Literature review
2. REVIEW OF THE LITERATURE

Study on natural products is always an interesting target for scientists over the decades, especially on plants. Historically, plants (fruits, vegetables, medicinal herbs, etc.) have provided a good source of a wide variety of compounds, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other secondary metabolites, which are rich in valuable bioactivities, e.g., antioxidant, anti-inflammatory, antitumour, antimutagenic, antibacterial, or antiviral activities. In many oriental countries (China, Japan, India etc), the traditional herbal medicines are being widely used for thousands of years. Herbal plants have become the main object of chemists, biochemists, and pharmacists. Their research plays an important role for discovering and developing new drugs, which are having hopefully more effectiveness and no side effects as compared to most of the modern drugs. Besides focusing on chemistry of compounds from any plants, the studies of herbal plants are based on folkloric reputation and traditional uses. In addition, the isolation and identification on these plants are due to the activities of their extracts and fractions. There is a large amount of plentiful sources for pharmacists developing herbal drugs, only about 35,000 on over 2,50,000 plant species identified. Particularly, tropical rainforests are a vital source of medicines. Only about 1% plants from tropical forest in the world have been subjected for pharmaceutical tests. (Kong et. al., 2003)

This shows clearly that the studies on herbal plants are to be focussed to meet the demand of natural products. From natural products, a number of herbal drugs have been developed into many forms of food supplements, nutraceuticals, and complementary and alternative medicine. For instance, ‘Paclitaxel’ (Taxol®) is an important drug used in the treatment of cancer. In a study of cancer program, over 30,000 plants collected to test for anticancer properties. It has been observed that the bark extract from the Pacific yew tree (Taxus brevifolia) had possessed antitumour properties. Soon after, the active compound was isolated and named as ‘Paclitaxel’, which is having an extremely complex sturcture. It took nearly 30 years for investigating the active ingredient to develope into drugs and bringing it to trade market. Now, Paclitaxel has become an effective tool of doctors for
treating patients with lung, ovarian, breast cancer. Similarly, ‘Mefloquine’ (Lariam®), an alkaloid, was developed in a program to find an antimalarial property (Kayser et al.). From *Cephaelis ipecacuanha* and related species, the isoquinoline alkaloid emetine obtained has been used for many years for the treatment of abscesses due to the spread of *Escherichia histolytica* infections. Therefore, studies an herbal plant profoundly not only to discover active compounds but also to find the effective mechanism of action to develop into drugs for the treatment of diseases. Furthermore, the studies also provided general constituents and effects that can encourage the use of herbal plants as “food” for intensifying health and prevent diseases.

In India, Karnataka state is endowed with rich natural resources especially along the Western Ghats ranges. The Western Ghats range arises abruptly in the west from the Arabian Sea coast and descends gradually towards the dry Deccan plains in the east. The total area of the Western Ghats is estimated to be about 20,000 sq. kms. The good climatic conditions and altitudinal gradients have resulted in the development of a variety of forests from evergreen to semi evergreen, moist deciduous to dry deciduous and scrub jungles. It is one of the richest biodiversity centers and is considered as one among the eighteenth hot spot of the world. This region comprises about 4000 species of angiosperms of which 2,280 species are endemic to this region (Pascal et al., 1982).

The plant selected for the study of *Echinops echinatus* Roxb roots belongs to family Compositae, commonly known as Brahmadandi, is a pubescent annual herb of 1-3 ft height with branches widely spreading from the base. The species is found practically throughout India, Pakistan, Afghanistan, etc, (Anonymous, 1952). This plant is being used traditionally for large number of diseases, disorders and also in many Ayurvedic and siddha formulations. The morphological characteristics of the plant have been given in the next chapter of ‘Materials and Methods’.

### 2.1 Traditional uses of *Echinops echinatus* Roxb

The Plant is bitter, it increases the appetite and stimulates liver; used in diseases of the brain, pains in the joints, inflammations, etc. Roots and root bark of the plant are
used in various indigenous systems of medicine for treating different ailments. The root is used as abortifacient and aphrodisiac (Kirtikar K.R. 1975), infusion of the root is given in seminal debility, impotence, hysteria, cancer and its decoction is given in dyspepsia, scrofula, syphilis and fevers (Nadkarni A.K., 2000). According to an ethnomedicinal survey carried out by Kakrani et al., (2005) the rural population of Kutch region in Gujarat state, India, uses the suspension of root bark powder in milk (100g/250ml) for the treatment of diabetes. The traditional healers of Chhattisgarh in India use this herb in different ways both internally and externally for the treatment of sexual disorders. An aqueous paste of the root is applied in the lower abdominal region to hasten the process of delivery; also, the patients are advised to take the paste internally for quick and safe delivery. In case of patients having poor sexual vitality, aqueous paste of the root bark powder is applied externally on the male genitals one hour before intercourse; pure honey can be used in place of water for better results. A paste prepared by mixing the root bark powder with the juice of Datura stramonium and Blumea lacera leaves is used to avoid premature ejaculation. The patients suffering from respiratory troubles, particularly asthma, are advised to inhale the fumes obtained by burning the leaves and roots of E. echinatus Roxb in order to get quick and permanent relief (Somashekar et al., 2007).

The present investigation is emphasizing on the investigation of phytochemical constituents and evaluation of hepatoprotective, wound healing, anti-inflammatory, antioxidant, antimutagenic and anti-tumour properties of E. echinatus Roxb. roots.

2.2 Medicinal Properties of Genus Echinops:

The genus Echinops belongs to the family Compositae and comprises over 120 species and they represent untouched natural resources for man to exploit. Traditionally, many species of this genus have been exploited for various ailments from decades. They are widely used in the herbal medicine for the treatment of various diseases and illnesses such as, migraine, diarrhea, heart pain, different forms of infections, intestinal worm infestation and hemorrhoid.

The phytochemical investigation on the root of E. grijissi demonstrated the presence of thiophenes (Lu et al., 1989; Guo et al., 1992; Koike et al., 1999; Lin et al.,
1999; Liu et al., 2002) and essential oil (Guo et al., 1994). Bioactive flavonoids, acylflavone-glycoside, echinacin, echinaticin and tamarixetin have been isolated from the *Echinops niveus* by Singh and Pandey (1994). The essential oil, exclusively tricyclic sesquiterpenes, *viz.* silphiperfol-6-ene, presilphiperfol-7(8)-ene, silphiperfolan-6-ol, were isolated from the roots of *Echinops giganteus* by Menut et al., (1997). A new neolignan glycoside, 1-(3-methoxy-4-hydroxyphenyl)-3-hydroxy-2-(2-O-beta-D-glucopyranosyl-3-methoxy-5-((1E)-3-hydroxypropenyl)phenyl) propanone has been isolated from the root of *Echinops grijissii* (Koike et al., 2002).

Hymete et al., (2005) studied the hydroalcoholic extracts of the root, flower head, leaf and stem of *E. ellenbeckii* and *E. longisetus* were investigated for their chemical constituents and biological activities. The presence of alkaloids, saponins, phytosterols, polyphenols and carotenoids in the different parts of the plants was observed. The leaf extracts of both plants and stem extract of *E. longisetus* showed strong inhibitory activity against cultures of *Staphylococcus aureus*. None of the extracts were found to be active against Gram-positive organisms. The flower extract of *E. ellenbeckii* showed strong inhibitory activity against *Candida albicans*. Root and flower extracts of the plants investigated showed lethal activity against earthworms. Moreover, the extracts of the roots of both plants showed molluscicidal activity against schistosome-transmitting snail hosts. The biological activities observed were dose dependent.

Nikolas et al., (2006) found the dichloromethane extract of the radix of *Echinops ritro* (Asteraceae) was the most biologically potent. Bioassay-guided fractionation of this extract led to the isolation of eight thiophenes. Antifungal activities of isolated compounds together with a previously isolated thiophene from *Echinops transiliensis* were first evaluated by bioautography and subsequently evaluated in greater detail using a broth microdilution assay against plant pathogens such as, *Colletotrichum acutatum*, *Colletotrichum fragariae*, *Colletotrichum gloeosporioides*, *Botrytis cinerea*, *Fusarium oxysporum*, *Phomopsis viticola*, and *Phomopsis obscurans*. 
2.3 Medicinal properties of *Echinops echinatus* Roxb.:

The plant has been reported for many biological activities like anti-inflammatory (Singh *et al.*, 1989), hypoglycemic and diuretic (Abraham *et al.*, 1986), antibacterial and antifungal (Savitasharma and Metha, 1989), antispasmodic (Bhakuni *et al.*, 1969), antifertility (Padashetty *et al.*, 2007) etc. However, very little less has been reported on the chemical composition of the roots (Sharma *et al.*, 1988). A recent study showed the presence of different flavonoids in the whole plant (Singh *et al.*, 2006). Shukla (2003) reported the presence of various classes of bioactive pharmacologically potent compounds *viz.* alkaloids, terpenoids, flavonoids, lipids, polyacetylenes and steroids in *Echinops echinatus*.

Singh *et al.*, (2006) have also reported the isolation of a new isoflavone glycoside, echinoside, together with 7-hydroxyisoflavone, kaempferol-4′-methylene, kaempferol-7-methyl ether, myrecetin-3-O-α-l-rhamnoside, kaempferol and kaempferol-3-O-α-l-rhamnoside, from the whole plant of *Echinops echinatus*. The structure of echinoside has been established by chemical and spectral data. This is the first report of the occurrence of these flavonoids in *E. echinatus*.

The bioactive principle constituents present in the flowers of *E. echinatus* have been identified as n-hentriacontane, n-hentriacontanol, lupeol, lupeol acetate, β-amyrin, β-amyrin acetate, β-sitosterol, palmitic acid, betulinic acid, betulin, apigenin, luteolin, quercetin, apigenin-7-O-glucoside, luteolin-7-O-glucoside, apigenin-7-O-β-D-(4″-p-coumaroyl)-glucoside and echinopsine (Chaudhuri, 1988).

The methodology and the various parameters examined in the present study to evaluate the different pharmacological activities were based on the previous research works. In the light of earlier literature, the review in the subsequent section will emphasize on the available reports of physiological, biochemical and pathological aspects of the systems under study.
Based on the traditional claims the following pharmacological parameters have been selected and investigations were conducted to evaluate the therapeutic potency of roots of *Echinops echinatus*. Roxb.

1. Antioxidant activity
2. Hepatoprotective protective activity
3. Wound healing activity
4. Anti-inflammatory activity
5. Anti-mutagenic activity and
6. Anti-tumour activity

A. Antioxidant activity

i. Oxidation:

Oxidation involves the transfer of electrons from one atom to another. The oxidized molecule loses an electron while the receiving molecule is reduced. Oxidation reactions are an essential part of aerobic metabolism, since oxygen is an electron acceptor in the electron flow system that produces energy (Lee et al., 2003). Oxidation becomes a problem when reactions become uncoupled and free radicals are formed.

Free radicals are molecules that are highly reactive and unstable because they contain an unpaired electron. Electrons are most stable in pairs, hence the free radicals tend to attach to or receive hydrogen ions from molecules with lower bond dissociation energy like unsaturated fatty acids or phenolic antioxidants.

Biological systems interact with external environment to maintain an internal environment that favours survival, growth and reproduction. or all but the most fastidious anaerobes, oxygen is vital. However, the paradox of aerobic life is that oxidative damage occurs to key biological sites, threatening their structure and function. Oxygenic threat is met by an array of antioxidants that evolved in parallel with our oxygenic atmosphere. The human body uses various antioxidants, largely on some of the plant based dietary antioxidants, some of which are dietary derived, for defense. The plant based dietary antioxidants are believed to have an important role in the maintenance of human health
and the search of drugs of plant origin with antioxidant activity has become a central focus.

