Chapter 1
Introduction

India is very popular for its traditional medicine treatment like Ayurveda, Siddha, and Unani, as mentioned even in the ancient Vedas and other scriptures. (Subhose *et al.,* 2005). Ayurveda means "science of life" and "science of longevity", because ancient Indian system of health care focused on views of human and their illness. It offers a complete system to live a long healthy life. In India, during the Buddhist period plant resources were used for treating many diseases. Ayurvedic medicine has a vast literature in Sanskrit covering various aspects of diseases, therapeutics, and pharmacy. *Rig veda*, an ancient Indian sacred collection of Vedic Sanskrit hymns, and the *Atharvaveda*, the fourth and last Veda of Hindu literature, are the earliest references to such plants, minerals, and animal products with their usage for medical purposes (Pan *et al*., 2014). India possesses almost 8% of the estimated biodiversity of the world including 126,000 species. Among 400 families of flowering plants in the world, at least 315 of these can be found in India (Pan *et al*., 2014). Currently, about 45,000 species (nearly 20% of the global species) are found in the Indian subcontinent (Singh, 2006). The western part of the Himalayan region possesses about 80% of herbal drugs in Ayurveda, 46% of Unani, and 33% of allopathic systems (Figure 1) (Baragi *et al*., 2008). The Indian Herbal Medicine (IHM) is derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, and so forth. Some drugs are also prepared from excretory plant products such as gum, resins, and latex and commonly used spices, herbs, and herbal formulae are used for the treatment of about 28 kinds of chronic diseases in humans (Sharma *et al*., 2007).

Herbal drugs still have their place in day-to-day therapy in spite of the presence of modern synthetic drugs and antibiotics. Indian Herbal Medicines are popular because of ease in access, low-cost and comparative freedom from serious side-effects. There are many adverse side-effects like hepatotoxicity, hypersensitivity, immunosuppression and allergic reactions associated with the synthetic drugs and antibiotics. Thus, bioactive compounds or chemicals derived from the plants have drawn the main

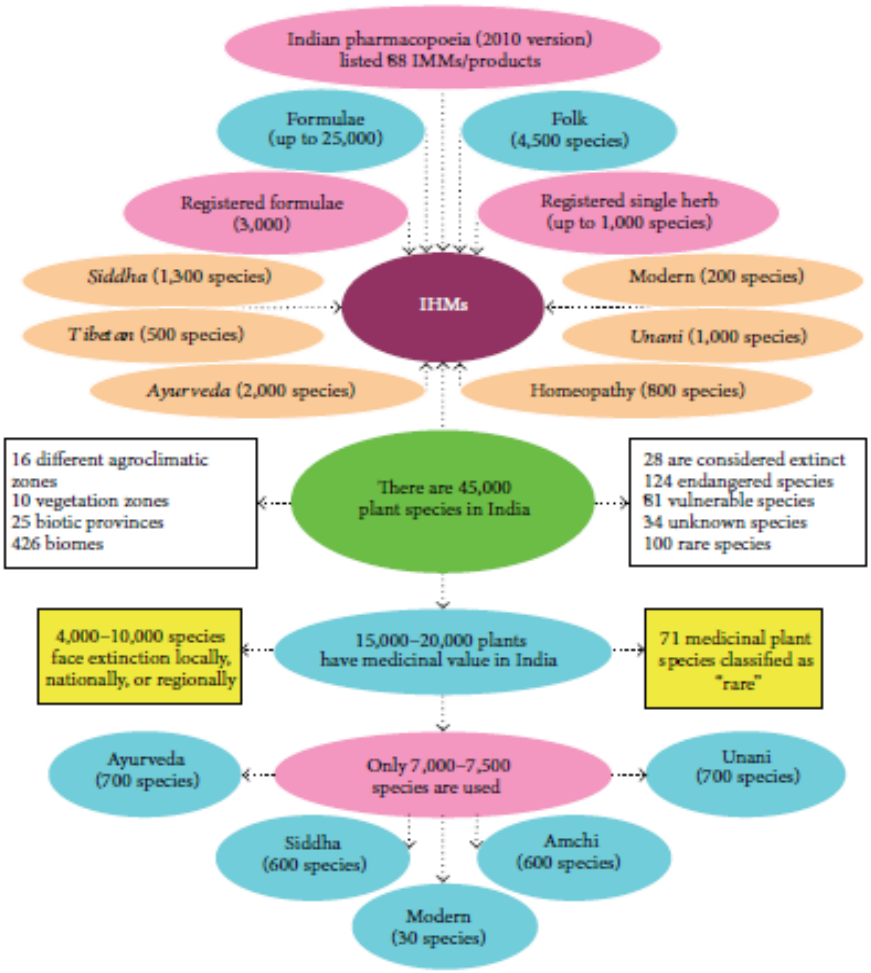

Figure 1: Plant species in India and Indian herbal medicine (IHM) (Pan *et al.*, 2014)

attention as a source of alternative medicines.

