylorus ligation. BMJ 100-300 mg/kg produced significant antiulcer activity in all the experimental gastric ulcer models except in case of ethanol-induced ulcers where 100 mg/kg was not found to decrease it significantly. BMJ (100-300 mg/kg) was found to have little or no effect on the offensive acid-pepsin secretion, while cell shedding (microgram DNA/mg of protein) and mucin secretion in terms of total carbohydrates: protein ration (TC:P), the two important parameters of defensive factors were significantly decreased and increased respectively indicating enhancement of protective mucosal factors. Thus, ulcer protective effect of BMJ may be due to its effect on mucosal defensive factors like enhanced mucin secretion, mucosal glycoprotein and decreased cell shedding rather than on offensive factors such as acid and pepsin (Rao et al., 2000).

The antioxidant activity of *B.monniera* was evaluated by determining $FeSO_4$ induced lipid peroxidation in rat brain homogenate (*in vitro*). Significant protection was observed at the dose of 500 µg/ml. The

mechanism of action could be through metal chelation at the initiation level and also as a chain breaker (Tripathi et al., 1996).

Indications

The entire plant constitutes the well-known drug Brahmi. It is astringent, bitter and cooling, and, is reported to improve the intellect. It is used in indigenous systems of medicine for the treatment of asthma, hoarseness, insanity, and epilepsy and as a potent nerve tonic, cardiotonic and diuretic. A clinical report showed that this drug is an anti-anxiety agent having adaptogenic effect. It exhibits a barbiturate hypnosis-potentiating effect in albino rats. The leaves are also useful as a diuretic and aperient and also used as a remedy for rheumatism. Both stem and leaves are used in snakebite.

Product Range

Mentat (MindCare), Mentat syrup, Anxocare, Brahmi.

1.7.5 *CENTELLA ASIATICA*

Latin Names: *Centella asiatica* (Linn.) Urban /
Hydrocotyle asiatica Linn. (Apiaceae)
English Names: Indian Pennywort, Centella and Gotu Kola
Sanskrit Names: Mandukaparni, Brahmi, Mandukig, Divya
Hindi Names: Brahma-manduki, Khulakhudi, Mandookaparni

History

Gotu kola is a pillar of herbal medicine in Ayurveda. In Sanskrit texts, this plant was called Brahmi, which means 'god-like', a reference to its anti-aging properties and to its use as an aid to medication. It is considered to be the most spiritual of all herbs. It was first used in India and is popular as a nerve tonic to promote relaxation and enhance memory. Indian healers use this herb to treat skin inflammations and as a mild diuretic. Oriental healers rely on Centella to treat emotional disorders such as depression. The leaves were used for pediatric complaints in bowel

problems, fever and applied externally for blows and bruises in the Coromandel Coast. In Java they were considered diuretic and on the Malabar Coast, the plant is one of the remedies for leprosy.

Habitat

It is commonly found as a weed in crop fields and other waste places throughout India up to an altitude of 600 m.

Morphology Description

C.asiatica is a prostrate, perennial herb. The stem is glabrous, pink and striated, rooting at the nodes; the leaves are fleshy, orbicular-reniform, crenate, base cordate and often lobed and long-petioled; the flowers are red, pink or white, in fascicled umbels; the fruits are oblong, dull brown, laterally compressed; the pericarp hard, thickened, woody and white.

Principal Constituents

Samples of the Indian plants collected from different places showed the presence of the following glycosides: indocentelloside, brahmoside, brahminoside, asiaticoside, thankuniside and isothankuniside. The corresponding triterpene acids obtained on hydrolysis of the glycosides are indocentoic, brahmic, asiatic, thankunic and isothankunic. These acids, except the last two, are also present in free form in the plant apart from isobrahmic and betulic acids. Mesoinositol, a new oligosaccharide, centellose, kaempferol, quercetin and stigmasterol are also present.

Pharmacology

Different fractions of the drug 'Mandukaparni' have shown barbiturate hypnosis potentiation effect in growing albino rats. It has also anticonvulsive activity, besides producing significant alterations in the neurochemistry of the brain.

The extract of the fresh plant significantly inhibits gastric ulceration induced by cold restraint stress (CRU) in rats (Chatterjee et al., 1992). It also prevented ethanol-induced gastric mucosal lesions by strengthening

the mucosal barrier and reducing the damaging effects of free radicals (Cheng and Woo, 2000). It was also found to provide significant protection against aspirin and pylorus-ligation induced gastric ulcers in rats (Sairam et al., 2001).

In pharmacological tests the plant has exhibited sedative, antidepressant activity in albino rats The asiaticoside, a glucoside, has given encouraging results for the treatment of leprosy (Chatterjee et. al., 1992). Darnis et al. (1979) has mentioned the use of a titrated extract of *Centella asiatica* in chronic hepatic disorders

Other studies have indicated an antioxidant effect of asiaticoside. When applied topically, asiaticosides derived from Centella was found to enhance induction of antioxidant (SOD, catalase, glutathione peroxidase, vitamin E and ascorbic acid) levels at an initial stage of healing wounds, which may be an important contributory factor in the healing properties of this substance (Shukla et al., 1999). Studies also indicated that *C. asiatica* has cognitive enhancing effect and an antioxidant mechanism is involved (Veerendra Kumar and Gupta, 2002).

Clinical Studies

Clinical trials have demonstrated that the herbal drug possesses an Ayurvedic Medhya Rasayana effect (brain invigorating). It was found that the extract increases the intelligence quotient in mentally retarded children. In a comparative clinical and instrumental trial with a placebo, the plant extract was found to improve venous disorders of the lower limbs (Allegra et al., 1981).

A double blind clinical trial conducted on 43 normal adults showed that the plant increased the mean level of R.B.C., blood sugar, serum cholesterol, vital capacity and total protein. The drug also decreased the mean blood urea level and a moderate decrease in the serum acid phosphate was observed.

A double blind clinical trial, conducted on 30 mentally retarded children who were free from epilepsy and other neurological conditions, showed significant improvements in both general ability and behavioral patterns when the drug was administered for a short period of 12 weeks.

Toxicology

The drug was found to be nontoxic up to a dose of 350mg/kg.

Indications

The plant is valued in indigenous medicine for treatment of leprosy and skin diseases and also to improve memory. The plant is used as an antidote to cholera. A cold poultice of the fresh herb is used as an external application in rheumatism, elephantiasis and hydrocele. For treating leprosy and other skin diseases it is given as an ointment or dusting powder. Internally it has been valued as a tonic and is used in bronchitis, asthma, gastric catarahh, leucorrhoea, liver and kidney troubles, urethritis and dropsy. A decoction of very young shoots is given for haemorrhoids. It may be used for better circulation, wound healing, cancer, vitality, general tonic, respiratory ailments, detoxifying the body, revitalizing connective tissue, burn and scar treatment, slimming and edema, arthritis, blood purifier, high blood pressure, sedative, anti-stress, anti-anxiety, an aphrodisiac, immune booster, anabolic and adaptogen.

Product Range

Abana (HeartCare), Geriforte (GeriCare/StressCare), Menosan, Mentat (MindCare), Mentat syrup, Anxocare, Geriforte Vet.

1.7.6 *EMBLICA OFFICINALIS*

Latin Names: *Emblica officinalis* Gaertn./
Phyllanthus emblica Linn. (Euphorbiaceae)

English Names: Indian Gooseberry, Emblic Myrobalan
Sanskrit Names: Amalaki, Dhatriphala, Shriphala
Hindi Names: Amla, Aovla

Habitat

The tree is commonly found in the mixed deciduous forests of India ascending to 4,500 ft. in the hills.

Morphology Description

E.officinalis is a small or medium-sized deciduous tree with smooth, greenish grey, exfoliating bark. The leaves are feathery with small narrowly oblong, pinnately arranged leaflets. The fruits are depressed, globose, fleshy and obscurely 6-lobed, containing 6 trigonous seeds.

Principal Constituents

Amla is highly nutritious and is an important dietary source of Vitamin C, minerals and amino acids. The edible fruit tissue contains protein concentration 3-fold and ascorbic acid concentration 160-fold compared to that of the apple. The fruit also contains a high concentration of most minerals and amino acids (Glutamic acid, proline, aspartic acid, alanine and lysine). The pulpy portion of fruit contains: gallic acid 1.32%, tannin, sugar 36.10%; gum 13.75%; albumin 13.08%; crude cellulose 17.08%; mineral matter 4.12% and moisture 3.83%. Amla fruit ash contains chromium, 2.5; zinc, 4; and copper, 3 ppm. Presence of chromium is of therapeutic value in diabetes. Fruit also contains phyllemblin and curcuminoides. The fruit contained 482.14 units of superoxide dismutase/g fresh weight and exhibited antisenescent activity. The seed oil contains 64.8% linolenic acid.