**ii. Antioxidants:**

Free radicals are electrically charged molecules, which seek out and capture electrons from other substances to finally neutralize themselves. Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, resulting in a chain reaction. Until subsequent free radicals are deactivated, thousands of free radical reactions may occur within only a few seconds. The term 'antioxidant' refers to any molecule capable of stabilizing or deactivating free radicals before they attack cells. These are in particular the primary antioxidants. There are also molecules deserving the 'antioxidant' term, because they act as chelating agents binding metal ions (redox activity). To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly complex antioxidant protection system. It actually involves a variety of components, endogenous and exogenous, that function interactively and synergistically to neutralize free radicals. These include antioxidant enzymes that catalyse free radical quenching reactions, metal binding proteins that sequester free iron and copper ions that are capable of catalyzing oxidative reactions, and diet-derived antioxidants like ascorbic acid, Vitamin E, carotenoids, polyphenols and other lowmolecular weight compounds such as lipoic acid. Dietary compounds that do not neutralize free radicals, however, enhance endogenous antioxidant activity also belong to antioxidants. Endogenous antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being. However, when under exposure to alcohol, medications, trauma, cold, infections, poor diet, toxins, radiation or strenuous physical activity, the endogenous antioxidant defense is not adequate to counteract the oxidative stress; protection against it is dependent upon the adequacy of antioxidants that are derived from the diet (Kaliora et al., 2005).

**iii. Free radicals and their role in diseases:**

Free radicals are natural by-products of our own metabolism. These are electrically charged molecules that attack our cells, tearing through cellular membranes to
react and create havoc with the nucleic acids, proteins, and enzymes present in the body. These attacks by free radicals, collectively known as oxidative stress, are capable of causing cells to lose their structure, function and can eventually destroy them. They are continuously produced by our body’s use of oxygen such as in respiration and some cell-mediated immune functions. They are also generated through environmental pollutants, cigarette smoke, automobile exhaust, radiation, air-pollution, pesticides, etc. (Li and Trush, 1994). Normally there is a balance between the amount of free radicals generated in the body and the antioxidant defense systems that scavenge/quench these free radicals preventing them from causing deleterious effects in the body (Nose, 2000). The antioxidant defense systems in the body can only protect the body when the amount of the free radicals is within the normal physiological level. But when this balance is shifted towards more of free radicals, increasing their burden in the body either due to environmental condition or produced within the body, it leads to oxidative stress, which may result in tissue injury and subsequent diseases (Finkel and Holbrook, 2000). Since free radicals play such an important role in the disease scenario of an individual, a thorough understanding of the various physiologically significant free radicals is of paramount importance before the search of the radical scavengers or the antioxidant principles to treat the physiological disorders caused by them. Free radicals may be designated as molecular sharks that damage molecules in cell membranes, mitochondria (the cell’s energy plants), DNA (the cell’s intelligence) and are very unstable, tend to rob electrons from the molecules in the immediate surrounding in order to replace their own losses. Reactive oxygen species (ROS) is a collective term, which includes not only the oxygen radicals (O2−, and •OH) but also some non-radical derivatives of oxygen. These include hydrogen peroxide (H2O2), hypochlorous acid (HOCl) and ozone (O3) (Bandhopadhyay et al., 1999). Over about 100 disorders like rheumatoid arthritis, hemorrhagic shock, cardiovascular disorders, cystic fibrosis, metabolic disorders, neurodegenerative diseases, gastrointestinal ulcerogenesis and AIDS have been reported as ROS mediated. Some specific examples of ROS mediated diseases include Alzheimers disease, Parkinson’s disease, Atherosclerosis, Cancer, Down’s syndrome and ischemic reperfusion injury in different tissues including heart, liver, brain, kidney and gastrointestinal tract.
iv. Free Radicals and Reactive Oxygen Species

Each atom is made of a nucleus consisting protons and neutrons and electrons, which revolve round the core. Since some electrons are not paired, that creates a lack in the molecule which will try to fill it by stealing an electron from another molecule. A free radical in contrast, is a molecule, or a molecular fragment that contains one or more unpaired electrons in its outer orbital, and is capable of independent existence, which is conventionally represented by \([R^*]\). Examples of free radicals are trichloromethyl \((\text{CCl}_3^*)\), superoxide \((\text{O}_2^*)\), hydroxyl \((\text{OH}^*)\), peroxyl \((\text{ROO}^*)\) and nitric oxide \((\text{NO}^*)\). Examples of some non-radical derivatives of oxygen molecules are hydrogen peroxide \((\text{H}_2\text{O}_2)\) and hypochlorous acid \((\text{HOCl})\) (Halliwell and Gutteridge, 1999). Ironically, these reactive species are derived from normal physiological and metabolic processes that are essential to the cell. All of these reactive oxygen species participate in the chain reaction of free radicals.

v. Reactive Oxygen Species (ROS)

Oxygen plays a vital role in diverse biological phenomena. It is essential for life but it can also provoke damaging oxidative events within cells. It is established that free radicals and other reactive species are continuously produced in human body. The term reactive oxygen species is a collective one that includes not only oxygen central radicals such as superoxide radicals, singlet oxygen and hydroxyl radicals, but also, some potentially dangerous non-radicals such as hydrogen peroxide and hypochlorous acid (Southorn and Powis, 1998).

ROS are unstable intermediates derived from molecular oxygen \(\left(\text{^1O}_2\right)\), a biradical. In the case of molecular oxygen, the triplet (biradical) state is more stable than the singlet oxygen \(\left(\text{^3O}_2\right)\). Singlet oxygen can be produced by several biochemical oxidations involving peroxidases and lipoxygenase, by reaction between various ROS or in the presence of light, oxygen and a photosensitizer such as porphyrins, as in the case of erythropoietic porphyria (Kanofsky, 1989, Halliwell and Gutteridge, 1990; Mathews-Roth, 2000).
Light + Photosensitizer

\[
3\text{O}_2 \xrightarrow{\text{Light + Photosensitizer}} \text{O}_2
\]

\[
\text{H}_2\text{O}_2 + \text{ONOO}^- \rightarrow \text{O}_2 + \text{NO}_2^- + \text{H}_2\text{O}
\]

\[
\text{H}_2\text{O}_2 + \text{ONOO}^- \rightarrow \text{O}_2 + \text{Cl}^- + \text{H}_2\text{O}
\]

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

Other ROS is derived from the secondary reduction of \(^3\text{O}_2\) in the inner membrane of mitochondria. Where oxygen receives one electron in the place of a metalloprotein of the respiratory chain (Liu, 1997; Raha and Rabinson, 2000). The superoxide radical anion thus formed (\(\text{O}_2^-\)) can dismutate into oxygen (\(^3\text{O}_2\)) and hydrogen peroxide (\(\text{H}_2\text{O}_2\)), either spontaneously at acidic pH or in a reaction catalyzed by superoxide dismutase (SOD). Superoxide and hydrogen peroxide can react with transition metals such as iron or copper to from the oxidant hydroxyl radical (Winterbourn, 1995; Buettner and Jurkiewicz, 1996).

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \xrightarrow{\text{Fe(II)/Cu (II)}} \text{Fe}^{3+} + \text{OH}^- + \text{OH}^-
\]

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

Superoxide and hydrogen peroxide are also enzymatically formed by NADPH oxidase in activated neutrophil and macrophages during inflammatory processes (Babior et al., 2002; Segal and Abo, 1993).

\[
\text{NADPH} + 2\text{O}_2 \rightarrow \text{NADP}^+ + \text{H}^+ + \text{O}_2^-.
\]

Myeloperoxidase, an enzyme present in the neutrophils, but not in macrophages, can produce hypochlorous acid (HOCl) by oxidizing chloride ions with hydrogen peroxide (Winterbourn, 1995), hypochlorous acid (or its conjugated base hypochlorite, depending of the pH), is a powerful oxidant towards various biomolecules, especially amino groups; furthermore, in acidic pH it readily decomposes to liberate the highly toxic chlorine gas \(\text{Cl}_2\) (Kelbanoff, 1999).

\[
\text{H}_2\text{O}_2 + \text{Cl}^- \xrightarrow{\text{Myeloperoxidase}} \text{HOCl} + \text{OH}^-
\]

\[
\text{HOCl} + \text{H}^+ + \text{Cl}^- \rightarrow \text{H}_2\text{O} + \text{Cl}_2
\]
vi. Reactive Nitrogen Species (RNS)

Nitric oxide ($\text{NO}^\cdot$) is an abundant reactive radical that acts as an important oxidative biological signaling molecule in a large variety of diverse physiological processes, including neurotransmission, blood pressure regulation, defence mechanisms, smooth muscle relaxation and immune regulation (Alderton et al., 2001; Bergendi et al., 1999). $\text{O}^\cdot$ is generated in biological tissues by specific nitric oxide synthases (NOSs), which metabolises arginine to citrulline with the formation of $\text{NO}^\cdot$ via a five-electron oxidative reaction. Due to its extraordinary properties, in 1992 $\text{NO}^\cdot$ was acclaimed as the “molecule of the year” in Science Magazine. Overproduction of reactive nitrogen species is called nitrosative stress. This may occur when the generation of reactive nitrogen species in a system exceeds the system’s ability to neutralise and eliminate them. Nitrosative stress may lead to nitrosylation reactions that can alter the structure of proteins and so inhibit their normal function.

Cells of the immune system produce both the superoxide anion and nitric oxide during the oxidative burst triggered during inflammatory processes. Under these conditions, nitric oxide and the superoxide anion may react together to produce significant amounts of a much more oxidatively active molecule, peroxynitrite anion ($\text{ONOO}^-$), which is an oxidising free radical that can cause DNA fragmentation and lipid oxidation.

\[
\text{NO}^\cdot + \text{O}_2^\cdot - \rightarrow \text{ONOO}^-
\]

vii. Oxidative Stress

All living organisms require molecular oxygen as an electron acceptor for efficient production of energy. The cellular metabolism of oxygen in human beings continuously produces small amounts of reactive oxygen species. Under normal circumstances, the major source of reactive oxygen species produced in the body occurs from leakage of electrons from the mitochondrial and microsomal electron transport chains. All aerobically respiring cells reduce molecular oxygen to create superoxide
radical (Sardesai, 1995). Electrons normally pass down a non-radical generating, cytochrome- catalyzed reduction of oxygen to water during oxidative phosphorylation (i.e., adenosine triphosphate, ATP, production). About 1-3% of the oxygen we consume generates superoxide (Halliwell, 1994). Normally, a balance between oxidative events and antioxidative force maintains status quo within the cells. A variety of enzymes help to maintain cells in a reduced state despite the presence of aerobic environment. Thus, major cellular reducing agents such as, ascorbate, glutathione and tocopherol are present predominantly in the reduced forms. In addition, a number of enzymes such as, superoxide dismutase, catalase and glutathione peroxidase are present in cytosol, mitochondria and peroxysomes, and remove these reactive chemical species (Slater, 1984).