Evaluation of antioxidant and free radical scavenging activities of medicinal herbs are the first and highly preferred approaches for the screening of herbal medicines for their therapeutic purpose. Oxygen is indispensable for the survival of most of the life forms. However, most of the unused oxygen is transformed into various reactive species. This reactive oxygen species (ROS) are mainly produced in the cell by the mitochondrial respiratory chain which imparts an oxidative stress producing super oxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH•) etc. during endogenous metabolic reactions. ROS are also produced by myeloperoxidase (MPO) - Halide - H_2O_2 system where, in the presence of chloride ion H_2O_2 is converted to hypochlorous acid, a potent oxidizing agent (Balasaheb and Pal, 2015). ROS in low concentration is essential for our physiological functions like cellular growth, gene expression and this may provide defense against infection too. However, inability to detoxify excess ROS by our body may cause oxidative stress. The oxidative stress may in turn increases

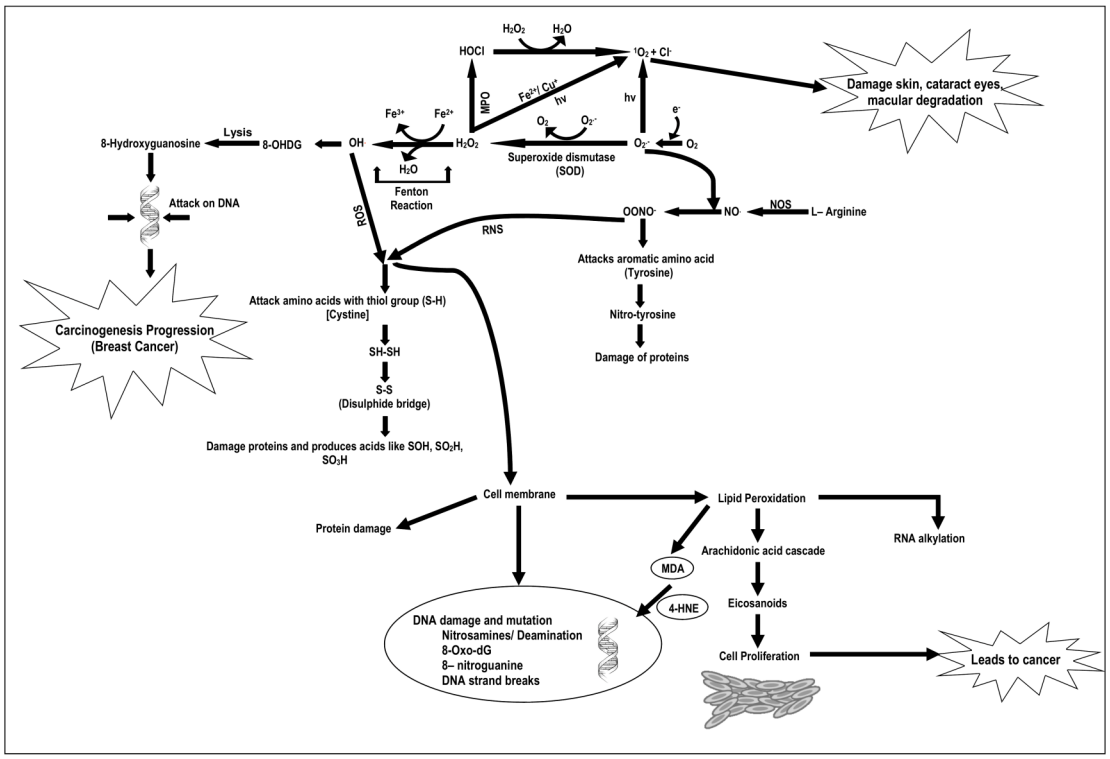

Figure 2: Schematic representation showing the free radical generation followed by the chain of by-product (ROS/RNS) formed due to oxidative stress and how they affect biological systems by cellular stress and even leading to carcinogenesis. [HOCl: Hypochlorous acid; H_2O_2: Hydrogen peroxide; $^1O_2$: Singlet oxygen; $O_2^{\cdot-}$: Superoxide; OH•: Hydroxyl radical; 8-OHdG: 8-hydroxy2-deoxy guanosine; $OONO^-$: Peroxynitrate; $NO^{\cdot}$: Nitric oxide; Fe^{2+}: Iron ion; Cu^+: Copper ion; Cl^-: Chlorine ion; ROS: Reactive oxygen species; RNS: Reactive nitrogen species; NOS: Nitric oxide synthase; MPO: Myeloperoxidase; MDA: Malondialdehyde; 4-HNE: 4-hydroxynonenol].

the risk of various diseases like diabetes, cancer, obesity, rheumatoid arthritis, cognitive disorders and ageing (Durackova, 2010; Poyton *et al*., 2009). On the other hand, reactive nitrogen species (RNS) as well as nitric oxide (NO) are generated under hypoxic condition during mitochondrial respiratory chain (Poyton *et al*., 2009). RNS induces excessive lipid peroxidation which may lead to the production of other reactive species like reactive aldehydes and malondialdehyde. These 'oxyradical overload' may lead to a variety of diseases including cellular inflammation, cancer by way of DNA damage, protein modification and by changing transcriptional parameters. All these may lead to the increased cellular homeostatis. Besides, accumulation of ROS by soluble mediators like arachidonic acid, cytokines and chemokines secreted by inflammatory cells, activate several signal transduction cascades including changes in transcription factors such as NF-κβ, AP-1, Nrf2, p53, SP1, HIF-1α, STAT3 and PPARγ. The activation of these transcription factors by ROS may lead to sustained inflammation