Pharmacology

Effects of alcoholic extract of fruits were studied in albino rats after isoproterenol-induced myocardial necrosis. Cardiac glycogen levels significantly increased in the group treated with *P.emblica.* Levels of serum GOT, GPT and LDH were significantly less in groups treated with it (Tariq et al., 1977).

Feeding of amla to hypercholesterolemic rabbits reduced serum, aortic and hepatic cholesterol in rabbits (Thakur, 1985).

The relative effects of a crude aqueous extract from the fruit and an equivalent amount of synthetic ascorbic acid (vitamin C) in reducing the clastogenic action of cesium chloride (CsCl) *in vivo* on mice bone marrow cells were compared. CsCl-induced chromosomal aberrations were observed in the mice 24 hours after exposure in frequencies that were

directly proportional to the dose administered even after treatment for seven days. On the other hand, oral administration of either ascorbic acid or *E. officinalis* extract for seven days prior to exposure to CsCl for 24 hours reduced the frequency of chromosomal aberrations.

Oral administration of *E. officinalis* juice (50mg/kg) was found to produce a dose-dependent protective effect against gastric damage induced by ethanol, indomethacin and histamine. The protection afforded by *E. officinalis* fruits was found to be better than that of ranitidine (50mg/kg). The results of the study suggested the novel cytoprotective activity of *E. officinalis* fruits on gastric mucosal cells (Rajeshkumar et al., 2001).

The ulcer protective potential of methanolic extract of *Emblica officinalis* Gaertn. (EOE) was assessed by Sairam et al. (2002) in different acute gastric ulcer models in rats induced by aspirin, ethanol, cold restraint stress and pyloric ligation and healing effect in chronic gastric ulcers induced by acetic acid in rats. EOE, 10-50 mg/kg administered orally, twice daily for 5 days showed dose-dependent ulcer protective effects in all the above acute ulcer models. Further study on gastric mucosal factors showed that it significantly decreased the offensive factors like acid and pepsin and increased the defensive factors like mucin secretion, cellular mucus (TC:P) and life span of mucosal cells. EOE also showed significant antioxidant effect in stressed animals (Al-Rehaily et al , 2002).

Hepatoprotective activity of *Emblica officinalis* has also been studied by Jose and Kuttan (2000).

The active tannoid principles of emblica, which have vitamin C-like properties, were found to induce an increase in both frontal cortical and striatal concentrations of the oxidative free radical scavenging enzymes, SOD, catalase and glutathione peroxidase and concomitant decrease in lipid peroxidation in these brain areas (Bhattacharya et al., 1999).

Clinical Studies

Clinical studies were conducted to investigate the effect of amalaki in amlapitta (gastritis syndrome). Amalaki churna was given in 20 cases in a dose of 3g, thrice a day for seven days. The drug was found effective in

85 per cent of cases. Cases of hyperchlorhydria with burning sensation in abdominal and cardiac regions and epigastric pain were benefited.

The therapeutic efficacy of Amalaki in cases of dyspepsia was evaluated and the results clearly indicate the efficacy of *Emblica officinalis* in relieving the dyspeptic symptoms as well as in promoting healing of ulcers (Chawla et. al., 1982).

Indications

This famous ayurvedic herb is one of the most useful medicines in the Indian Pharmacopoiea and is considered to be one of the strongest rejuvenates (rasayana), particularly for the blood, bones, liver and heart.

The fruit is acrid, cooling, refrigerant, diuretic and laxative. The dried fruit is useful in hemorrhage, diarrhea and dysentery. In combination with iron, it is used as a remedy for anemia, jaundice and dyspepsia. The fruit is useful in both acid-peptic ulcer and non-ulcer dyspepsia. Amla fruits are anabolic, anti-bacterial and resistance building. They possess expectorant, cardiotonic, antipyretic, antioxidative, anti-inflammatory, antiviral and anti-emetic activities. They are also used in the treatment of leukorrhea and atherosclerosis. It is considered as one of the foremost rejuvenating drugs imparting a long healthy life and weight gain.

Product Range

Abana (HeartCare), Bonnisan, Mentat (MindCare), Mentat syrup, Ophthacare, Pilex (VeinCare), Septilin (ImmunoCare), Septilin syrup, Styplon, Daily Health Capsules, Gentle Face Wash Cream, Hair Oil, Anxocare, Digyton, Regurin, Styplon Vet, Amalaki, Chyavanaprasha.

1.7.7 *GLYCYRRHIZA GLABRA*

Latin Name: *Glycyrrhiza glabra* Linn. (Fabaceae)
English Names: Liquorice, Licorice
Sanskrit Names: Yashti-madhu, Yashti-madhuka
Hindi Names: Mulhathi, Jethi-madh

Habitat

It grows in the sub- tropical and warm temperate regions of the world, chiefly in Mediterranean countries and China.

Morphology Description

It is a hardy herb or undershrub; the leaves are multifoliolate, imparipinnate; the flowers are in axillary spikes, papilionaceous, lavender to violet in colour; the pods are compressed and contain reniform seeds. The rootstock, which is stout, throws off a large number of perennial roots. The dried, peeled or unpeeled underground stems and roots constitute the drug known in the trade as Licorice.

Principal Constituents

The principal constituent of liquorice to which it owes its characteristic sweet taste is glycyrrhizin, which is present in different varieties in a concentration of 2-14%. Other constituents present in liquorice are: glucose (up to 3.8%), sucrose (2.4-6.5%), mannite, starch (30%), asparagine, bitter principles, resins (2-4%), a volatile oil (0.03-0.035%) and coloring matter. A steroid estrogen, possibly estriol, is also reported to be present in liquorice. The presence in the inner bark of a hemolytically active saponin has been reported. The plant contains phytoestrogens in the form of isoflavones such as formononetin; glabrone, neoliquiritin and hispaglabridin A & B (Paris and Guillot, 1955).

Pharmacology

The antiulcer activity of *Glycyrrhiza glabra* has been demonstrated both experimentally and clinically. Intraperitoneal, intraduodenal or oral

administration of aqueous or alcoholic extracts of *Glycyrrhiza glabra* reduced gastric secretions in rats, and it inhibited the formation of gastric ulcers induced by pyloric ligation, aspirin, and ibuprofen. Glycyrrhizin and its aglycone (glycyrrhetic acid, enoxolone), two of the active constituents of *Glycyrrhiza glabra*, both have antiphlogistic activity and increase the rate of mucus secretion by the gastric mucosa. Deglycyrrhizinated liquorice (97% of glycyrrhizin is removed) effectively treated stress-induced ulcers in animal models. The mechanism of antiulcer activity involves acceleration of mucin excretion through increasing they synthesis of glycoprotein at the gastric mucosa, prolonging the life of the epithelial cells, and antipepsin activity (De et al., 1997). Licorice root is very effective in the treatment of peptic ulcers. Carbenoxolone, a popular drug for treating peptic ulcers, is a semisynthetic derivative of glycyrrhizin, developed over 30 years ago. The mechanism underlying the anti-ulcer effects of licorice appears to involve the ability of GL and GA (glycyrrhetinic acid - a partially hydrolyzed form of glycyrrhizin) to inhibit the enzymes 15-hydroxyprostaglandin dehydrogenase and D-13-prostaglandin reductase, which inactivates the protective prostaglandins (PG) in the gastric mucosa.

The protective action of Glycyrrhiza flavonoids (GF), the major components in the radix of Glycyrrhiza, on carbon tetrachloride-induced hepatotoxicity was investigated. The carbon tetrachloride-induced increases of serum glutamic-pyruvic transaminase and lactate dehydrogenase were significantly inhibited by GF dose-dependently. Carbon tetrachloride-induced necrosis in mice was ameliorated by GF pretreatment. Concomitantly, the carbon tetrachloride-induced elevation of MDA in the liver was lowered by GF. GF neither reduced the activities of the two enzymes in normal mouse sera nor directly inhibited the activities of the two enzymes in the serum. These findings suggest that the anti-lipid peroxidation effect of GF was contributed to its protective action against carbon tetrachloride-induced hepatotoxicity (Wang and Han, 1993).