Large increase in the amount of oxygen species leads to oxidative stress, which is defined as a 'disturbance in the pro oxidant – antioxidant balance in favour of the pro oxidants leading to potential damage'. Reactive oxygen species are capable of chemically altering virtually all major classes of biomolecules (lipids, proteins, nucleic acids) with concomitant changes in structure and function. Oxidative stress is involved in the generation or worsening of more than a hundred pathogenic conditions (Siess, 1991).

viii. Reactive Oxygen Species and Biochemical Consequences

Oxygen radical is capable of reversibly or irreversibly damaging compounds of all biochemical classes, including nucleic acids, proteins, lipids, carbohydrates and connective tissue macromolecules.

ix. DNA Damage

DNA is readily attacked by oxidizing radicals if they are formed in its vicinity. Free radical damage of DNA of living organisms may be due to breakage of the main strand, degradation of bases, or cleavage of hydrogen bonds. All compounds of nucleic acids may be exposed to free radical damage, which may become permanent, or may be repaired by special mechanism (Barzilai and Yamamoto, 2004).
x. Damage of Enzymes and Proteins

Proteins are membrane constituents, and therefore, their damage explains the membrane damaging effect of free radicals, while the loss of specific activity of enzymes may also have severe consequences. 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 (Friguet, 2006).

xi. Lipid Damage

Lipids are by far the most susceptible target for free radical attack. The detection and measurement of lipid peroxidation is most frequently cited to support the involvement of free radical reaction on toxicology and human diseases. Extensive lipid peroxidation in biological membranes causes alterations in fluidity, falls in membrane potential, increases permeability to ions eventual rapture of the membrane leading to release of cell and organelle contents such as lysosomal hydrolytic enzymes. Some end products of metal ion dependent lipid peroxidation are cytotoxic. Lipid peroxides and/or cytotoxic aldehydes derived from them can block macrophage action, inhibit protein synthesis, kill bacteria, inactivate enzymes, cross link proteins, generate thrombin and act as chemotoxin for phagocytes (Halliwell and Chirico, 1993).

xii. Carbohydrate Damage

Sugars including glucose, mannitol and deoxy sugars react readily with hydroxyl radicals. 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 (Moller et al., 1996).

xiii. Gene Activation

The ability of free radical to activate transcription is now recognized. The immediate early genes C-fos, C-myc and beta-actin are induced rapidly hydrogen free
radical, possibly through the induction of DNA strand breaks. These genes encode transcription factors which participate in the induction of cell growth, differentiation and development. Free radicals have been also involved in the activation of transcription factor NF-κB, which is an important factor, which controls the transcription of a number of cytokine genes (Ryter and Tyrrell, 1998).

xiv. **Antioxidant defense mechanisms**

The harmful effects of ROS and RNS are balanced by the antioxidant action of non-enzymatic antioxidants in addition to antioxidant enzymes. These antioxidant defenses are extremely important as they represent the direct removal of free radicals (pro-oxidants), thus providing maximal protection for biological sites.

xv. **Plants as potent antioxidants:**

The most efficient enzymatic antioxidants involve superoxide dismutase, catalase and glutathione peroxidase (Mates et al., 1999). Non-enzymatic antioxidants involve Vitamin C, Vitamin E, carotenoids, thiol antioxidants (glutathione, thioredoxin and lipoic acid), natural flavonoids, a hormonal product of the pineal gland, melatonin and other compounds (Re et al., 1999). As plants produce a number of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity.

The predominant phenolic compound vanillic acid was characterized from *Tunisian nigella sativa* shoots and roots were assayed for the antioxidant activities by DPPH scavenging reducing and chelating activites by Bourgou et al., (2008).

A study was carried by Suresh et al., (2008) to determine the antioxidant activity of selected medicinal plants namely *Albizia amara, Achyranthes aspera, Cassia fistula, Cassia auriculata* and *Datura stramonium* by inhibition of lipid peroxidation technique. The highest inhibition of lipid peroxidation activity was observed in *A. amara* (96%) followed by *C. fistula* (89%) and *C. auriculata* (89%). The potency of protective effect of *A. amara* was about 4 times greater than the synthetic antioxidant butylated...
hydroxytluene, (BHT). This study indicates that the antioxidant activity of *A. amara* could be harnessed as a drug formulation.

Tevfik Özen and Kadir Kinalioğlu (2008) reported the antioxidant activity of *Parmelia saxatilis* by different analytical methods. Water and methanol were used as solvents and antioxidative effects were measured by a ferric thiocyanate method (FTC) and thiobarbituric acid test (TBA). The antioxidant activity increased with the increasing amount of extracts (from 50 to 250 µg) added to linoleic acid emulsion. The methanol extract of PS exhibited high antioxidative activity that was not significantly (*P*<0.05) different from *α*-tocopherol, while aqueous extracts of *P. saxatilis* showed low antioxidative activity. Similar trends of antioxidant activity were observed using either the FTC or TBA methods. Antioxidant activity, reducing power, free radical scavenging (DPPH), superoxide anion radical scavenging, metal chelating and hydrogen peroxide scavenging activities of PS extracts showed dose dependence and increased with concentration of PS extract. The results obtained in this study indicate that the PS might be a potential source of natural antioxidant.

Prateek *et al.*, (2008) have reported the antioxidant properties of methanol extract of three medicinal plants *viz.* *Withania somnifera, Asparagus racemosus* and *Centella asiatica*, which were evaluated by the following methods, namely the ABTS (2,2’-azinobis-3-ethylbenzothiozoline- 6-sulphonic acid) radical scavenging assay, Trolox equivalent antioxidant capacity assay (TEAC), Oxygen radical absorbance capacity (ORAC) assay, DPPH free radical scavenging activity assay, Super oxide scavenging activity assay and Nitric oxide radical scavenging activity assay. The methanol extract from *Centella asiatica* was most active in the ABTS assay with followed by *Withania somnifera*. The minimum activity showed by *Asparagus racemosus*. The Trolox equivalent antioxidant capacity, DPPH (1, 1-diphenyl-2-picryl hydrazyl) and Superoxide radical scavenging activity of the methanolic extracts was in order of *Withania somnifera* > *Centella asiatica* > *Asparagus racemosus*. In the Oxygen radical absorbance capacity assay the ORAC value of *Withania somnifera* was found maximum and followed by *Centella asiatica* and *Asparagus racemosus*. The Nitric oxide radical activity was found
in order of *Asparagus racemosus* > *Withania somnifera* > *Centella asiatica*. These studies suggest that all the tested plants have moderate to potent antioxidant activity.

Nooman *et al.*, (2008) evaluated the methanolic crude extracts of some commonly used medicinal plants for their free radical scavenging properties using ascorbic acid as standard antioxidant. Free radical scavenging activity was evaluated using DPPH free radical. The overall antioxidant activity of green tea (*Camellia sinensis* Linn.) was the strongest, followed in descending order by black tea (*Camellia sinensis* Linn.), *Eugenia aryophyllus* (Spreng.) Bullock and Harrison, *Piper cubeba* Linn., *Zingiber officinale* Roscoe and *Piper nigrum* Linn. *Trigonella foenum graecum* Linn. and *Elettaria cardamomum* (Linn.). Maton (2008) showed weak free radical scavenging activity with the DPPH method. All the methanolic extracts exhibited antioxidant activity significantly. The IC$_{50}$ of the methanolic extracts ranged between 6.7 ± 0.1 and 681.5 ± 8.4 µg/ml and that of ascorbic acid was 8.9 ± 0.1 µg/ml. The study reveals that the consumption of these spices would exert several beneficial effects by virtue of their antioxidant activity.

Stanjneer *et al.*, (2009), reported the antioxidant and free radical scavenging activities of red and yellow forms of *Melampyrum barbatum* L. In their study, they reported the results concerning flower and leaf antioxidant enzyme activities (superoxide dismutase, catalase, guaiacol peroxidase and glutathione peroxidase), reduced glutathione quantity, flavonoids, photosynthetic pigments and soluble protein contents and quantities of malondialdehyde, (•)OH and (•)O(2) radicals; total antioxidant capacity was determined by FRAP (Ferric Reducing Antioxidant Power) method and scavenger activity by DPPH (1,1-diphenyl-2-pycril-hydrasil radical) method. The flowers of the red form of *M. barbatum* exhibited the highest antioxidant property.

Similarly, several investigators have worked on various plants which have been found to possess potent antioxidant activity viz., *Rubus idaeus, Rubus occidentalis*, and *Fragaria ananassa* (Shiow and Hsin-Shan, 2000); *Cordyceps sinensis* (Li *et al.*, 2001); *Emblica officinalis* (Kaur and Kapoor, 2002); *Satureja hortensis* (Güllüce *et al.*, 2003); *Allium cepa, Illicium religiosum, Fagopyrum esculentum, Origanum officinalis,*
Rosmarinus officinalis, Pyrus pyrifolia, Acanthopanax senticosus, Eugenia caryophyllata and Erigeron annuus (Young and Kyong, 2003); Ardisia compressa (Sonia and de Mejia, 2004); Theobroma cacao (Osman et al., 2004); Aframomum danielli, Allium cepa, Allium sativa, Capsicum frutescens, Citrus sinensis, Curcuma longa, Justicia flava, Ocimum gratissimum, Piper guineense (Odukoya et al., 2005); Fagopyrum esculentum (Ting and Chi-Tang, 2005); Rhodiola sacra, Polygonum multiflorum and P. multiflorum (Chi-Chun et al., 2006); Garcinia mangostana (Weecharangsan et al., 2006); Gentiana lutea (Kussar and Zupaneie et al., 2006); Lycium barbarum (Li et al., 2007); and Zanthoxylum piperitum (Yamazaki et al., 2007)

B. Hepatoprotective activity:

i. Liver

Liver serves multiple functions in the body that include protein synthesis and drug detoxification. It also plays an important role in metabolism. Owing to this function, liver is vulnerable to damage from various causes like viral and bacterial infections and drug overdose. The malfunction of liver after damage can cause fatal diseases.

ii. Liver related diseases

Liver failure due to various diseases can be divided into two major types known as acute and chronic liver failure. The etiology and subsequent progression of liver failure are quite different in acute and chronic stages of damage.

iii. Acute liver failure

Acute liver failure, also known as fulminant hepatic failure, it is a rare liver disorder. It is defined by the development of hepatic encephalopathy and coagulopathy in a short period of time after a certain kind of insult to the liver without previous history of any kind of liver diseases. Short-term administration of CCl4 causes acute hepatocellular injury with centrolobular necrosis and steatosis (O'Grady, 2005; Sass and Shakil, 2005).

The damage in the liver cells during acute liver injury can be divided into two stages (Riordan and Williams, 2000; Palmes et al., 2005). In the first stage, the damage is directly due to the hepatic noxious agents that cause necrosis and apoptosis and finally
cell death. The second stage of liver cell damage involves various cytokines and other cytotoxic mediators, for example interleukins and tumor necrosis factor-α (TNF-α), released from Kupffer cells and other cell types. These proinflammatory mediators then attract vast amount of neutrophils to the liver (Galun and Axelrod, 2002). A further damage of the liver cells is caused by oxidative stress as a result of a large amount of free radicals (e.g. nitric oxide, reactive oxygen species, lipid peroxides and other prooxidants) produced from the activated neutrophils in the presence of pro-inflammatory mediators (Losser and Payen, 1996). Kupffer cells are believed to play an important role in mediating the secondary stage of acute liver failure due to the release of various cytokines, proinflammatory factors and free radicals (Rosser and Gores, 1995).

iv. Chronic liver failure

Chronic liver failure is a prolonged and repeated process of damage to the liver tissue. Most of the chronic liver diseases are in conjunction with fibrogenesis and the subsequent complications brought about by fibrosis (e.g. cirrhosis, hepatocellular carcinoma, and portal hypertension). There are different types and etiology of chronic liver disease namely viral hepatitis infection, liver cirrhosis, hepatocellular carcinoma, alcoholic liver disease, non-alcoholic steatohepatitis, hepatic ischemia-reperfusion injury and liver transplantation.

The time to onset of experimental cirrhosis depends on species, route of administration, dose, interval between doses, and use of enzymatic inducers. The development of liver fibrosis involves expression of cellular receptors for several growth factors that stimulate the proliferation of activated lipocytes and the synthesis of extracellular matrix (Gressner, 1995; Dooley et al., 2000). Regression of hepatic fibrosis has been reported in a number of studies, but as yet no substance has been shown to be safe and effective enough for the treatment of hepatic fibrosis (Friedman, 1993; Schuppan et al., 1998).

v. Liver regeneration

A number of crucial factors were identified that initiate the regeneration process including the cytokines TNF-α (Yamada et al., 1997) and IL-6 (Clavien, 1997) as well as
the transcription factors including nuclear factor-kappa B (NF-kB) (Cressman et al., 1994), signal transducers and activators of transcription-3 (STAT3) (Cressman et al., 1995).