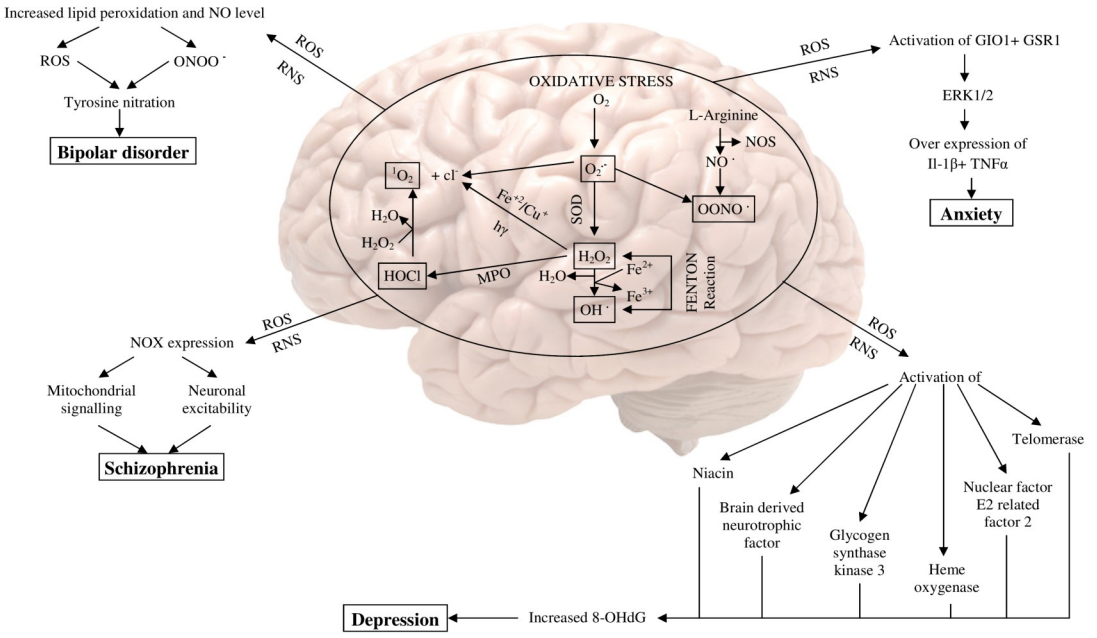

Figure 3: Schematic representation showing the free radical generation followed by the chain of by-product (ROS/RNS) formed due to oxidative stress and how they affect biological systems by cellular stress and even leading to different types of mental disorders. [HOCl: Hypochlorous acid; H_2O_2: Hydrogen peroxide; $^1O_2$: Singlet oxygen; $O_2^{\cdot-}$: Superoxide; OH•: Hydroxyl radical; 8-OHdG: 8-hydroxy2-deoxy guanosine; $OONO^-$: Peroxynitrate; $NO^{\cdot}$: Nitric oxide; Fe^{2+}: Iron ion; Cu^+: Copper ion; Cl^-: Chlorine ion; ROS: Reactive oxygen species; RNS: Reactive nitrogen species; NOS: Nitric oxide synthase; MPO: Myeloperoxidase; MDA: Malondialdehyde; 4-HNE: 4-hydroxynonenol; NOX: Nitrous oxide; TNF α: Tumor necrosis factor α; ERK: Extra cellular signal regulated kinases; SOD: Super oxide dismutase].

or an oxidative environment which in turn might lead to the damage of healthy neighbouring epithelial and stomatal cells over a long period of exposure which might lead to cancer (Figure 2) (Hussain and Harris, 2007; Reuter *et al.*, 2010).

"Everybody knows what stress is, and nobody knows what it is", once noted by Hans Selye (1973). According to current knowledge, it is safe to define stress as "a physical, chemical, or an emotional factor that causes bodily harm often causing disease". The association between stress, anxiety, depression and cognitive dysfunction comes to mind, if we focus on stress as an "emotional factor". Now-a-days, 'stress' can be defined as altered biochemical process produced or enhanced by psychological, physiological or environmental stimuli. High oxygen consumption makes the brain vulnerable to redox imbalance. Oxidative stress in the brain causes the nervous system impairment and kick off depression, anxiety disorder and high anxiety level. Anxiety is an unpleasant emotional state, in which the feeling of fear is incommensurate to the nature of the threat

(Weinberger, 2001). There are many factors like emotional stress, behavioral, expressive and physiological features enhance the subjective features of anxiety. Anxiety is very common in US populations and constitutes approximately 28.8% of the total population (Kessler *et al*., 2005). In India, about 29% of girls and 23% of boys among school going kids were found to suffer from Psychiatric disorders (Jayanthi *et al*., 2015). A population based study