Glycyrrhizin reduces the toxic action of carbon tetrachloride- and galactosamine-induced cytotoxicity in cultured rat hepatocytes, through its antioxidant activity. Glycyrrhizin inhibited histamine release from rat

mast cells and prevented carbon tetrachloride-induced liver lesions and macrophage-mediated cytotoxicity. Intragastric administration of a flavonoid fraction isolated from *G.glabra* to mice protected against carbon tetrachloride hepatotoxicity. Glycyrrhizin protected the liver apparently through its membrane stabilization effects.

Kilgore et al. (1998) analyzed glycyrrhizin and glycyrrhetinic acid, a natural structural glycomimetic, for their ability to decrease myocardial infarct size after regional myocardial ischemia/reperfusion. The data suggested that both, glycyrrhizin and glycyrrhetinic acid which contain carbohydrate moieties, were effective in reducing the degree of myocardial injury after an acute period of ischemia/reperfusion.

The spasmolytic activity of Glycyrrhiza has been demonstrated in vivo (guinea-pig, rabbit, and dog), and appears to be due to the flavonoids liquiritigenin and isoliquiritigenin.

Vaya and Belinky (1997) isolated the antioxidant constituents from licorice roots and studied their antioxidative capacity toward LDL oxidation. Haraguchi et al. (2000) also studied the protection of mitochondrial functions against oxidative stresses by isoflavans from *Glycyrrhiza glabra.*

Glycyrrhizin, a glycoside obtained from *G. glabra* was studied for its anti-arthritic and anti-inflammatory effect on formaldehyde induced rat-paw edema in adrenalectomised rats. It was found to potentiate the anti-arthritic action of hydrocortisone in rats (Gujral et al., 1961).

The anti-inflammatory and antiallergic actions of the drug have been attributed to the corticosteroid-like activity of glycyrrhizin and glycyrrhetic acid (enoxolone). These compounds act indirectly by potentiating the activity of corticosteroids. *In vitro*, glycyrrhetic acid inhibits β-reductase, an enzyme that competitively inactivates steroid hormones, and 11β-hydroxysteroid dehydrogenase, the enzyme that deactivates cortisol. Glycyrrhizin given intraperitoneally suppressed contact dermatitis in mice, and was more effective than prednisolone, but no effects were observed after oral administration.

Clinical Studies

The oral administration of the powdered root of *G.glabra* in 5 cases of pemphigus, which had been kept free from the bullae with prednisolone, could considerably reduce the dose of prednisolone without the reappearance of the lesions. The potentiating effect of *G.glabra* appeared to be due to its inhibitory effect on the metabolic degradation of prednisolone. A controlled clinical trial on 92 randomly selected cases of postoperative traumatic inflammation following tonsillectomy with powdered *G.glabra* given in a dose of 3g t.d.s in 28 cases. In another series of 24 cases, oxyphenbutazone 2 tabs t.d.s were given. On sequential analysis, the anti-inflammatory response of *G.glabra* was found to be equivalent to that of oxyphenbutazone. *G.glabra* appeared to possess a more potent antipyretic and anti-exudative activity in comparison to oxyphenbutazone.

Oral administration of *Glycyrrhiza glabra* to 15 patients with peptic ulcer reduced symptoms and improved healing in 75% of the cases. Glycyrrhetic acid (enoxolone), the active constituent, produced its antiulcer activity inhibiting 15-hydroxyprostaglandin dehydrogenase and -prostaglandin reductase. Inhibition of these two enzymes stimulated an increase in the concentration of prostaglandins E and F, in the stomach, which promoted the healing of peptic ulcers owing to a cytoprotective effect on the gastric mucosa. Carbenoxolone, a derivative of glycyrrhetic acid, has been used clinically for years in the treatment of gastric and duodenal ulcers.

Oral administration of deglycyrrhizinated liquorice (380 mg, 3 times daily) to 169 patients with chronic duodenal ulcers was as effective as antacid or cimetidine treatments. These results indicate that, in addition to glycyrrhetic acid, other unidentified constituents of *Glycyrrhiza glabra* contribute to its antiulcer activity.

Toxicology

Excessive amounts of the root, herbal teas or candy derived from *G. glabra* may be harmful. Licorice increases salt retention and depletes the potassium in the body, causing lack of energy, weakness and even death.

People with hypertension or heart problems should avoid licorice (Anonymous, 1986).

Indications

Licorice is used for the treatment of asthma, acute and chronic bronchitis and chronic cough. It is a mild anti-inflammatory for arthritis and rheumatism and is used to treat gastric, duodenal and oesophageal ulceration or inflammation, heartburn and mouth ulcers.It is used to improve eyesight, strength, sexual potency and libido. It is considered, as adaptogens generally do, to enhance the effects of other herbs in a formula, so it is widely used. It is a good hepatoprotective agent. It also possesses antioxidant activity.

Product Range

Abana (HeartCare), Diabecon (GlucoCare), Diakof (CoughCare Sfree), Geriforte (GeriCare/ StressCare), Herbolax (LaxaCare), Koflet (CoughCare), Menosan, Rumalaya forte, Septilin (ImmunoCare), Septlin syrup, Antacid Suspension, Anti-Wrinkle Cream, Cough Syrup, Daily Health Capsules, Geriforte Vet, Himpyrin Vet, Regurin, Yashti-madhu.

1.7.8 *MANGIFERA INDICA*

Latin Name: *Mangifera indica* (Anacardiaceae)
English Name: Mango
Sanskrit / Indian Names: Amra, Chuta

Morphology Description

It is a large evergreen tree, with a heavy, dome-shaped crown. The mango is the most popular fruit in India.

Principal Constituents

The unripe, fully developed mangoes of pickling varieties contain citric, malic, oxalic, gallic, succinic and two unidentified acids. The ripe fruits constitute a rich source of vitamin A; some varieties contain fairly

good amounts of vitamin C also. β-carotene, carotenoids and xanthophyll are the principal pigments in ripe mango. The leaves contain the glucoside mangiferine. The bark of the mango tree contains tannin (16-20%). Mangiferine has been isolated from the bark.

Pharmacology

It possesses anti-viral, anti-parasitic, antiseptic, antitussive, anti-asthmatic, ascaricide, expectorant, cardiotonic, contraceptive, hypotensive, laxative, parasiticide, stomachic and vermifuge properties.

The extract of *M.indica* reduces ischemia-induced neuronal loss and oxidative damage in the gerbil brain most probably due to the antioxidant activity of the extract (Martinez Sanchez et al., 2001).

The antioxidant activity of the extract was also studied on hydroxyl-mediated oxidation of bovine serum albumin (BSA) and in a hepatic microsome system and was found to reduce the oxidation of BSA and inhibited lipid peroxidation, which was, initiated enzymatically by NADPH. The results suggested that the extract has an antioxidant activity probably due to its ability to scavenge free radicals involved in microsome lipid peroxidation. In addition, the extract's antioxidant profile *in vitro* is probably similar to its principal polyphenolic component, mangiferin, a glycosylated xanthone (Martinez et al., 2001).

The analgesic and anti-inflammatory effects of Vimang, an aqueous extract of *M.indica* was also studied in acetic acid-induced abdominal constriction and formalin-induced licking, and carrageenan- and formalin-induced edema, respectively. The different polyphenols found in Vimang could account for these actions (Garrido et al., 2001).

In another study, Vimang was found to provide significant protection against 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced oxidative damage in serum, liver, brain as well as against hyperproduction of ROS by peritoneal macrophages. Thus Vimang could be useful to prevent the production of ROS and the oxidative tissue damages *in vivo* (Sanchez et al., 2000).

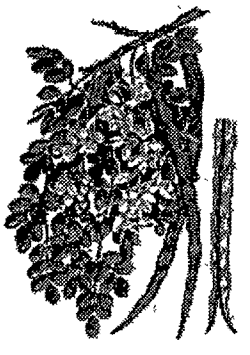
Indications

The bark is astringent; it is used in diphtheria and rheumatism; it is believed to possess a tonic action on the mucous membrane. It is astringent, anthelmintic, useful in hemoptysis, hemorrhage, nasal catarrh, diarrhea, ulcers, diphtheria, rheumatism and for lumbrici. The leaves are given in the treatment of burns, scalds and diabetes. Mangiferin from the leaves has been reported to possess antiinflammatory, diuretic, chloretic and cardiotonic activities and displays a high antibacterial activity against gram positive bacteria. It has been recommended as a drug in preventing dental plaques. Mangiferin shows antiviral effect against type I herpes simplex virus (HSV-I).

Product Range

It is used in Daily Health Capsules, Regurin.