When there is tissue damage in the liver, the proliferation of the surviving hepatocytes will be triggered (Koniaris et al., 2003). Once the regeneration process is stimulated, the quiescent hepatocytes move from G0 phase to the proliferative stage in cell cycle. The liver cells are now sensitized to various growth factors, for example hepatocyte growth factor and transforming growth factor-α (TGF-α). After the liver mass and function are restored, the replication of liver cells is inhibited by the presence of growth inhibitors, transforming growth factor-β and activin (Koniaris et al., 2003). The repairing process in damaged liver also involves the extracellular matrix, which is the structural material for the scaffolding of the liver. Extracellular matrix in nature is a network of groups of structural molecules including collagens, non-collagenous glycoproteins and proteoglycans (Schuppan et al., 2001). However, when hepatic injury persists, this regeneration process will turn into a scarring process known as fibrosis (intermediate stage) and cirrhosis (late stage). Both conditions are due to excessive accumulation of extracellular matrix (Bataller and Brenner, 2005).

vi. Liver fibrosis

Liver fibrosis is a common phenomenon in many chronic liver diseases as a result of over accumulation of extracellular matrix in the liver (Bataller and Brenner, 2005). There is usually a 3 to 5-fold increase in the amount of extracellular matrix in fibrotic liver when compared with normal liver (Gressner, 1992). This formation of extracellular matrix is triggered by the inflammatory responses during liver injury (Bedossa and Paradis, 2003). The initial step of the remodeling process includes the activation and transformation of the hepatic stellate cells into fibroblast-like cells (Hautekeete and Geerts, 1997).
vii. Liver cirrhosis

Liver cirrhosis is the end stage of fibrogenesis of the liver injury. In human cirrhotic liver, there is an observable morphological change of the formation of regenerative nodules (Roncalli, 2004). Portal hypertension is one of the common complications in liver cirrhosis due to an increase in intrahepatic resistance to blood flow (Gines et al., 2004).

viii. Carbon tetrachloride as a hepatotoxin

During the early part of this century, carbon tetrachloride was first found to produce hepatic injury in man and experimental animal (Meyer and Samuel, 1923). The intervening years have seen thousands of reports devoted to this agent (Meyer and Samuel, 1923; Recknagel and Jr. Glende, 1973). Poisoning with CCl₄ has been a well-accepted and widely used model to study the pathophysiology of inflammation, liver injury or hepatic inflammation (Recknagel and Jr. Glende, 1973).

CCl₄ can be used to study both acute and chronic liver failure. When a high toxic dose is injected, the hepatocellular necrosis masks the regenerative ability of the liver and results in liver failure. However, a repeated low dose administration of CCl₄ will lead to fibrosis, cirrhosis, fatty degeneration and hepatocellular carcinoma (Weber et al., 2003).

In the course of unraveling the mechanism by which it produces fatty liver, CCl₄ has served to elucidate the pathogenesis of fatty metamorphosis induced by other etiological factors (Recknagel and Jr. Glende, 1973). While it can lead to a damage of number of tissues it is particularly damaging to the liver and kidneys of many species (Von Oettingen, 1964). The agent is a potent hepatotoxin. Single doses lead promptly to centrizonal necrosis and steatosis (Von Oettingen, 1964; Recknagel and Jr. Glende, 1973). Within a few minutes, there is an injury to the endoplasmic reticulum, which leads to functional defects of the hepatocyte and multiple biochemical manifestations of hepatic injury (Von Oettingen, 1964; Recknagel and Jr. Glende, 1973).
Carbon tetrachloride (Range and Dale, 1987) because of its high lipid solubility is well distributed in the body, but produces toxic effects that are largely confined to the liver and kidneys. The toxicity is increased by agents (e.g. phenobarbitone), which induce microsomal drug metabolizing enzymes and reduced by the inhibitors of microsomal enzymes. The microsomal mixed function oxidase system withdraws an electron from CCl₄ leaving the reactive trichloromethyl radical CCl₃⁻. This free radical has lifetime of only about 100 microseconds and so has time to diffuse for only a short distance within the liver cell before undergoing secondary reactions. The secondary reactions, which are responsible for the biochemical damage, are of various kinds.

a) Oxidation of thiols to disulphide bonds.

b) Saturation of double bonds in lipids, proteins or nucleotides, resulting covalent attachment of free radical group of those sites.

c) Lipid peroxidation reaction in which polyunsaturated membrane lipids are converted to peroxide derivative and eventually to aldehydes and other products leading to a further cascade of reaction, which results in irreversible membrane damage.

The experimental intoxication induced by carbon tetrachloride (CCL₄) is widely used for modeling liver injury in rats. Hepatotoxicity is connected with severe impairment of cell protection mechanisms. The location of liver injury is defined mainly by the biotransformation of CCL₄, which is cytochrome P-450 dependent. Free radicals initiate the process of lipid peroxidation, which is generally caused of inhibition of enzyme activity (Mac Cay et al., 1984., Poli et al., 1985). It is now generally accepted that the hepatotoxicity of CCL₄ is the result of reductive dehalogenation, which is catalysed by enzyme system P450, and which forms the highly reactive trichloromethyl free radical. This then readily interacts with molecular oxygen to form the trichloromethyl peroxy radical. Both trichloromethyl and its peroxy radical are capable of binding to proteins or lipids, or of abstracting a hydrogen atom from an unsaturated lipid, initiating lipid peroxidation and liver damage and by doing so playing a significant role in pathogenesis of diseases (Recknagel et al., 1989).
ix. Silymarin

Silymarin is a flavonoid isolated from *Silybum marianum* that kindled widespread world research on hepatoprotective agents (Wagner *et al*., 1968; Abraham *et al*., 1970; Pelter and Hansel, 1975). Other important antihepatotoxic drug discoveries from plant sources include cynarin from *Cynara scolymus* (Panizzi and Scarpti, 1954) and schizandrin from *Schisandra sphenanthera* (Liu *et al*., 1978). The discovery of diverse chemical compounds from the natural products and synthetic compounds used in protective liver therapy such as phospholipids, sugar alcohols, pyrimidine, purine derivatives, vitamins, cysteine, glutathione, corticoids, androgens, penicillamine, ricinin etc., does not confine the activity to any particular class of compounds (Shirwaikar *et al*., 1991), but emphasizes once again the complexity of liver disorders in addition to the different action, mechanisms of different pharmaceutical preparations.

x. Chemistry of silymarin

Silymarin is a complex mixture of four flavonolignan isomers, namely silybin, isosilybin, silydianin and silychristin with an empirical formula $C_{25}H_{22}O_{10}$. The structural similarity of silymarin to steroid hormones is believed to be responsible for its protein synthesis facilitatory actions. Among the isomers silybin is the major and most active component and represents about 60-70 per cent, followed by silychristin (20%), silydianin (10%), and isosilybin (5%). Silipide is the silybin - phosphatidylcholine complex which ensures a large increase in the bioavailability of silybin (Vailati *et al*., 1993).

In the present study also Silymarin was used as a reference standard drug for the evaluation of hepatoprotective effect of root extracts and their constituents of *Echinops echinatus*.

xi. Plants as Hepatoprotective agents

Valcheva-Kuzmanova *et al*., (2004) studied the effect of the natural fruit juice from *A. melanocarpa* (NFJAM) on carbon tetrachloride (CCL₄)-induced acute liver
damage in rats. Histopathological changes such as necrosis, fatty change, ballooning degeneration and inflammatory infiltration of lymphocytes around the central veins occurred in rats following acute exposure to CCl₄ (0.2 ml/kg 1, 2 days). The administration of CCl₄ increased plasma aspartate transaminase (AST) and alanine transaminase (ALT) activities induced lipid peroxidation (as measured by malondialdehyde content in rat liver and plasma) and caused a depletion of liver reduced glutathione (GSH). NFJAM (5, 10 and 20 ml/kg 1, 4 days) dose-dependently reduced the necrotic changes in rat liver and inhibited the increase of plasma AST and ALT activities, induced by CCl₄. NFJAM also prevented the CCl₄-induced elevation of malondialdehyde formation and depletion of GSH content in rat liver.

Dubey and Batra (2008) evaluated hepatoprotective potential effect of ethanolic fraction of *Thuja occidentalis* (EFTO) has been assessed against CCL₄ induced liver damage in rats. A dose of EFTO 400 mg/kg p.o.exhibited significant protection from liver damage in acute and chronic CCL₄ induced liver damage model. Histopathological examination was carried out after the treatment to evaluate hepato protection. The fraction was found to possess good hepatoprotective property.

Manokaran *et al.*, (2008) evaluated the hepatoprotective activity of hydroalcoholic extract of *Aerva lanata* against paracetamol induced liver damage in rats. The hydroalcoholic extract of *Aerva lanata* (600mg/kg) was administered orally to the animals with hepatotoxicity induced by paracetamol (3gm/kg). Silymarin (25mg/kg) was given as reference standard. All the test drugs were administered orally by suspending in 0.5% Carboxy methyl cellulose solution. The plant extract was effective in protecting the liver against the injury induced by paracetamol in rats. This was evident from significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin. It was concluded from the result that the hydroalcoholic extract of *Aerva lanata* possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rat.

Vadivul *et al.*, (2009) evaluated of hepatoprotective and *in-vitro* cytotoxic activity of alcoholic extract of leaves of *Premna serratifolia* Linn. Hepatoprotective
activity is studied by carbon tetrachloride induced hepatotoxicity in rats and the in vitro cytotoxic activity is carried out by trypane blue exclusion method using EAC cell lines. The degree of protection in hepatoprotective activity has been measured by using biochemical parameters such as SGOT, SGPT, ALP, bilirubin and total protein. The results suggest that the alcoholic extract at the dose level of 250mg/kg has produced significant hepatoprotection by decreasing the activity of serum enzymes, bilirubin, and lipid peroxidation which is comparable to that of standard drug silymarin. The alcoholic extract also does exhibit the IC₅₀ value of 75µg/ml which indicates the significant in vitro cytotoxic activity of the extract. It is concluded that alcoholic extract of leaves of *Premna serratifolia* Linn. is not only an effective hepatoprotective agent, but also possesses significant antitumour activity.

Chaudhari *et al.*, (2009) evaluated the hepatoprotective activity of hydroalcoholic extract of *Momordica charantia* Linn. leaves against carbon tetrachloride induced hepatotoxicity in albino wistar rats. Hydroalcoholic extract of *Momordica charantia* leaves (100mg/kg and 200mg/kg p.o.) were administered to the experimental rats for seven days. The hepatoprotective activity of hydroalcoholic extract of *Momordica charantia* leaves were evaluated by estimation of SGOT, SGPT, ALP and total bilirubin. Histopathology of the liver was also studied. In the hydroalcoholic extract of *Momordica charantia* leaves treated animals, the toxic effect of carbon tetrachloride was controlled significantly by restoration of the increased levels of SGOT, SGPT, ALP and total bilirubin as compare to the toxicant control. The hydroalcoholic extract of leaves of *Momordica Charantia* showed significant hepatoprotective activity.

The ethanol and aqueous extracts of *Acacia ferruginea* leaves were tested for their efficacy against carbon tetrachloride (CCL₄) induced hepatotoxicity in Wistar albino rats. The different groups of animals were administered with CCL₄ (1ml/kg, s.c.). The ethanol and aqueous extracts at the dose of 200mg/kg were administered to CCL₄ treated rats. The result of present study demonstrated that ethanol extract significantly decreases the level of alanine aminotransferase, aspartase aminotransferase, total bilirubin and direct bilirubin in blood, as compare to aqueous extract (Akare *et al.*, 2009).
Rosa et al., (2009) evaluated the Hepatoprotective activity of methanol, hexane and chloroform extracts of Prostechea michuacana (EMM) were studied against carbon tetrachloride (CCl4) induced hepatic injury in albino rats. Pre-treatment with methanolic extract reduced biochemical markers of hepatic injury like ALT and AST levels demonstrated dose dependant reduction in the in vivo peroxidation induced by CCl4. Likewise, pretreatment with extracts from EMM on paracetamol induced hepatotoxicity and the possible mechanism involved in this protection were also investigated in rats. The extracts of EMM at 200, 400 and 600 mg/kg were administered. The degree of protection was measured by using biochemical parameters such as serum transaminase (GOT and GPT), alkaline phosphatase (ALP) and bilirubin. The methanol extract of orchid produced significant hepatoprotective effect by decreasing the activity of serum enzymes, and bilirubin. These results suggest that EMM could protect from paracetamol-induced lipid peroxidation eliminating the deleterious effects of toxic metabolites from paracetamol. Hexane and chloroform extracts did not show any effect. This hepatoprotective activity was comparable with sylmarin. The results obtained in the present study indicate that the MEMC can be a potential source of natural hepatoprotective agent.