revealed that anxiety disorder goes almost untreated (de Graaf *et al*., 2002; Issakidis *et al*., 2002). Oxidative stress causes the lipid peroxidation in the brain and result in decrease of the membrane fluidity, damage in membrane protein inactivating receptors, enzyme and ion channels (Valko *et al*., 2007; Delattre *et al*., 2005; Lebel, 1991). As a result, neurotransmission, neural function and overall brain activity can be altered by 'oxidative stress' (Delattre *et al*., 2005; Cardozo *et al*., 1999). ROS generated oxidative stress has been linked with several diseases which are specific for nervous system impairment including neurodegenerative and neuropsychiatric diseases, such as schizophrenia, bi-polar disorder and major depressive disorder (Figure 3) (Valko *et al*., 2007; Delattre *et al*., 2005; Bilici *et al*., 2001; Yao *et al*., 2001).

In our biological system, excessive reactive species derived from oxygen and nitrogen may lead to the oxidative damage tissues and organs. Oxidative stress can be considered as a combined pathological mechanism, and it induces the initiation and progression of liver injury. The liver is associated with most of the physiological and metabolic functions in our body system. Bio-transformation and metabolism of xenobiotic compounds are enhanced by liver, which in some cases cause liver damage. Liver is a crucial organ attacked by ROS (Sanchez-Valle *et al*., 2012). Parenchymal cells may get damaged by oxidative stress and cause injury in liver. ROS are produced by mitochondria, microsomes and peroxisomes in parenchymal cells, regulating on PPARα, a fatty acid oxidation gene expression. Moreover, oxidative stress molecules are potentially more sensitive for kupffer cells, hepatic stellate cells and endothelial cells. Oxidative stress is produced by variety of cytokines like TNF-α secreted by kupffer cells. Drug induced hepatotoxicity has become the leading cause behind the acute liver failure among the patients of United States (Kaplowitz, 2005). Among all the organs in our body, liver is one of the most vital organs which safeguards from the harmful chemicals. Continual exposure of xenobiotics causes serious damage of liver due to its portal location in the circulation and central role in detoxification (Jones, 1996; Dey *et al*., 2013). Halogenated alkenes are the organic xenobiotics which hold the potentialities to cause liver injury