1.7.9 *MORINGA PTERYGOSPERMA*



Latin Names: *Moringa pterygosperma* Gaertn./ *Moringa oleifera* (Moringaceae)
English Names: Horse Radish, Drumstick
Sanskrit Names: Shigru, Shobhanjana
Hindi Names: Sahijna, Sainjna, Munaga

Habitat

It grows wild in the sub-Himalayan tract, from Chenab eastwards to Sarda and is cultivated all over the plains of India.

Morphology Description

M.pterygosperma is a small or medium-sized tree. The bark is thick, soft, corky and deeply fissured; the leaves, usually tri-pinnate; the leaflets, elliptic; the flowers, white, fragrant in large panicles; the pods, pendulous, greenish, triangular and ribbed with trigonous, winged seeds.

Principal Constituents

The roots contain an active antibiotic principle, pterygospermin. The root bark contains two alkaloids (total alkaloids, 0.1%), viz. moringine which is identical with benzylamine and moringinine belonging to the sympathomimetic group of bases. It also contains traces of an essential oil with a pungent smell, phytosterol, waxes and resins. An alkaloid, named spirochin, has been isolated from the roots. Hypotensive principles niazinin A, niazinin B, niazimicin, and niaziminin A and B were obtained from ethanolic extracts of the fresh leaves. These compounds are mustard-oil glycosides and are very rare in nature. They are rare examples of naturally occurring thiocarbamates

Pharmacology

The leaves of *M.oleifera* were used by the Indians in their herbal medicine as a hypocholesterolemic agent in obese patients. The scientific basis for their use in hypercholesterolemia was examined by Ghasi et al. (2000). They tested the crude extract of leaves of *M.oleifera* and showed that it possessed hypocholesterolemic activity.

Hot water infusions of flowers, leaves, roots, seeds and stalks or bark of *Moringa oleifera* were screened to detect three pharmacologic activities in experimental models in rats. The antispasmodic activity was demonstrated using isolated duodenum, oral anti-inflammatory activity by carrageenan-induced hindpaw edema and oral diuretic activity by urine output in metabolic cages. The seed infusion showed a significant inhibition of acetylcholine-induced contraction with an ED50 of 65.6-mg/ml bath concentration, inhibition of carrageenan-induced edema at 1000 mg/kg and diuretic activity at 1000 mg/kg. Some activity was also demonstrated in the roots (Caceres et al., 1992).

Aqueous extract of *M.pterygosperma* was given orally at a dose of 200mg/kg to different groups of rats for 6, 12, 18 and 24 days. Parallel controls were run with each group, which received gum acacia suspension as vehicle. Throughout the experimentation the vaginal smear of each rat was examined daily at a regular interval of 24 hr. At the end of the experiment the entire record of different stages of the oestrous cycle of

each rat was analyzed. It was found that the dose of 200mg/kg disturbed the normal regular estrous cycle in all the animals; however, the response was duration dependent (Shukla et. al., 1988a). Aqueous extracts of the roots and of the root bark of *M.oleifera* were effective in preventing implantation. The anti-implantation activity of *M.oleifera* root was consistent regardless of its time and place of collection (Shukla et. al., 1988b). The thiocarbamate glycosides from *Moringa oleifera* showed hypotensive activity (Faizi et. al., 1995).

The methanol fraction of *M.oleifera* leaf extract (at 100 mg/kg and 150mg/kg) was found to possess significant protective actions in acetylsalicylic acid, serotonin and indomethacin induced gastric lesions in experimental rats. A significant enhancement of the healing process in acetic acid-induced chronic gastric lesions was also observed with the extract-treated animals.

The antimicrobial activities of *Moringa oleifera* leaves, roots, bark and seeds were investigated in vitro against bacteria, yeast, dermatophytes and helminths pathogenic to man. By a disk-diffusion method, it was demonstrated that the fresh leaf juice and aqueous extracts from the seeds inhibit the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Caceres et al., 1991).

Toxicity

In cardiovascular profile at lower concentrations (1-10mg) it produced a dose dependent positive inotropic effect and at higher concentrations (0.1-1μg) a dose dependent negative inotropic effect on the isolated frog heart.

Indications

Biological activity studies have confirmed the anti-inflammatory, antispasmodic and diuretic activities of the seeds. The seeds are used as antibacterial, anticholeric and anti-viral agents.

Product Range

Rumalaya (JointCare), Septilin (ImmunoCare), Pain Massage Oil, Rumalaya Vet.

1.7.10 *MUCUNA PRURIENS*

Latin Names: *Mucuna pruriens* Baker non DC. / *Mucuna prurita* Hook. (Fabaceae)
English Names: Cow-Itch Plant, Cowhage, Velvet bean
Sanskrit Names: Kapikachhu, Atmagupta
Hindi Names: Kaunch, Kevanch

Habitat

It grows all over India and in the Andaman and Nicobar Islands.

Morphology Description

M.pruriens is a herbaceous twining annual. The leaves are trifoliolate; the leaflets, broadly ovate, elliptic or rhomboid ovate and unequal at the base; the flowers, purple and in axillary, pendulous racemes; the pods, curved, longitudinally ribbed, turgid and densely clothed with persistent pale brown or grey, irritant bristles; the seeds, black, 4-6 in a pod and ovoid.

Principal Constituents

M.prurita has been found to contain L-DOPA, 40mg/g of the plant. The plant/seeds contain the bioactive alkaloids mucunine, mucunadine, mucuadinine, pruriendine and nicotine, besides β-sitosterol, glutathione, lecithin, oils, venolic and gallic acids. The seeds with seed coat showed the presence of a number of bioactive subtances including tryptamine, alkylamines, steroids, flavonoids, coumarins and cardenolides.

Pharmacology

This herb helps to not only burn body fat, but protects lean muscle as well. It has been shown to increase testosterone levels, leading to

deposition of protein in the muscles and increased muscle mass and strength. Mucuna has been used for over a thousand years as an aphrodisiac and research shows that this extract is also beneficial for enhancing mental alertness, reducing cholesterol and lowering blood sugar levels (Akhtar et al., 1990), all without stimulating the CNS.

Treatment of Parkinson's disease with the cowhage plant-*Mucuna pruriens* has been reported (Vaidya et al., 1978).

The alcohol extract of seeds of *M.pruriens* has an antilipid peroxidation property, which is mediated through the removal of superoxides and hydroxyl radicals (Tripathi and Upadhyay, 2002).

It significantly decreased the sleeping time, increased the motor activity and gave equivocal results in rotarod test in experimental animals. The high dose (3 times the clinical dose) significantly increased the sleeping time, decreased the motor activity and reduced the time for falling from the rod. Thus the drug possesses CNS stimulant effect at low doses and CNS depressant effect at high doses (Ahmad et. al., 1991).

Several *in vivo* studies have been conducted on the blood-sugar-lowering effect of *M.pruriens* (Rathi et al., 2002). These studies all validate the traditional use of the plant for diabetes. An ethanol-water extract (250 mg/kg) of the root, fruit and seed dropped blood sugar levels in rats by more than 30%. At 200 mg an ethanol extract produced a 40% fall in serum blood glucose within one month, and a 51% reduction at four months. Furthermore, decoction of the leaf (5 g/kg) reduced total cholesterol in rats; the seed had the same effect (Pant et al., 1968).

Traditionally the seed has been used by indigenous people throughout the world for snakebite. Several *in vivo* studies validate this traditional use. In rats, a water extract of the seed (21 mg/kg) inhibited venom-induced blood and coagulation alterations, and reduced lethality of the *Echis carinatus* (saw-scaled viper) venom. The antivenin effect of *M.pruriens* is thought to be due to an immune mechanism, as proteins in the seed are able to raise antibodies against the venom (Guerranti et al., 2001).

Clinical Studies

It is now being considered as an alternative to the pharmaceutical medication levodopa. In a case study it was given to a Parkinson's patient for 12 years instead of the pharmaceutical L-dopa medication. It was found to slow the progression of Parkinson's symptoms (such as tremors, rigidity, slurring, drooling, and balance), and to have none of the side-effects of the current pharmaceutical L-dopa. Numerous *in vivo* studies also have been conducted in rats and humans. In one human study, 45 mg of the bean powder was given to 60 patients (26 previously treated with L-dopa and 34 L-dopa naive). There were statistically significant reductions in Parkinson's symptoms in all subjects. In addition, a 2002 U.S. patent was awarded on *M.pruriens* citing its use for the treatment of disorders of the nervous system, including Parkinson's disease (Liebert, 1995).