Similarly, there are plenty of medicinal plants on which the hepatoprotective screening has been carried out, for instance, Berberis aristata (Janbaz and Gilani 2000); Fumaria Vailantii and Gentiana olivieri (Goknur et al., 2000); Cassia angustifolia Vahl. (Ilavarasan et al., 2001); Coffee (He et al., 2001); Apium graveolens Linn. and Croton oblongifolius Roxb. (Ahmed et al., 2002); Thespesia populnea (Havarasan et al., 2003); Ailanthus excelsa Roxb. (Hukkeri et al., 2003); Sarcostemma brevistigma (Sethuraman, et al., 2003); Vitex negundo (Srinivas, et al., 2004); Wrightia tinctoria Roxb. (Chandrashekar, et al., 2004); Annona reticulata (Hukkeri, et al., 2004); Coscinium fenestratum (Venukumar and Latha, 2004); Glycyrrhiza glabra Linn. (Rajesh and Latha, 2004); Boerhaavia erecta and B. rependa L (Krishna and Shanthamma, 2004a; 2004b); Diospyros cordifolia (Krishna, et al., 2005); Adhatoda vasica (Bhattacharyya, et al., 2005); Polygala arvensis (Dhanabal et al., 2006); Beta vulgaris (Agarwal et al., 2006) and Pergularia daemia (Sureshkumar and Mishra, 2007).
C. Wound Healing activity:

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal state. Wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. Wound healing proceeds via an overlapping pattern of events including haemostasis, inflammation, proliferation, and tissue remodeling. The normal healing response begins the moment the tissue is injured.

Wound healing is a fundamental response to tissue injury that results in restoration of tissue integrity. It has 3 phases; inflammatory, proliferative and maturational, and is dependent upon the type and extent of damage, the general state of the host's health and the ability of the tissue to repair. The inflammatory phase is characterized by haemostasis and inflammation, followed by epithelization, angiogenesis, and collagen deposition in the proliferative phase. In the maturational phase, the final phase of wound healing the wound undergoes contraction resulting in a smaller amount of apparent scar tissue.

Wound healing is a complex but orderly phenomenon involving a number of processes:

- Induction of an acute inflammatory process by the initial injury
- Regeneration of parenchymal cells
- Migration and proliferation of both parenchymal and connective tissue cells
- Synthesis of ECM proteins
- Remodeling of connective tissue and parenchymal components
- Collagenation and acquisition of wound strength.
i. Biology of wound healing

Wound healing is a dynamic, interactive process involving soluble mediators, blood cells, extracellular matrix, and parenchymal cells. Wound healing has three phases; inflammation, tissue formation, and tissue remodeling; that overlap in time.

ii. Inflammatory phase

Tissue injury causes the disruption of blood vessels and extravasation of blood constituents. The blood clot reestablishes hemostasis and provides a provisional extracellular matrix for cell migration. Platelets not only facilitate the formation of a hemostatic plug but also secrete several mediators of wound healing, such as platelet-derived growth factor, that attract and activate macrophages and fibroblasts (Heldin and Westermark, 1996). However, in the absence of hemorrhage, platelets are not essential to wound healing. Numerous vasoactive mediators and chemotactic factors are generated by the coagulation and activated-complement pathways and by injured or activated parenchymal cells. These substances recruit inflammatory leukocytes to the site of injury (Clark, 1996).

Infiltrating neutrophils cleanse the wounded area of foreign particles and bacteria and are then extruded with the eschar or phagocytosed by macrophages. In response to specific chemoattractants, such as fragments of extracellular-matrix protein, transforming growth factor β, and monocyte chemoattractant protein 1, monocytes also infiltrate the wound site and become activated macrophages that release growth factors such as platelet-derived growth factor and vascular endothelial growth factor, which initiate the formation of granulation tissue. Macrophages bind to specific proteins of the extracellular matrix by their integrin receptors, an action that stimulates phagocytosis of microorganisms and fragments of extracellular matrix by the macrophages (Brown, 1995). Adherence to the extracellular matrix also stimulates monocytes to undergo metamorphosis into inflammatory or reparative macrophages. Adherence induces monocytes and macrophages to express colony-stimulating factor 1, a cytokine necessary for the survival of monocytes and macrophages; tumor necrosis factor α, a potent
inflammatory cytokine; and platelet-derived growth factor, a potent chemoattractant and mitogen for fibroblasts.

iii. Epithelialization

Re-epithelialization of wounds begins within hours after injury. Epidermal cells from skin appendages such as hair follicles quickly remove clotted blood and damaged stroma from the wound space. At the same time, the cells undergo marked phenotypic alteration that includes retraction of intracellular tonofilaments (Paladini et al., 1996); dissolution of most inter-cellular desmosomes, which provide physical connections between the cells; and formation of peripheral cytoplasmic actin filaments, which allow cell movement (Goliger and Paul, 1995; Gabbiani et al., 1978). Furthermore, epidermal and dermal cells no longer adhere to one another, because of the dissolution of hemidesmosomal links between the epidermis and the basement membrane, which allows the lateral movement of epidermal cells. The expression of integrin receptors on epidermal cells allows them to interact with a variety of extracellular-matrix proteins (e.g., fibronectin and vitronectin) that are interspersed with stromal type I collagen at the margin of the wound and interwoven with the fibrin clot in the wound space (Clark, 1990). The migrating epidermal cells dissect the wound, separating desiccated eschar from viable tissue. The path of dissection appears to be determined by the array of integrins that the migrating epidermal cells express on their cell membranes.

One to two days after injury, epidermal cells at the wound margin begin to proliferate behind the actively migrating cells. The absence of neighbor cells at the margin of the wound (the “free edge” effect) may signal both migration and proliferation of epidermal cells. Local release of growth factors and increased expression of growth-factor receptors may also stimulate these processes. Leading contenders include epidermal growth factor, transforming growth factor α, and keratinocyte growth factor (Nanney and King Jr., 1996; Werner et al., 1994; Abraham and Klagsbrun, 1996). As re-epithelialization ensues, basement-membrane proteins reappear in a very ordered sequence from the margin of the wound inward, in a zipper like fashion (Clark et al.,
1982). Epidermal cells revert to their normal phenotype, once again firmly attaching to
the reestablished basement membrane and underlying dermis.

iv. Formation of Granulation tissue

New stroma, often called granulation tissue, begins to invade the wound space
approximately four days after injury. Numerous new capillaries endow the new stroma
with its granular appearance. Macrophages, fibroblasts, and blood vessels move into the
wound space at the same time (Hunt, 1980). The macrophages provide a continuing
source of growth factors necessary to stimulate fibroplasia and angiogenesis; the
fibroblasts produce the new extracellular matrix necessary to support cell in growth; and
blood vessels carry oxygen and nutrients necessary to sustain cell metabolism.

The structural molecules of newly formed extracellular matrix, termed the
provisional matrix (Clark et al., 1982), contribute to the formation of granulation tissue
by providing a scaffold or conduit for cell migration. These molecules include fibrin,
fibronectin, and hyaluronic acid (Greiling and Clark, 1997; Toole, 1991). The fibroblasts
are responsible for the synthesis, deposition, and remodeling of the extracellular matrix.

Cell movement into a blood clot of cross-linked fibrin or into tightly woven
extracellular matrix may require an active proteolytic system that can cleave a path for
cell migration. A variety of fibroblast derived enzymes, in addition to serum-derived
plasmin, are potential candidates for this task, including plasminogen activator,
collagenases, gelatinase A, and stromelysin (Mignatti et al., 1996; Vaalamo et al., 1997).

After migrating into wounds, fibroblasts commence the synthesis of extracellular
matrix. The provisional extracellular matrix is gradually replaced with a collagenous
matrix (Clark et al., 1995; Welch et al., 1990), perhaps as a result of the action of
transforming growth factor β1 (Clark et al., 1995).

Once an abundant collagen matrix has been deposited in the wound, the
fibroblasts stop producing collagen, and the fibroblast-rich granulation tissue is replaced
by a relatively acellular scar. Cells in the wound undergo apoptosis (Desmouliere et al., 1995) triggered by unknown signals.

v. Wound contraction and extracellular-Matrix

a. Reorganization

Wound contraction involves a complex and superbly orchestrated interaction of cells, extracellular matrix, and cytokines. During the second week of healing, fibroblasts assume a myofibroblast phenotype characterized by large bundles of actin-containing microfilaments disposed along the cytoplasmic face of the plasma membrane of the cells and by cell–cell and cell–matrix linkages (Welch et al., 1990; Desmoulière and Gabbiani, 1996). The appearance of the myofibroblasts corresponds to the commencement of connective-tissue compaction and the contraction of the wound.

Collagen remodeling during the transition from granulation tissue to scar is dependent on continued synthesis and catabolism of collagen at a low rate. The degradation of collagen in the wound is controlled by several proteolytic enzymes termed matrix metalloproteinases, which are secreted by macrophages, epidermal cells, and endothelial cells, as well as fibroblasts (Mignatti et al., 1996).

b. Tensile strength of wound

This is the strength of a healing wound and is measured experimentally by the amount of force required to disrupt it. In the beginning a wound will be having little tensile strength because the clot alone will be holding the edges together. Thereafter, tensile strength increases rapidly as collagen deposition increases and cross linkages are formed between collagen fibers.

Wounds gain only about 20 percent of their final strength in the first three weeks, during which time fibrillar collagen has accumulated relatively rapidly and has been remodeled by contraction of the wound. Thereafter, the rate at which wounds gain tensile strength is slow, reflecting a much slower rate of accumulation of collagen and, more important, collagen remodeling with the formation of larger collagen bundles and an
increase in the number of intermolecular cross-links (Bailey et al., 1975). Nevertheless, wounds never attain the same breaking strength (the tension at which skin breaks) as uninjured skin. At maximal strength, a scar is only 70 percent as strong as normal skin (Levenson et al., 1965).

Extracellular matrix involves five main components which include collagen, adhesive glycoproteins, basement membrane, elastic fibers and proteoglycans which are responsible for wound strength.

vi. Wound healing activity in plants

Mrityunjoy et al., (2007) evaluated the effect of hydrochloric extract of Tectona grandis on experimentally induced wounds in rats and compare the effects observed with a known wound healing agent, Aloe vera. The models selected were excision wound, incision wound, burn wound and dead space wound. In the excision wound and burn wound models, animals treated with Tectona grandis leaf extract showed significant reduction in period of epithelisation and wound contraction 50%. In the incision wound model, a significant increase in the breaking strength was observed. Tectona grandis leaf extract treatment orally produced a significant increase in the breaking strength, dry weight and hydroxyproline content of the granulation tissue in dead space wound. It was concluded that Tectona grandis leaf extract applied topically (5% and 10% gel formulation) or administered orally (250 mg and 500 mg/kg body weight) possesses wound healing activity.

Shetty et al. (2008) reported the potential of alcoholic and aqueous extracts of Ocimum sanctum in wound healing in Wistar albino rats. The wound healing parameters were evaluated by using incision, excision and dead space wounds in extract-treated rats and controls. Both the doses of alcoholic and aqueous extract significantly increased wound breaking strength, hydroxyproline, hexuronic acid, hexosamines, superoxide dismutase, catalase, reduced glutathione and significantly decreased percentage of wound contraction and lipid peroxidation when compared with the control group. The results suggest that O. sanctum has antioxidant properties, which may be responsible and
favorable for faster wound healing and this plant extract may be useful in the management of abnormal healing and hypertropic scars.

Mahesh et al., (2009), evaluated the wound healing potential of aqueous extract of leaves of Ocimum kilimandscharicum at two different doses (200 and 400 mg/kg) in three types of wound models on rats: the excision, the incision and dead space wound model. Significant increase in skin breaking strength, granuloma breaking strength, wound contraction, dry granuloma weight and decreased in epithelization period was observed in animals of both the treatment groups compared to control.

Agarwal et al., (2009) evaluated wound healing activity of Plantain banana (M. Sapientum Var, paradisiaca,) in terms of (i) percentage wound contraction, epithelisation period and scar area; (ii) wound breaking strength and; (iii) on granulation tissue antioxidant status (estimation of superoxide dismutase and reduced glutathione), free radical (lipid peroxidation, an indicator of tissue damage) in excision, incision and dead space wound models. Both extracts when studied for incision and dead space wound parameters, increased wound breaking strength and levels of enzymes in granulation tissue and decreased percentage of wound area, scar area and lipid peroxidation when compared to control group. Thus Plantain banana favoured wound healing which could be due to its antioxidant effect.

Similarly, many investigators reported the wound healing effect of the various plant extracts such as Tridox procumbens (Udupa, et al., 1995); Trigonella foenum graecum (Taranalli and Kuppast, 1996); Hypericum mysorensce (Mukherjee and Suresh, 2000); Nelumbo nucifera (Mukherjee, et al., 2000); Ginkgo biloba (Bairy and Rao, 2001); Aegle marmelos (Jaswanth, et al., 2001); Opuntia ficus-indica (Park and Chun, 2001); Gmelina arborea Roxb. (Shirwaikar, et al., 2002); Bryophyllum pinnatum (Mahamood and Patil, 2002); Eucalyptus globulus (Hukkeri, et al., 2002); Terminalia arjuna (Madhura and Sushma, 2003); Cinnamomum zeylanicum (Kamath, et al., 2003); Eucalyptus globulus (Kusum, et al., 2004); Oxalis corniculata (Taranalli, et al., 2004b); Merremia tridentata (Hatapakki, et al., 2004); Diospyros cordifolia (Mankani, et al., 2004); Saussurea lappa (Ganachari, et al., 2005); Plagiochasma appendiculatum
D. Anti-inflammatory activity

i. Introduction to inflammation:

Inflammation is as the local response of living mammalian tissues to the injury due to any agent. It is a body’s defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosed cells and tissues (Harsh Mohan, 2003).

The agents causing inflammation may be as under:

1. Physical agents like heat, cold, radiation, and mechanical trauma.
2. Chemical agents like organic and inorganic poisons.
3. Infective agents like bacteria, viruses, and their toxins.
4. Immunological agents like cell mediated and antigen mediated antibody reactions.

Signs of Inflammation are Rubor (Redness), Tumor (Swelling), Calor (Heat) and Dolor (Pain).

ii. Types of Inflammation

Depending upon the defense capacity of the host and duration of response, inflammation can be classified as Acute and Chronic.

a. Acute inflammation: It’s of short duration and represents the early body reaction and is usually followed by repair. The main features of acute inflammation are accumulation of fluid and plasma at the affected area, intravascular activation of platelets, and polymorphonuclear neutrophils as inflammatory cells.

b. Chronic inflammation: It’s of long duration and occurs either after causative agent of acute inflammation persists for a long time, or the stimulus is such that it induces the chronic inflammation. The main features of chronic inflammation are...
the presence of chronic inflammatory cells as lymphocytes, plasma cells, and macrophages.

Anti-inflammatory drugs have been evaluated by studying inflammatory response produced in animals by injecting the foreign or noxious agents. These responses mostly comprise of the development of oedema/or the formation of exudate and granuloma.

Drugs, which suppress any of these components, are designated as anti-inflammatory agents. The term anti-inflammatory has so many connotations that multiplicity of assay is required to this property in any chemical compound (Goodman et al., 2004)

The complete process of inflammation contains of three phases,

1) Dilatation of increased permeability of small blood vessels resulting in the formation oedema and swelling.

2) Emigration of leukocytes from venules and capillaries, cellular infiltration and a general mopping up reaction.

3) Proliferation of fibroblasts and synthesis of new connective tissue to repair the injury.

A number of mediators have been identified which initiate the early development (first phase) of certain in sequential manner. Thus, there is an initial rele of release of histamine, and 5-hydroxytryptamine(5-HT)producing an increased vascular permeability, followed by release of kinins, further contributing to the increased vascular permeability, lastly, the prostaglandins and slow reacting substance(SRS) are released to maintain the increased vascular permeability produced by histamine, 5-HT and kinins.

The biochemical events accompanying the second phase are not well understood. Many factors are implicated as the regulators of phagacytosis including the calcium, chemotoxin, leukocyte-promoting factor, complement factor, etc. These chemotoxin factors play a major role in the process of leukocytic migration. Polymorphonuclear leukocytes predominate in the cellular infiltrate during the early stages; their number
diminishes and granular mononuclear leukocytes (monocytes) become the predominating leukocyte in the inflammed tissue.

As the exudates phase of inflammation subsides, the initial stages of the reparative or third phase are set in motion. The fibroblast, which is most dominant cell in the wounded zone, first proliferates then synthesis extracellular material including new collagen fibers and acidic mucopolysacharides, which are laid down to form the new connective tissue matrix (Vogel et al., 1997).

The principle underlying the testing of anti-inflammatory activity does injecting irritants, inflammatory substance; induce the reduction of the local oedema.

The inflammatory process involves a series of events that can be elicited by numerous stimuli (e.g. infectious agents, ischemia, antigen-antibody interactions and thermal of other physical injury). Each type of stimulus provokes on a theme. At a macroscopic level, the response usually is accompanied by the familiar clinical signs of erythema, edema, tenderness and pain.

Many different mechanisms are involved in the inflammation process (Bosca et al., 2005). The ability to mount an inflammatory response is essential to survive in the face of environmental pathogens and injury, although in the same situations and diseases the inflammation response may be exaggerated and sustained for no apparent beneficial reason. Several classes of leukocytes play an important role in inflammation.

Recent studies have examined the role of the endothelial cells and cell adhesion molecules including E-, P-, and L-selectins intercellular adhesion molecules I (ICAM-1), vascular cell adhesion molecules I (VCAM-1) and leukocyte integrins in the adhesion of leukocytes, platelets and endothelium at a sites of inflammation. Activated endothelial cells play a important role in targeting circulatory cells to inflammatory sites (Heumann and Roger, 2002)

Thus, endothelial activation results in adhesion of leukocytes by their interaction with newly expressed L-selectin and P-selectin, where endothelial expressed E-selectin
interacts with sialyated Lewis X and other glycoproteins on the leukocyte surface; endothelial ICAM-1 interacts with leukocyte integrins. NSAIDs may inhibit expression or activity of certain of these cells adhesion molecules. Such effects have been described for some NSAIDs and not others, suggesting that interference with action of cell adhesion molecules is not a common mechanism of action of all NSAIDs (Smith et al., 2000; Ferrario et al., 2005).

Several different cytokines also appear to play an essential role in orchestrating the inflammatory process especially interleukin I (IL-1) and tumour necrosis factor (TNF). Both IL-1 and TNF are derived from mononuclear cells and macrophages (as well as other cell types) and induce expression of numerous genes to promote the synthesis of a variety of proteins that contribute to inflammation events IL-1 and TNF are considered principal mediators of biological response to bacterial lipopolysaccharides (endotoxins) and may other infectious stimuli. IL-1 and TNF appears to work in concert with each other and with growth factors (such as granulocytes/macrophage colony stimulating factor, GM-CSF and other cytokines, such as IL-8 and related chemotactic (chemkines), which can promote neutrophil infiltration and activation.

Prostaglandin P2 (PGE2) and prostacyclin (PG12) cause erythema and an increase in local blood flow. PGE1 and PGE2 cause edema when injected into the hind paw of rats, it is not clear if they can increase vascular permeability (leakage) in the post capillary and collecting venules without the participation of other mediators (e.g. bradykinin, histamine, leukotriene C4) (Harbige, 2003).

on the basis of the different aspects of inflammation, the following experimental models are generally selected to investigate the anti-inflammatory activity of the chemical compound or for the plant extract (Ghosh, M.N. 2000).

c. Rat paw oedema:

Oedema represents the early phase of inflammation. Carrageenan induced paw oedema is the simplest and most widely used model for the study the antiinflammatory activity of new compound. Similarly, the paw edema induced by histamine, 5-HT,
bradykinin, dextran, hyaluronidase and prostaglandin E1 have been used for studying the antagonists to these mediators. These agents can be injected in a 0.1 ml volume of suitable, concentration in sterile saline into the supplantar tissue of the rat hind foot, and the paw volume can be measured immediately and then at predetermined intervals by the plethysmometeric method.

d. **Granuloma pouch**

Granuloma represents the exudative and proliferation phases of inflammation. The cotton pellet method described by Meir *et al.*, (1950) and croton oil method by Selye (1953) have been most widely used for producing granuloma. In the cotton pellet method the inflammation is measured by weighing the capsular granuloma together with the cotton which is often contaminated with the exudates, weight of the pouch wall and the accumulation of collagen in the granuloma can be satisfactorily studies.

e. **Pleurisy test**

Pleurisy is well known phenomena of exudative in-inflammation in man. In experimental animals pleurisy can be induced by several irritants. Carragennan induced pleurisy in rats is considered to be an excellent acute inflammatory models in which fluid extravasations, leukocyte migration and the various biochemical parameters involved in the inflammatory response can be measured easily in the exudates.

f. **Urate-induced synovitis**

The important of urate in gout and the deposition of sodium urate in suffering patient is well known. Fairs and McCarty (1962) reported that they were performed a study by injection 20 mg sodium urate crystals suspension in their own knee-joint. They developed as experimental model in dogs for testing anti-inflammatory compounds by inducing the formation of urea crystals on synovial cavity.
g. **Sponge implantation technique**

The sponge implantation technique was described first by Saxena (1960) for short term experimentalism but was used subsequently to study the formation of granuloma using term implantation.

h. **Glass rod granuloma**

The glass rod granuloma as first described by Vogel (1970) reflects the chronic proliferate inflammation of the newly formed connective tissue not only wet and dry weight, but also chemical composition and mechanical properties can be measured.

iii. **Plants as anti-inflammatory agents:**

Pandurangan *et al.*, (2008) reported the anti-inflammatory activity of methanol extract of *Solanum trilobatum* Linn. (Solanacea) root in animal models of inflammation. Crude alkaloidal fraction (CAF) at doses of 25, 50 and 100 mg/kg significantly reduced carrageenan induced rat paw volume at 3 h after carrageenan challenge as compared to control group of animals. CAF (25, 50 and 100 mg/kg) significantly and dose dependently suppressed cotton pellet induced granuloma formation. Results indicated that CAF of methanol extract of the Solanum trilobatum has anti-inflammatory activity in acute and chronic inflammation.

Mahesh *et al.*, (2009) evaluated the anti-inflammatory activity in rodents for ethanolic extract of male flowers (inflorescences) of *Borassus flabellifer* using acute inflammatory models like; carrageenan induced paw oedema and chronic models like; cotton-pellet induced granuloma and carrageenan-induced air-pouch model in rats along with the biochemical parameters like SGPT, SGOT, lipid per oxidation and ALP were also estimated as supportive studies. Oral administration of the extract at the doses 150 and 300 mg/kg b.w. exhibited dose dependent and significant anti-inflammatory activity in acute (carrageenan-induced hind paw oedema) and chronic (cotton pellet granuloma and carrageenan-induced air-pouch models) of inflammation. The extract also showed significant results for biochemical parameters.
The anti-inflammatory activity of many medicinal plants have been similarly evaluated viz., *Curcuma amada* (Mujumdar et al., 2000) *Gochnatia polymorpha* (Moreira, 2000); *Goniothalamus andersonii* (Shigeo et al., 2001); *Cassia angustifolia, Rheum palmatum, Coptis chinensis, Phellodendron amurense* and *Scutellaria baicalensis* (Cuéllar et al., 2001); *Leucas aspera* (Goudgaoen et al., 2003); *Calendula officinalis, Hypericum perforatum, Plantago lanceolata and Glycyrrhiza glabra* (Herold et al., 2003); *Alchornea cordifolia* (Mavar et al., 2004); *Erigeron floribundus* (Asongalem, 2004); *Synurus deltoids* (Park et al., 2004b); *Securidaca longipedunculata* (Okoli, 2005); *Bacopa monniera* (Shabana, 2006); *Andrographis Paniculata* (Sheeja et al., 2006); *Ruta graveolens* (Ratheesh and Helen, 2007).