Clinical studies in India have validated that the plant does indeed have aphrodisiac activity. It also has anabolic and growth hormone stimulant properties. The anabolic effect of the seed is due to its ability to increase testosterone. In 2002, a U.S. patent was filed on the use of *Mucuna pruriens* to stimulate the release of growth hormone in humans. The high levels of l-dopa in the mucuna seed are converted to dopamine, which stimulates the release of growth hormone, by the pituitary gland. L-dopa and dopamine are also effective inhibitors of prolactin. Prolactin is a hormone released by the pituitary gland; increased levels are considered responsible for 70–80% of erection failure in males. In one study, oral intake of the seeds in 56 human males was able to improve erection, duration of coitus, and post-coital satisfaction after only four weeks of treatment. The seed also has fertility promoting and spermatogenic effects in human males, being able to improve sperm count and motility.

Toxicity

Adverse effects were mild and were mainly gastro-intestinal in nature. No adverse effects were seen in clinical laboratory reports.

Indications

L-DOPA is a neurotransmitter precursor, an effective drug for relief in Parkinson's disease. The seed is a prophylactic against oligospermia, useful in increasing sperm count, ovulation in women, etc. It prevents male and female sterility and acts as a nervine tonic. It is anabolic, analgesic, androgenic, anthelmintic, anti-inflammatory, antioxidant, antiparkinson, antipyretic, antispasmodic, aphrodisiac, ascaricide, carminative, diuretic, hypocholesterolemic, hypotensive, hypoglycemic, nervine, spermatogenic, taenicide, teratogenic, vermifuge. It is considered a diuretic, nerve tonic, and aphrodisiac. Externally it is applied to ulcers. It has a long history of use in Indian Ayurvedic medicine, where it is used for worms, dysentery, diarrhea, snakebite, sexual debility, cough, tuberculosis, impotence, rheumatic disorders, muscular pain, gonorrhea, sterility, gout, delirium, dysmenorrhea, diabetes, and cancer. In India it is considered an aphrodisiac, emmenagogue, uterine stimulant, nerve tonic, diuretic, and blood purifier. Leaves and roots are useful in ulcers.

Product Range

Confido (Speman forte), Geriforte (GeriCare / StressCare), Mentat (MindCare), Mentat syrup, Speman (ProstaCare), Tentex forte (VigorCare for Men), Anxocare, Geriforte Vet, Speman Vet, Tentex forte Vet.

1.7.11 *PIPER LONGUM*

Latin Name: *Piper longum* Linn. (Piperaceae)
English Name: Indian Long Pepper
Sanskrit Names: Pippali, Magadhi, Kana, Ushana
Hindi Names: Pipal, Pipar

Habitat

It occurs in the hotter parts of India, from the Central Himalayas to Assam, the Khasi and the Mikir hills, the lower hills of Bengal and the evergreen forests of the Western Ghats from Konkan to Travancore. It has also been seen growing in the Nicobar Islands.

Morphology Description

P.longum is a slender aromatic climber with perennial woody roots. The stems are joined; the leaves are ovate and cordate with broad rounded lobes at the base, entire and glabrous; the spikes are cylindrical, male spikes are larger and slender; the fruits are ovoid and yellowish orange.

Principal Constituents

Its main constituents are piperine and piplartine.

Pharmacology

P.longum showed significant protection against gastric ulcers induced by 2 h cold restraint stress, aspirin (200 mg/kg, 4 h) and 4 h pylorus-ligation. The anti-ulcerogenic effect seemed to be due to the augmentation of mucin secretion and decreased cell shedding rather than offensive acid and pepsin secretion which however were found to be increased by them (Agrawal et al , 2000).

Piperine, an active alkaloidal constituent of the extract obtained from *P.longum* was evaluated for its antihepatotoxic potential in order to validate its use in traditional therapeutic formulations. This active principle exerted a significant protection against tert-butyl hydroperoxide and carbon tetrachloride hepatotoxicity by reducing both *in vitro* and *in vivo* lipid peroxidation, enzymatic leakage of GPT and AP, and by preventing the depletion of GSH and total thiols in the intoxicated mice (Koul and Kapil, 1993).

Indications

The fruits are used for diseases of the respiratory tract, viz. cough, bronchitis, asthma, etc.; as counter-irritant and analgesic when applied locally for muscular pains and inflammation and as general tonic and hematinic. It is known to enhance the bio-availability of food and drugs as well as being a carminative.

Product Range

Abana (HeartCare), Bonnisan, Geriforte (GeriCare / StressCare), Cough Syrup, Digyton, Geriforte Vet, Chyavanaprasha.

1.7.12 *SOLANUM NIGRUM*

Latin Name: *Solanum nigrum* (Solanaceae)
English Name: Black Nightshade, Petty Morel, Poisonberry, Garden nightshade
Sanskrit Name: Kakamachi
Hindi Name: Gurkama

Habitat

It is found in all states of Australia, in waste areas and damp shady spots. It is sometimes called garden nightshade because it appears so often in cultivated grounds.

History

In Bohemia the leaves are placed in the cradles of infants to promote sleep. In the islands of Bourbon and Mauritius the leaves are eaten in place of spinach; and the fruit is said to be eaten 'without inconvenience' by soldiers in Kaffraria.

Description

It is a brittle-stemmed weed that grows up to a metre tall. It has ovate leaves, tiny white star shaped flowers and small round black berries that are green when unripe.

Constituents

The green berries and leaves contain glyco-alkoloids including solasodine (Ikeda et al., 2000).

Pharmacology

An antiulcerogenic activity of *Solanum nigrum* was studied against aspirin-induced gastric ulcers in rats. In addition, its effect on output of

gastric acid and pepsin and hexosamine concentrations in gastric fluid was recorded in ulcerated and non-ulcerated rats. *Solanum nigrum* (aerial parts) powder and its methanolic extract decreased the ulcer index significantly. The activity may be due to inhibition of acid and pepsin secretions and/or their *in vitro* ability to bind these (Akhtar and Munir, 1989).

Effect of ethanol extract of dried fruits of *Solanum nigrum* Linn was investigated for its hepatoprotective activity against CCl_4-induced hepatic damage in rats and showed remarkable hepatoprotective activity (Raju et al., 2003). Studies by Sarwat et al. (1995) suggest that the hepatoprotective effect of crude plant extract of *Solanum nigrum* may be due to its ability to suppress the oxidative degradation of DNA in the tissue debris.

It inhibits gentamicin-induced kidney cell damage, prolongs pentobarbital-induced sleeping time, suppresses aggressive behavior, reduces spontaneous motility, inhibits free radical-induced DNA damages, antiulcerogenic activity, hypotensive activity.

Indications

It is diaphoretic, purgative, narcotic, poison, and anti-inflammatory (topical). Green berries are recommended for herpes simplex. Leaves can be used as a poultice for ulcers or skin conditions. It is used as an ointment for abscesses, sores, and in a douche for leucorrhoea, eczema, nappy rash and wounds.

1.7.13 *STRYCHNOS NUX-VOMICA*

Latin Name: *Strychnos nux-vomica* Linn. (Loganiaceae)
English Names: Snake Wood, Nux-vomica,
Poison Nut, Quaker Buttons.
Sanskrit Names: Kupilu, Vishamushti
Hindi Names: Kuchla, Kuchila

History

There is no mention of nux-vomica in the earliest sanskrit and medical writing. There can be little doubt that nux-vomica was not used medicinally by ancient Hindus. However, the Hindi name 'Kuchila' or 'Kuchula' occurs in ancient Persian and Arabian literature.

Habitat

It grows throughout tropical India upto an altitude of 1360 meters.

Morphology Description

It is an evergreen or deciduous tree. The leaves are glabrous, 8-15 cm. long, broadly elliptic obtuse or acute, entire and with prominent central nerves. The flowers are greenish white in terminal compound cymes. Corolla tube is cylindrical. The style is filiform and stigma is undivided. The berries are globose. Seeds are discoid (coin-like), covered with fine and silky hair, embedded in white and bitter pulp.

Principal Constituents

The seeds contain strychnine, brucine and vomicine as major alkaloids and a -colubrine, β-colubrine, pseudostrychnine and sec-pseudobrucine as minor alkaloids. A glucoside Loganin, about 3 per cent fatty matter, caffeotannic acid and a trace of copper is also present. The pulp of the fruit contains about 5 % of loganin together with the alkaloid strychnicine.

Pharmacology

Strychnine has powerful stimulant action on the motor-cells of the central nervous system.