However, there are a few reports on the anti-inflammatory activity of *E. echinatus*. Singh et al., (1989) reported the anti-inflammatory studies of ethanol extract of *E. echinatus* whole plant. Ethanol extract of *E. echinatus* inhibited the acute inflammation induced in rats by carrageenan, formaldehyde and adjuvant and the chronic arthritis induced by formaldehyde and adjuvant. The extract was more effective parenterally than orally. Funda and Bilge (1995) reported a significant anti-inflammatory activity of *E. echinatus*. Tunon et al., (1995) also reported a potent a significant anti-inflammatory activity of *E. echinatus*. They evaluated the anti-inhibitory activity based on prostaglandin biosynthesis and platelet activating factor (PAF)-induced exocytosis in vitro.

A new anti-inflammatory active flavanone namely, glycoside 5,7-dihydroxy-8,4-dimethoxyflavanone-5-O-a-l-rhamno-pyranosyl-7-O-b-d-arabinopyranosyl-(1,4)-O-b-d-glucopyranoside and a known compound dihydroquercetin-4'-methyl ether have been isolated from the leaves of *E. echinatus* (Yadava and Singh, 2006)

Eventhough, there are few reports suggesting the presence of anti-inflammatory activity in *E. echinatus*, in these investigations as well this parameter was selected to confirm and strengthen the earlier works.
E. Antimutagenic activity

i. Mechanism of action of antimutagens:

A large number of antimutagens of plant origins are known. The mechanisms through which these antimutagenes decrease the level of mutations are different due to specificity to influence certain stages of the mutation process. Antimutagenic effects may result from inhibition of metabolic activation of promutagen inactivation of activated genotoxicants (Weisburger et al., 1990) as well as from chemical and enzymatic inactivation of genotoxic agents as explained by Wattenberg et al., (1985). According to Kuroda et al., (1990) these antimutagenic agents can also correct the mutation process by acting during the DNA replication and repair process.

There are several reports on the genotoxic effects of synthetic chemicals, such as drugs, pesticides and many chemicals which we use in our day to day life. Dunipace et al., (1987) have studied the genotoxic effects of fluoride in mouse bone marrow micronuclease test. A potential influence of sodium fluoride (NaF) was examined on mammalian cells by means of mouse bone marrow micronuclease test. The animals treated with various dose of NaF, the humera were analysed for fluoride content for monitoring of absorption of fluoride. The bone fluoride content increased as the dose of NaF was increased.

Rita et al., (1991) studied the antimutagenic effect of glutathione (GSH) on mitomycin-C (MMC) induced micronuclei in bone marrow erythrocytes of Swiss albino mice. Plewa et al., (1991) have evaluated the suppression of mutagenic activity of aromatic amine compound by diethylthiocarbamate. Grover et al., (1992) have reported the antimutagenic activity of acetone extract of bark and fruit powder of *Terminalia chebula* in *Salmonella typhimurium* test system. The mutagenic and antimutagenic activities of extracts and chromatographic fractions of *Uncarea tomentosa* (Tobacco) bark has been reported by Rizzi et al., (1993). The plant extract showed protective antimutagenic effect *in vivo* against the mutations was induced by 8-methoxy-psoralen (8 MOP) plus UVA in *S. typhimurium* TA 102 strain.
Yen et al., (1996) had undertaken the comparative study on antimutagenic potential green, black and decaffeinated teas, and also studied the contribution of flavonols to the antimutagenic effect. All selected types of tea inhibited the NADPH-dependent reduction of cytochrome C and the O-dealkylations of ethoxy, methoxy and pentoxy resorufin. Concentration dependent suppression of mutagenic response finally concluded that flavonols may be the components responsible for antimutagenic properties.

Sreekumar and Hosono (1998) have studied the antimutagenic properties of polysaccharide produced by Bifidobacterium longum, in skim milk. The increase in fermentation time significantly increased antimutagenicity with all strains tested against the mutagenecity of 3-amino-1, 4-dimethyl-5H-Pyrido [4, 3-b] indole (Trp-P-1) and 3-amino-1-methyl-5H-Pyrido [4, 3-b] indole Trp-P-2). The isolated crude polysaccharide showed a dose dependent inhibition on the mutagenecity of Trp-P-1.

Centchtroman, a non-steroidal oral contraceptive was used to evaluate the antimutagenic effect in vivo in Swiss albino mice by measuring chromosome aberrations against three positive mutagen dimethylbenz(a) anthracene, cyclophosphamide and mitomycine, results indicated that compound can reduce the mutagenic effects of known genotoxic compounds Giri et al., (1999). Kimura et al., (1999) have reported the antimutagenic effect of Glycine betaine in beer against 2-chloro 4-methylthioburanoic acid, the sanma fish mutagen in Salmonella typhimurium TA-100 and TA-1535.

Krikova et al.,(1999) have studied the antimutagenic acitivity of suberin extract from Quercus suber (cork) in Euglena gracilis, which showed protection against acridine orange, ofloxacin and UV radiation induced mutations in Euglena gracilis. Arriaga–Alba et al., (2000) studied the antimutagenic acitivity of β-carotene to quinolone induced mutation in Salmonella typhimurium. The protective effects of a plant compound caffeine has been shown to protect the mutagenic effects of a known mutagen ethylmethane sulfonate in mouse bone marrow cells (Harish et al., 2000)

Lena et al., (1999) have reported antimutagenic acitivity in Salmonella typhimurium by the protein of Propionbacterium freudenreichii sub species Shermanii
against 4-nitroquinoline-1-oxide, N-methyl-N'-nitro-N-nitrosoguanidine, sodium azide and 9-aminoacridine. The efficiency of Coleus aromaticus extract in modifying cyclophosphamide and mitomycin-C induced clastogenesis in mouse bone marrow cells. The anticlastogenic activity of the Ethanolic extract of leaves of Coleus aromaticus was investigated by taking bone marrow chromosomal aberration assay and micronucleus test as the parameters (Prasad et al., 2002). Ferguson et al., (2003) have evaluated the antimutagenic activity of hydroxycinnamic acids from plant cell walls on simple bacterial model.

Hamss et al., (2003) have reported the antimutagenic properties of Bell pepper (Capsium annum) and Black Pepper (Piper nigrum) on Drosophila melanogaster. The results have reported that Bell pepper was effective in reducing the mutational events, induced by ethyl carbamate and the methyl methanesulfonate and Black pepper was only effective against ethyl carbamate.

Suriyo et al., (2004) have studied the protective action of ascorbic acid as an antioxidant against genotoxicity and cytotoxicity in mice during p-DAB induced hepatocarcinogenises in mice. Nersesyan and Muradyan (2004) have reported the antigenotoxicity effect of Sea-Buckthron juice on micronuclease frequency in bone marrow cells induced by cisdichloroamine platinum-II. Saenphet et al., (2005) have investigated the antimutagenic activity of aqueous extract of Thubergia laurifolia against the mutagenicity induced by Pureraria micrifica at a dose of 600mg/kg in rats PCEs. Jelena et al., (2007) have reported antimutagencity potential of volatile terpenes from sage oil against UV-induced mutations.

Halder et al., (2005) have evaluated the antimutagenic activity of black tea polyphenols, theaflavins and thearubigins in vivo in bone marrow cells of mice in terms of chromosomal aberration against benzo(a)pyrene, which indicated a significant decrease in mutagenicity. Smerak et al., (2006) have reported the antimutagenic effects of epigallacatechin gallate by using Ames test, micronuclease test and comet assay.

Ginseng exhibited antioxidant and antimutagenic activity. The concentrated root extract of panax ginseng and its lyophilized powder tested against mutagenesis using
Ames test system. The extract exhibited antimutagenic activity against mutations incurred by hydrogen peroxide, 4-Nitroquinoline-N-oxide and tert-butylhydroperoxide. Antimutagenic activity may be due to its property to initiate and promote DNA repair (Geetha et al., 2006). Santos et al., (2007) have evaluated the antimutagenic activity of aqueous extract of Randia echinocarpa fruit using Salmonella micro suspension assay and 1-nitropyrene as mutagen.

Recently, Aqil and Ahmdad (2008) have reported the antimutagenic activity of four ayurvedic medicinal plants. Methanolic extracts of Acorus Calamus (Rizome), Hemidesmus indicum (stem), Holarrhena antidysenterica (Bark) and Plumbago zeylanica (Root) exhibited the inhibition of His+ revertants from 18.5% to 82.66% against direct acting mutagens such as methyl methanesulphonate (MMS) and sodium azide (NaN3) induced mutagenicity in Salmonella tester strain TA 97a, TA100,TA 102 and TA 104. Hyun and Seung Shi Ham (2008) have evaluated the antioxidant and antimutagenic activities of 70% ethanol extract from masou salmon (Oncorhynchus masou).

In very recent reports by Parvathy and Srinivas (2009) the antioxidant, antimutagenic and antibacterial activities of curcumin–beta-diglucoside have been shown. The mutagenicity studies showed that high protection against the mutagenicity of sodium azide to Salmonella typhimurium tester strain TA 1531 and TA 98 system. Mialn Nagy et al., (2009) reported antimutagenic activity and radical scanenging activity of water extracts of Ligustrum plants leaves against ofloxacin induced genotoxicity in the unicellular flagellae Euglena gracilis. Similarly, Ham et. al, (2009) reported antimutagenic effects of subfractions of Chaga mushroom (Inonotus obliquus) extract in Salmonella typhimurium tester strain TA 98 and TA 100 system.

F. Anti-tumour activity

Cancer claims 6 million lives in each year on a world wide basis, cancer represent the single largest cause of death in both men and women. Cancer is a class of disease or disorders characterized by uncontrolled division of cells and the ability of these cells to invade other tissues, by implantation into distant sites by metastasis (Halliwell and
Cancer may effect people at all ages, but risk tends to increase with age, due to the fact that DNA damage becomes more apparent in aging DNA. It is the principle cause of death in developed countries. Statistics indicate that men are largely plagued by lung, colon, rectum, and prostate cancer, whilst women increasingly suffer from breast, colon, rectum, and stomach cancer (Abdulla and Gruber, 2000). Despite many therapeutic advances in the understanding of the processes in carcinogenesis, overall mortality statistics are unlikely to change until there is a reorientation of the concepts for the use of natural products as new chemopreventive agents.

ROS/RNS have been shown to possess many characteristics of carcinogens. Mutagenesis by ROS or RNS could contribute to the initiation of cancer, in addition to being important in the promotion and progression phases, causing structural alteration in DNA, e.g. base pair mutations, rearrangements, deletions, insertion and sequence amplification. They affect cytoplasmic and nuclear signal transduction pathways (Schreck et al., 1992; Burdon et al., 1995) modulate the activity of the proteins and genes that responds to stress and which act to regulate the genes that are related to cell proliferation, differentiation and apoptosis (Cerutti, 1994; Schreck et al., 1992; Burdon et al., 1995). There is considerable evidence that ROS/RNS are somehow involved in the link between chronic inflammation and cancer. Inflammation can accelerate the development of cancer (Rosin et al., 1994). A notable activity of tumour promoters is their ability to recruit inflammatory cells and to stimulate them to generate ROS/RNS.

Plants have a long history of use in the treatment of cancer (Hartwell, 1982). A contribution towards the chemotherapy of cancer, natural product secondary metabolites from plants and microbes in particular, play a very important role in the amelioration of this group of diseases. A major group of these products are the powerful antioxidants, others are phenolic in nature, and the remainder includes reactive groups that confer protective properties. Several plant-derived compounds are currently successfully employed in cancer treatment.