Strychnine, the major active principle in the alcoholic extract of the seeds of *Strychnos nux-vomica*, is responsible for its antilipid peroxidative property. The mechanism of action of this drug is through the chelation of the free iron in the system. It has also been observed that strychnine does not have any pro-oxidant property, because it does not convert Fe^{3+} to Fe^{2+}

and vice versa in the reaction system, as has been observed with several other antioxidants (Tripathi and Chaurasia, 2000).

Toxicology

Strychnine, one of the major alkaloids, is toxic in large doses.

Indications

It is stimulant, tonic and prescribed for nervous disorders. It is used as an aphrodisiac. The powdered seeds are employed in atonic dyspepsia. The tincture of nux vomica is often used in mixtures - for its stimulant action on the gastro-intestinal tract. In the mouth it acts as a bitter and increases appetite. It stimulates peristalsis in chronic constipation due to atony of the bowel and so is often combined with cascara and other laxatives with good effects. It improves the pulse and raises blood pressure and is of great value as a tonic to the circulatory system in cardiac failure.

Product Range

Tentex forte (VigorCare for Men), Tentex forte Vet.

1.7.14 *SYZYGIUM AROMATICUM*

Latin Names: *Syzygium aromaticum* (Linn.) Merrill. & Perry. (Myrtaceae)/ *Caryophyllus aromaticus* Linn./*Eugenia caryophyllata* Thunb /*E. aromatica* Kuntze.

English Name: Clove

Sanskrit Name: Lavangaha

Hindi Name: Laung

History

Cloves appear to have been cultivated in China as early as BC 266. Sanskrit writing regarded it as light and cooling on the stomach when used as a digestive. It was also useful in relieving thirst, vomiting, flatulence and colic. A paste of clove was applied on the forehead for relief from colds. A clove roasted in the flame of a lamp and held in the mouth

was used as a popular remedy for sore throat. It was also recommended for strengthening the gums and freshening the breath. It was known for its pectoral, cardiac, tonic and digestive qualities. In India, clove fruits were considered male cloves and buds were female cloves. It was believed that one male clove eaten daily prevents constipation.

Habitat

It is cultivated in Tanzania, Indonesia, Penang and to a lesser extent in the Seychelles, Reunion, Mauritius and Sri Lanka. In India, it is grown and cultivated in Tamilnadu and Kerala. It thrives in all situations ranging from the sea level up to an altitude of 900 m but is unable to withstand long summers.

Morphology Description

It is a large shrub or a medium sized tree with pyramidal or conical crown 9-12 m. high and sometimes taller. The main stem is erect and often forking at a height of 1.5-1.8 m. The bark is smooth and grey. Leaves are lanceolate, in pairs, acute at both ends, gland-dotted and fragrant. Flower buds are borne in small clusters at the ends of branches, greenish, turning pink at the time of maturity and aromatic. Drupes (mother-of-clove) are fleshy, dark pink, 2.5 cm. long x 1.5 cm. thick. Seeds are oblong, soft, grooved on one side and 1.5 cm. long.

Principal Constituents

The cloves contain 13 % tannin (gallotannic acid). Oleanolic acid has been isolated from spent cloves (residue from the distillation of essential oil. Clove buds contains a higher percentage (97%) of eugenol. The clove bud oil contains free eugenol, eugenol acetate (2-17%) and caryophyllene (chiefly α and β-form) as its main constituents. Among the other constituents present, the most important is methyl-n-amyl ketone, to which the oil owes its fresh and fruity aroma. Other substances present in traces are methyl salicylate, methyl benzoate, methyl alcohol, benzyl alcohol, furfuryl alcohol, furfural, α-methyl furfural, dimethyl furfural, α-pinene, methyl-n-heptyl ketone and vanillin.

The oil, obtained by solvent extraction of cloves, contains little or no caryophyllene, but contains epoxydihydrocaryophyllene (Goldstein, 1953). The oil contains eugenone, eugenine, eugenitine and iso-eugenitol. The oil also contains sesquiterpenes like a-cubebene, β-caryophyllene, β-caryophyllene oxide besides eugenol. The leaves yield an essential oil (3%) on steam distillation. It contains a-cubebene, a-copaene, b-caryophyllene, eugenol, isoeugenol acetate, eugenol acetate and farnesol. Presence of ethyl acetate, ethanol, limonene, α & β-pinenes, p-cymene, 1,8-cineole, carvone, etc. are also reported in the clove leaf oil. The leaves also contain 3,4-dihydroxy phenethyl alcohol and 3,4-dihydroxy benzoic acid having anti-inflammatory action.

Pharmacology

The compounds of clove show significant activity as inducers of detoxifying enzyme-glutathione S-transferase in mouse liver and intestines. This ability is correlated with their activity in inhibiting the chemical carcinogenesis and as anticancer agents. The methanolic extract of clove exhibits anti-tumour activity. The methanolic extract showed remarkable induction of differentiation of myeloid leukaemia (MI) cells into macrophage like cells.

The aroma extracts and aroma components isolated from clove were found to inhibit malondialdehyde formation from blood plasma oxidised with Fenton's reagent (Lee and Shibamoto, 2001).

Dorman and Deans (2000) showed that volatile oil of clove possesses anti-bacterial activity against 25 different genera of bacteria. Antimicrobial and antioxidant properties of *Syzygium aromaticum* were also studied by Deans et al. (1995). Kim et al. (1998) investigated the effect of aqueous extract of *Syzygium aromaticum* flower bud on immediate hypersensitivity in rats and suggested that the inhibition was due to inhibition of histamine release from mast cells *in vivo* and *in vitro*. Srivastava (1993) showed that eugenol and acetyl eugenol, the two components of oil of cloves inhibited arachidonate-, adrenaline- and collagen-induced platelet aggregation.

Toxicology

There is no adverse effect reported on use of this herb as drug.

Clinical Studies

Clove forms an ingredient of pharmaceutical powder used in treatment of gastronomic disorders caused due to alcohol consumption. A mixture of 120mg cysteine, 300mg $NaHCO_3$, 0.2ml coptis rhizome tincture and 0.1ml clove tincture when administered orally before drinking is effective in treating hangover. Clove is also an ingredient in anti-inflammatory and analgesic preparation containing cinnamaldehyde and skin irritants. The preparation accelerates blood circulation and does not cause pain at the application site.

Indications

The oil is used in sustained release topical analgesic preparations and used in dentifrices for removal of stain in teeth, in root canal filling material, as salivary stimulator in sublingual pharmaceutical compositions. The oil is also used in buccal deodarant tapes and in dental bandages for protection to gingival tissue. The oil is used in topical formulations for use in cryotherapy for treating circulatory diseases such as posttraumatic edema. It may also be used in the treatment of AIDS patients. The eugenol and acetyl eugenol components of the oil inhibit arachidonate-adrenaline and collagen induced platelet aggregation. The oil should be used in restricted concentration because of phototoxic and photo-irritant activity. Clove oil is also an ingredient of hair and body shampoos. It has insulin-potentiating activity. The oil exhibits anti-implantation activity. The cloves are highly esteemed as a flavouring material and are extensively used, whole or in ground form, as a culinary spice. They impart a warming quality to food, and are used for flavouring ham, roasts, pickles, preserves, ketchups and sauces, as seasoning for sausages, dressing for poultry and meat, and in specialized spices for mincemeat and pastry. Because of their pungent and aromatic taste, cloves are favoured for making cakes, pies, puddings, cookies and candy.

They are used in making spiced wines and for scenting the chewing-tobacco; they form an ingredient of betel-chew.

Product Range

Abana (HeartCare), Geriforte (GeriCare / StressCare), Himcolin Gel, Koflet lozenge (CoughCare lozenge), Mentat (MindCare), Mentat syrup, Cough Syrup, Daily Health Capsules, ImmuneGuard Capsules & Syrup, Anxocare, Geriforte Vet, Regurin.

1.7.15 *TINOSPORA CORDIFOLIA*

Latin Name: *Tinospora cordifolia* (Willd.) Miers ex Hook. f. & Thoms. (Menispermaceae)
English Name: Tinospora
Sanskrit Names: Guduchi, Amrutha
Hindi Names: Giloya, Gurcha, Gulancha

Habitat

It grows throughout tropical India, ascending to an altitude of 300m.