In the 1970s, perhaps one of the significant break-through in the field of anticancer drugs comes from the Madagascan periwinkle. Vinblastine and Vincristine are the two
vinca alkaloids isolated from periwinkle *Catharanthus roseus*. They have proven to be effective agents against childhood leukemia, breast cancer, Hodgkin’s disease and choriocarcinoma. Vinblastine and Vincristine exert their anticancer properties by inhibiting mitosis by binding to tubulin, thus, preventing the cell from making spindles it needs to be able to move chromosomes around it and divide. The two clinically active agents etoposide and teniposide, which are semisynthetic derivatives of natural product, epipodophyllotoxin derived from *Podophyllum peltatum* (May apple) and *Podophyllum emodi* (Lee and Xiao, 2005). Extensive research led to the development of etoposide and teniposide as clinically effective agents which are used in the treatment of lymphomas, branchial and testicular cancers.

A more recent addition to the armamentarium of plant-derived chemotherapeutic agents is the taxanes (Kingston, 2005). Paclitaxel was isolated from *Taxus brevifolia*. Paclitaxel, as well as active paclitaxel analogs, such docetaxel, have provided a major, renewable natural source of this important class of drugs. In addition 23 taxanes are in preclinical development as potential anticancer agents. Topotecan and irinotecan are the clinically active agents derived from camptothecin, which is isolated from Chinese ornamental tree, *Camptotheca acuminata*. Topotecan is used for the treatment of ovarian and small cell lung cancers, while irinotecan is used for the treatment of colorectal cancers. Other plants-derived agents in clinical use are homoharringtonine, isolated from the Chinese tree, *Cephalotaxus harringtonia var drupacea* (Itokawa et al., 2005), and elliptinium, a derivative of ellipticine, isolated from species of several genera of the apocynaceae family, including *Bleekeria vitensis* A.C. Sm., a Fijian medicinal plant with reputed anticancer properties. Elliptinium is marketed in France for the treatment of breast cancer. A number of additional plant-derived agents are currently under investigation such as 4-Ipomeanol (Rehm and Devor, 1993; Rowinsky et al., 1993), β-lapachone (Li et al., 1999), etc.

**G. Antioxidants in cancer therapy**

The biological activities of medicinal plants are attributed mostly to their bioactive chemicals. Many of the common fruits and vegetables contain bioactive
compounds with antioxidant activities that may potentially be chemoprotective against a variety of cancers (Wargovich et al., 2001). Polyphenols such as resveratrol modulate the cell signaling pathways, inhibition of angiogenesis, induction of apoptosis and type II programmed cell death. Curcumin neutralizes ROS by induction of detoxifying enzymes, inhibition of Cyclooxygenase (COX-1 and COX-2) and phospholipase A2 (PLA2) enzymes, induction of apoptosis and down regulation of β-catenin and catechins show their antioxidant activity by modulation of cell signaling pathways, inhibition of COX-2 and iNOS enzymes, antiangiogenic, induction of apoptosis (Kris-Etherton et al., 2002). In polyphenols the hydroxyl groups attached to aromatic rings create an electron-rich environment that traps the ROS precluding them from reacting with nucleophilic centers in cellular proteins and DNA.

Studies in cell cultures show that vitamin E, vitamin C, selenium, and some polyphenols selectively induce apoptosis in cancer cells while sparing normal cells (Sigounas et al., 1997; Taper et al., 2004). Other findings, in a model of metastatic growth, show that vitamin C is an angiostatic factor and may have potential in aiding host resistance to tumour growth and invasiveness (Ashino et al., 2003). Antioxidants also show promise in cancer therapy by their palliative action, reducing painful side effects associated with treatment (Kennedy et al., 2001).

Kavimani et al., (1999) reported antitumour activity of the methanolic extract of *G. lotoides* against Dalton's ascitic lymphoma (DAL) in Swiss albino mice. A significant enhancement of mean survival time of tumour bearing mice and peritoneal cell count in normal mice was observed with respect to the control group receiving 5-Flourouracil (20 mg/kg). Tumour cell growth was inhibited in albino mice treated with extract of *Glotoide* (50 mg/kg) after intra peritoneal inoculation with DAL cells. After 14 days of inoculation, the extract of the plant is able to reverse the changes in the haematological parameters, such as total white blood cells count, protein and packed cellular volume consequent to tumour inoculation.

Gupta et al., (2000) studied effects of methanol extract (ME) of *Cassia fistula* seed on the growth of Ehrlich ascites carcinoma (EAC) and on the life span of tumour
bearing mice were studied. ME treatment showed an increase of life span, and a decrease in the tumour volume and viable tumour cell count in the EAC tumour hosts. Cytological studies have revealed a reduction in the mitotic activity, and the appearance of membrane blebbing and intracytoplasmic vacuoles in the treated tumour cells. Improvement in the hematological parameters following ME treatment, like hemoglobin content, red blood cell count and bone marrow cell count of the tumour bearing mice have also been observed. The results of the present study suggest that ME of C. fistula seed has an antitumour activity.

Rajkapoor et al., (2004) evaluated the antitumour activity of the ethanol extract of Indigofera aspalathoides (EIA) in mice against the (EAC) tumour model. The activity was assessed using survival time, peritoneal cell count, hematological studies, solid tumour mass and in vitro cytotoxicity. Oral administration of EIA increased the survival time and normal peritoneal cell count. Hematological parameters, protein and PCV, which were altered by tumour inoculation, were restored. Solid tumour mass was also significantly reduced. EIA was found to be cytotoxic in the in vitro model. EIA possesses significant antitumour activity.

Malaya et al., (2004) studied the antitumour effect and antioxidant role of Bauhinia racemosa, the antitumour activity and antioxidant status of methanol extract (50, 100 and 200 mg/kg) of B. racemosa stem bark against Ehrlich ascites carcinoma (EAC) tumour in mice. Acute and short-term toxicity studies were performed initially to ascertain the safety of methanol extract of B. racemosa (MEBR). After 24 h of tumour inoculation, the extract was administered daily for 14 days. After the administration of the last dose followed by 18-h fasting, mice were then sacrificed for observation of antitumour activity. The effects of MEBR on the growth of transplantable murine tumour, lifespan of EAC-bearing hosts and simultaneous alterations in the haematological profile and liver biochemical parameters (lipid peroxidation, antioxidant enzymes) were estimated. The MEBR showed decrease in tumour volume, packed cell volume and viable cell count, and increased the non-viable cell count and mean survival time thereby increasing lifespan of EAC tumour-bearing mice. The haematological profile reverted to more or less normal levels in extract-treated mice. Treatment with MEBR decreased the
levels of lipid peroxidation and increased the levels of glutathione, superoxide dismutase and catalase. The methanol extract of *B. racemosa* stem bark exhibited antitumour effect by modulating lipid peroxidation and augmenting antioxidant defence system in EAC-bearing mice.

Santoshkumar *et al.*, (2007) reported that the methanol extract of *H. hookerianum* Wight and Arnott stem (MEHH) exhibited potent *in vitro* cytotoxic activity against various cancerous cell lines. And also reported for *in vivo* antitumour properties against Ehrlich ascites carcinoma (EAC) tumor bearing mice at 100, 200, and 400 mg/kg body weight doses given orally once daily for 14 days. The results indicated that administration of the extract not only increased the survival of animals with ascites tumour, decreased the body weight induced by the tumour burden, and reduced packed cell volume and viable tissue cell count, but also altered many hematological parameters changed during tumour progression, indicating the potent antitumour nature of the extract. Among the three doses tested, the 200 mg/kg body weight dose was found to be the most potent.

Meenakshi *et al.*, (2008) evaluated the antitumour and antioxidant potential of the ethanol extract of *Tragia Plukenetii* R.Smith (ETP) on Ehrlich ascites carcinoma (EAC) tumour model. Tumour was induced in mice by intraperitoneal injection of EAC cells (2x10^6 cells/mouse). Ethanol extract of *T. Plukenetii* (ETP) was administered to the experimental animals at the dose levels of 100, 200 and 300 mg/kg/day after 24 h of tumour inoculation. The antitumour effect of ETP was evaluated by assessing *in vitro* cytotoxicity, survival time, hematological and antioxidant parameters. Oral administration of ETP increased the survival time of the EAC bearing mice. The ETP brought back the altered levels of the hematological and antioxidant parameters in a dose dependent manner in EAC bearing mice. The results were comparable to that of the result obtained from the animals treated with the standard drug 5-floururacil (20 mg/kg.bw). Thus present study revealed that ETP possessed significant antitumour and antioxidant activity.

Gupta, *et al.*, (2008) The methanol extract of *Caesalpinia bonducella* (*C. bonduc*) leaves (MECB) were evaluated for antitumour activity against Ehrlich ascites carcinoma
(EAC)-bearing Swiss albino mice. The extract was administered at doses of 50, 100, and 200 mg/kg body weight per day for 14 days after 24 h of tumour inoculation. After the last dose and 18 h fasting, the mice were sacrificed. MECB caused significant decrease in tumour volume, packed cell volume, and viable cell count; and it prolonged the lifespan of EAC-tumour bearing mice. Haematological profile converted to more or less normal levels in extract-treated mice. MECB significantly decreased the levels of lipid peroxidation and significantly increased the levels of GSH, SOD, and CAT. The MECB was devoid of conspicuous short-term toxicity in the mice when administered daily (i.p.) for 14 days at 50, 100, 200, and 300 mg/kg. The treated mice showed conspicuous toxic symptoms only at 300 mg/kg. MECB exhibited significant antitumour and antioxidant activity in EAC-bearing mice.

Raju Senthilkumar et al., (2008) examined the antitumour activity of *Pisonia aculeata* Linn. (Nyctaginaceae), leaves extract on Ehrlich Ascites Carcinoma (EAC) in mice. Tumour was induced in mice by intraperitoneal injection of Ehrlich Ascites Carcinoma cells (1X10^5 cells/mouse). Ethanol extract of *Pisonia aculeata* (EEPA) was administered to the experimental animals at the doses of 250 & 500 mg/kg/day, p.o. The antitumour effect of the extract was evaluated by using survival time, hematological parameters, body weight increase, solid tumour volume and peritoneal cell count. Oral administration of EEPA increased the survival time and inhibits the weight gain of the tumour bearing mice. After 14 days of inoculation, the extract also reduces the solid tumour volume developed by the EAC cells. The findings of this study indicate that the EEPA possesses significant antitumour activity on dose dependent manner.

Wenrong et al., (2008) isolated thiophenes from dichloromethane fraction of the crude ethanol extract of roots of *Echinops grijissi* Hance. The isolated thiophenes exhibited different cytotoxicity against a panel of four human tumour cell lines, HepG2, K562, HL60 and MCF-7, and they were assayed for their toxicity against the cell lines in order to compare their relative anti-tumour activity and find candidates of potential anti-tumour drugs.
Ashok Kumar et al., (2009) investigated the antitumour effect and antioxidant role of the methanol extract of *Oxystelma esculentum* R. Br. (Asclepiadaceae) (MEOE) on tumour growth and the host survival time with mice. The antitumour and antioxidant potential of *Oxystelma esculentum* were studied against Ehrlich’s ascites carcinoma cell line (EAC) treated mice. MEOE was administered at doses of 200 and 400 mg/kg body weight once a day for 9 days after 24 h of tumour inoculation. Among the treated animals, six animals were sacrificed for biochemical and tumour analysis, and the remaining four groups were kept to study lifespan. On day 10, the parameters of tumour volume, packed cell volume, viable, and non-viable cell count were studied. Hematological and liver biochemical parameters and antioxidant enzymes such as lipid peroxidation (LPO), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), etc. were estimated. Decreases in tumour volume, packed cell volume, and viable cell count were observed in MEOE-treated mice when compared to EAC-treated mice. Treatment with MEOE at doses of 200 and 400 mg/kg increased the mean survival time to 29.66 ± 0.71 and 34.33 ± 2.34 days, compared with EAC-treated mice at 19.16 ± 1.13 days. The extract also decreased the body weight of the EAC-bearing mice. Hematological profiles indicated a decrease in white blood cells (WBC), an increase in red blood cells (RBC), and, thereby, Hemoglobin (Hb). MEOE restored all the parameters of hematological profiles to approximately normal. Treatment with MEOE decreased the levels of LPO and increased the levels of GSH, SOD, and CAT. These data indicate the methanol extract of *Oxystelma esculentum* exhibits significant antitumour activity, which might be due to the antioxidant effects on EAC bearing hosts.