Morphology Description

T.cordifolia is a large, glabrous, deciduous climbing shrub. The stems are rather succulent with long filiform fleshy aerial roots from the branches. The bark is grey-brown and warty; the leaves are membranous and cordate; the flowers are small, yellow or greenish yellow, in axillary and terminal racemes or racemose panicles; the male flowers are clustered and females are usually solitary; the drupes are ovoid, glossy, succulent, red and pea-sized; the seeds are curved.

Principal Constituents

It contains tinosporine, tinosporide, tinosporaside, cordifolide, cordifol, heptacosanol, clerodane furano diterpene, diterpenoid

furanolactone tinosporidine, columbin, and β-sitosterol (Gangan et al., 1994; Jahfar, 2003).

Pharmacology

The anti-stress or anxiolytic activity of *Tinospora cordifolia* is well documented by researchers and clinically tested. The ethyl acetate extract showed CNS depressant and hypoglycemic activity in rats. The aqueous extract showed anti-inflammatory activity in rats against acute and chronic type of inflammations induced by carrageenan. The anti-cancer (Stanely Mainzen Prince et. al., 1998), adaptogenic (Rege et. al., 1999) and immunopotentiating (Kapil and Sharma, 1997) of *Tinospora cordifolia* were also studied.

Tinospora cordifolia extract quenched superoxide radicals and hydroxyl radicals in *in vitro* assays. The extract also inhibited lipid peroxidation in isolated liver microsomal fractions. The antioxidant activity was further studied in cell cultures. When incubated with activated macrophages, the extract inhibited the production of superoxide generated by the oxidative burst of these immune cells. The antioxidant activity of the extract was also found to be useful in the amelioration of cyclophosphamide-induced toxicity (Mathew and Kuttan, 1997).

Stanely and Menon (2001) showed that *Tinospora cordifolia* root extract exhibits antioxidant action in alloxan diabetes.

Effect of *Tinospora cordifolia* extract on modulation of hepatoprotective and immunostimulatory functions in carbon tetrachloride (CCl_4) intoxicated mature rats were reported by Bishayi et al. (2002). Treatment with *T. cordifolia* extract (100 mg/kg body weight for 15 days) in CCl_4 intoxicated rats was found to protect the liver, as indicated by enzyme level in serum. A significant reduction in serum levels of SGOT, SGPT, ALP, bilirubin was observed following *T. cordifolia* treatment during CCl_4 intoxication. Treatment with *T. cordifolia* extract also deleted the immunosuppressive effect of CCl_4. The results of the experiment suggested that treatment by *T. cordifolia* extract might be the critical remedy for the adverse effect of CCl_4 in liver function as well as immune functions.

The antioxidant activity of an arabinogalactan polysaccharide (TSP) isolated from *Tinospora cordifolia* was studied by Subramanian et al. (2002). The polysaccharide showed good protection against iron-mediated lipid peroxidation of rat brain homogenate as revealed by the thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxide (LOOH) assays. TSP also provided significant protection to protein against gamma ray induced damage. The protective action can possibly be explained by its very high reactivity towards DPPH, superoxide radicals and the most damaging of radicals, the hydroxyl radical.

Aqueous extract of *T. cordifolia* inhibited Fenton ($FeSO_4$) reaction and radiation mediated 2-deoxyribose degradation in a dose dependent fashion with an IC_{50} value of 700 µg/ml for both Fenton and radiation mediated 2-DR degradation. The results revealed that the direct and indirect antioxidant actions of *T. cordifolia* probably act in corroboration to manifest the overall radioprotective effects (Goel et al. 2002).

These experiments suggest that its ability to scavenge free radicals and to block free radicals and to inhibit radical-induced membrane damage may be the underlying feature of many of the biological activities of this herb.

Indications

The stem is used in general debility, dyspepsia, fevers and urinary diseases. The bitter principles present in the drug show antiperiodic, antispasmodic, anti-inflammatory and antipyretic properties. The plant is used in ayurvedic rasayanas to improve the immune system and the body's resistance against infections. It is used as an immunomodulator in immunosuppression of obstructive jaundice, hepatic fibrosis, peritonitis and sepsis. The plant has been found effective in preventing fibrous changes and promotes regeneration of the liver against CCl_4 induced hepato toxicity. Other indications include nephritis, gastritis, peptic ulcer, dyspepsia, arthritis, bronchitis, gout, food poisoning, angina pectoris, diabetes, psoriasis, eczema and dermatitis.

Product Range

Abana (HeartCare), Bonnisan, Diabecon (GlucoCare), Diakof (CoughCare Sfree), Diarex (DiarCare), Evecare (MenstriCare), Geriforte (GeriCare / StressCare), Koflet (CoughCare), Mentat (MindCare), Mentat syrup, Purim (HemoCare), Rumalaya (JointCare), Rumalaya forte, Septilin (ImmunoCare), Septilin syrup, Antacid Suspension, Anti-Stress Massage Oil, Blood Purifier Capsules and Syrup, ImmuneGuard Capsules and Syrup, Anxocare, Appetonic Vet, Diarex Vet, Digyton, Geriforte Vet, Himpyrin Vet, Immunol, Rumalaya Vet, Guduchi, Chyavanaprasha.

1.7.16 *TRIBULUS TERRESTRIS*

Latin Name: *Tribulus terrestris* Linn., Bulgaricum (Zygophyllaceae)
English Names: Small Caltrops, Land Caltrops, Puncture Vine
Sanskrit Names: Gokshura, Trikanta, Shvadamshtra
Hindi Name: Gokhru

Habitat

It is commonly found throughout India, upto an altitude of 5400 m.

Morphology Description

T.terrestris is a variable, prostrate annual. The roots are slender, cylindrical, light brown and faintly aromatic. The leaves are paripinnate; the leaflets are 5-8 pairs, subequal, oblong to linear-oblong. The flowers are leaf-opposed, solitary, pale-yellow to yellow; the fruits are globose, consisting of 5-12 woody cocci, each with 2 pairs of hard, sharp, divaricate spines, one pair longer than the other. Several seeds are seen in each coccus with transverse partitions between them.

Principal Constituents

It contains harmine, diosgenin, tribulusamine A1 and B2 (two lignanamides) as the major constituents. The active ingredient in *Tribulus terrestris* is called protodioscin, and is extracted specifically from the fruit

of the herb. The fruit itself contains several compounds, such as resins, alkaloids, natural oils and tannins (Gheorghiu and Ionescu-Matiu, 1968; Tomowa et al., 1977).

Pharmacology

Tribulus is Nature's master hormone regulator (a natural plant source of testosterone) and has been used for centuries to treat a wide variety of health problems including loss of libido (sex drive), impotence, infertility, edema, liver, kidney and heart problems.

Research has shown that tribulus, taken by men and women, dramatically and safely increases sex drive, increases estrogen and testosterone by 10% and 30% without side effects dramatically speeds muscle and energy recovery in athletes and in women relieves upto 99% of menopausal symptoms. The mechanism by which *T.terrestris* increases testosterone levels is believed to be through the stimulation of the secretion of leutinizing hormone. It has also been shown to reduce cholesterol and high blood pressure in clinical trials.

A study conducted in Japan in 1998 demonstrated that tribulusamine A1 and B2 acted as liver protectants and prevented apoptosis in certain instances (Li et al., 1998).

Wang et al. (1990) showed that *T.terrestris* is one of the ideal medicines to treat angina pectoris. It is shown that saponin of *T.terrestris* has the action of dilating coronary artery and improving coronary circulation, and thus has better effects on improving ECG of myocardial ischemia.

Indications

Tribulus terrestris is an herb that has been receiving greater acclaim in recent years as a libido enhancer and bodybuilding supplement. It is well known diuretic plant drug useful in urolithiasis, dysurea, impotence and kidney dysfunction. It is also used as a mood-enhancer, antiseptic, and anti-inflammatory. It is used for a variety of liver, kidney, and cardiovascular diseases.

Product Range

Bonnisan, Confido (Speman forte), Diabecon (GlucoCare), Geriforte (GeriCare / StressCare), Himplasia, Renalka, Rumalaya (JointCare), Rumalaya forte, Speman (ProstaCare), Tentex Royal, Digyton, Geriforte Vet, Nefrotec Vet, Rumalaya Vet, Speman forte Vet, Speman Vet, Gokshura, Chyavanaprasha.

1.7.17 *WITHANIA SOMNIFERA*

Latin Name: *Withania somnifera* Dunal (Solanaceae)
English Name: Winter Cherry, Indian Ginseng
Sanskrit Names: Ashvagandha, Hayahvaya, Vajigandha
Hindi Name: Asgandh

Morphology Description

W.somnifera is an erect, evergreen, tomentose shrub. The roots are stout, fleshy and whitish brown; the leaves are simple ovate, glabrous; the flowers are inconspicuous, greenish or lurid-yellow, in axillary, umbellate cymes; the berries are globose, orange-red when mature, enclosed in the persistent calyx and have yellow, reniform seeds.

Principal Constituents

Biochemically heterogeneous alkaloids including cuscohygrine, anahygrine, tropine, pseudotropine, anaferine are present. The plant has steroidal lactones-withanolides, withaferin, which are estrogenic compounds.

Pharmacology

Ashwagandha roots are known to possess restorative and adaptogenic properties. The free radical scavenging activity of *W.somnifera* and its protective effect on H_2O_2-induced cytotoxicity and DNA damage was studied by Russo et al. (2001). Administration of *W.somnifera* was found to reduce two-stage skin carcinogenesis induced by DMBA and croton oil. Studies indicated that it could reduce the papilloma induced

alterations to the antioxidant defence systems (Davis and Kuttan, 2001). The antioxidant activity of *W.somnifera* glycowithanolides was also assessed in chronic footshock stress induced changes in rat brain frontal cortex and striatum, lending support to the clinical use of the plant as an anti-stress adaptogen (Bhattacharya et al., 2001). Studies have also suggested that the ameliorating role of root extract of ashwagandha in the lead intoxicated mice could be the result of its antiperoxidative action (Chaurasia et al., 2000). Studies also showed that ashwagandha possesses adaptogenic, cardiotropic, cardioprotective and anticoagulant properties (Dhuley, 2000). Oral administration of ashwagandha prevented the rise in lipid peroxidation and showed better stress tolerance in stress-induced animals (Dhuley, 1998).

Indications

Ashwagandha is used in asthma and as a uterine sedative. The total alkaloids showed relaxant and antispasmodic effects against several spasmogens on intestinal, uterine, bronchial, tracheal and blood-vascular muscles. Studies indicate ashwagandha possesses anti-inflammatory, anti-tumor, anti-stress, antioxidant, immunomodulatory, hemopoietic and rejuvenating properties. It also appears to exert a positive influence on the endocrine, cardiopulmonary and central nervous systems (Mishra et al., 2000).

Product Range

Abana (HeartCare), Geriforte (GeriCare/StressCare), Mentat (MindCare), Mentat syrup, Reosto, Tentex forte (VigorCare for Men), Anti-Stress Massage Oil, Anxocare, Galactin Vet, Geriforte Vet, Immunol, Speman forte Vet, Tentex forte Vet, Ashvagandha.

1.7.18 BHUNIMBADI KWATH

Kwath are the preparations containing drugs or combination of drugs made to coarse powder and kept for preparation of Kasaya. Bhunimbadi kwath contains nimba (Azadirachta indica), visva, amrita (Tinospora cordifolia), daruharidra (Berberis aristata), sati (Curcuma

zerumbet), bhunimba, panksar, pippali (Piper longum), gajapippali (Scindapsus officinalis) and brhati.

The Ayurvedic formulary mentions the use of Bhunimbadi kwath in acid peptic disorders (Anonymous, 1978). It is also used in typhoid fever and for blood impurity.

1.7.19 KAPARDI BHASMA

It is a very fine powder obtained after incineration of covrie shells. It is known for its antacid property and used in acid peptic disorders (Anonymous, 1978). It is also used in earache, wound healing and intestinal colic

1.7.20 MOUKTIKA BHASMA

Sanskrit/Indian Names: Mouktika bhasma/Muktashukti/ Mouktika Sukti

English Name: Pearl Oyster Shell Calx

It is the ash obtained by calcinating the shells of Pinctada margaritifera. It is antacid, tonic and used in acid peptic disorders (Anonymous, 1978).

It is used in Himcocid, ImmuneGuard Capsules & Syrup.

1.7.21 PRAVAL BHASMA

Sanskrit / Indian Name: Praval bhasma

English Name: Coral Calx

It is a very fine powder obtained after incineration of corals. It is a rich, natural source of calcium, and due to appropriate ayurvedic processing, has the advantage of easy absorption in the intestine.

It is used in Diabecon (GlucoCare), Lukol.

1.7.22 SHANKH BHASMA

Sanskrit Names: Shankha / Shankh bhasma

English Names: Conch Shell Calx, Conch Shell Ash

It is the calcinated conch shell of *Turbinella pyrum* (Gastropoda, Class: Molusca). It mainly consists of calcium, iron and magnesium It is well known for its antacid and digestive properties. It is useful in hyperchlorhydria, ulcers, dysentry, dyspepsia, indigestion, jaundice, sprue, colic and hepato- splenomegaly.

Pandit et al. (2000) studied the anti-ulcer activity of Shankh bhasma in rats and found that it offered significant protection in indomethacin and cold restraint stress induced gastric ulcers. The study also exhibited a potent anti-peroxidative effect, suggesting that Shankh bhasma may act as gastric cytoprotective agent by modulating scavenging of free radicals.

Used in Abana (HeartCare), Diarex (DiarCare), Gasex (GastriCare), Rumalaya (JointCare), Septilin (ImmunoCare), Antacid Suspension, Diarex Vet, Rumalaya Vet.

1.7.23 SHRING BHASMA

It is a very fine powder obtained after incineration of horns of the deer and contains calcium and phosphate. Shringa bhasma is used for disorders of respiratory tract.

1.7.24 SHUDDHA GAIRIKA BHASMA

Sanskrit / Indian Name: Shuddha Gairika bhasma

English Name: Hematite Calx

It is an astringent, hematinic, which is useful for diarrhea.

It is used in Diarex Vet.

1.7.25 SUTASHEKHAR RAS

It is a herbomineral ayurvedic preparation. It consists of 17 ingredients, such as mercury, sulphur, swarna bhasma, tamra (copper) bhasma, ginger, black pepper, pippal, *Cassia cinnamon*, *Mesua ferrea*, *Cinnamomum tamala*, *Elettaria cardamomum*, datura seeds, shankh bhasma, all in equal proportions macerated in juice of *Eclipta alba* for 12 hours, with intermittent mixing. It is then prepared in the form of tablets. Clinical trial of Sutashekhar ras has proved its efficacy in the management of duodenal ulcers (Dash et al., 1987).

1.7.26 SWARNABHASMA

It is a very fine powder obtained after incineration of gold.

From ancient times, Swarnabhasma (gold ash) has been used in several clinical manifestations including loss of memory, defective eyesight, infertility, overall body weakness and incidence of early aging. Swarnabhasma has been used by Ayurvedic physicians to treat different diseases like bronchial asthma, rheumatoid arthritis, diabetes mellitus, nervous disorders, etc. Since 8th century AD, gold was used in the form of bhasma (ash) after proper purification and incineration as described in the Ayurvedic pharmacopoiea. The quantitative analysis of Swarnabhasma showed that the substance contained a significant amount of gold (20.34%) alongwith iron (39.09%) and some other elements like arsenic (0.17%), barium (0.33%), calcium (1.96%), magnesium (2.08%), strontium (1.28%), aluminium (8.60%), copper (0.18%), lead (0.03%), zinc (74 ppm), cobalt (70 ppm) and nickel (75 ppm).

According to the modern concept, the scientific basis for its application in degenerative diseases, atleast in part, may arise through enhancement in free radical concentrations. It was found to induce enhanced activity of SOD and catalase.

Swarnabhasma was found to produce no signs of toxicity indicating that the drug can be used safely in oral route for atleast a few months (Mitra et al., 2002).

Gold, widely used in modern medicine for the treatment of rheumatoid arthritis, is highly valued for various medicinal uses in Indian

systems of medicine. Traditional gold preparations are attributed with tonic/rejuvenating and antioxidant properties. Our earlier studies revealed interesting analgesic, immunostimulant, adaptogenic and glycogen sparing properties in these preparations. Shah and Vohora (2002) studied the antioxidant/restorative effects of calcined gold preparations used in Indian systems of medicine against global and focal models of ischaemia. Enzymatic parameters (lipid peroxidase, reduced glutathione, catalase, glutathione reductase, glutathione-S-transferase, glutatione peroxidase, superoxide dismutase, and glucose-6-phosphate dehydrogenase) were employed to assess ischaemic brain damage and its modulation. Significant restoration of altered values to near normal levels by Ayurvedic Swarna bhasma (25 mg/kg, orally for 10 days) suggest potentials for gold preparations in cerebrovascular diseases. The preparations deserve more scientific attention for possible therapeutic exploitation.