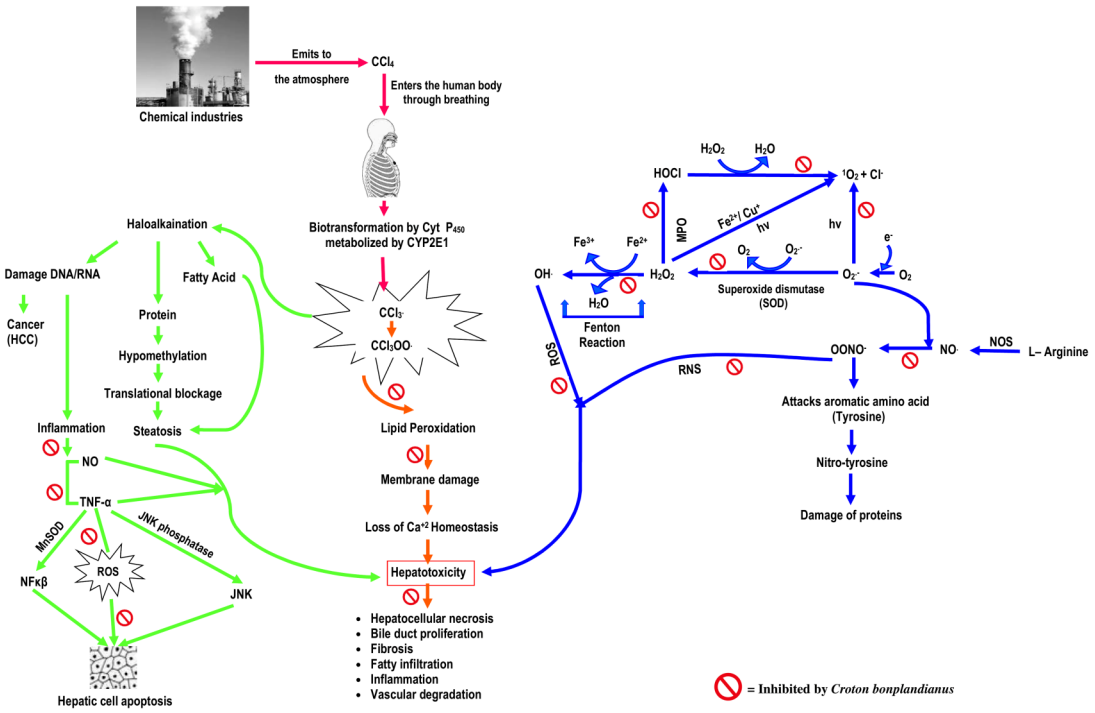

Figure 4: Schematic representation showing the free radical generation followed by the chain of by-product (ROS/RNS) formed due to oxidative stress and how they affect biological systems by cellular stress and CCl_4 induced hepatotoxicity. The pathway demonstrates the mechanism of CCl_4 induced hepatotoxicity which is predominantly mediated by oxidative stress and inflammatory injury due to the formation of reactive metabolic intermediates and the free radical formation cascade during xenobiotic induced hepatotoxicity causing oxidative and nitrosative stress. Cyt P450 = cytochrome P450, $CCl_3^{\bullet}$ = trichloromethyle radical, $CCl_3OO^{\bullet}$ = trichloromethylperoxy radical, TNF-α = tumor necrosis factor-α,; HOCl: Hypochlorous acid; H_2O_2: Hydrogen peroxide; $^1O_2$: Singlet oxygen; $O_2^{\bullet-}$: Superoxide; $OH^{\bullet}$: Hydroxyl radical; 8-OHdG: 8-hydroxy2-deoxy guanosine; $OONO^-$: Peroxynitrate; $NO^{\bullet}$: Nitric oxide; Fe^{2+}: Iron ion; Cu^+: Copper ion; Cl^-: Chlorine ion; ROS: Reactive oxygen species; RNS: Reactive nitrogen species; NOS: Nitric oxide synthase; MPO: Myeloperoxidase; MDA: Malondialdehyde.

(Dey *et al*., 2013; Subramonium and Pushpangadan, 1999).

In recent years, Drug Induced Hepatotoxicity (DIHT) has emerged as a tremendous concern in medicine where around 75% of idiosyncratic drug reactions result in either liver transplantation or proves to be lethal (Mehta *et al*., 2014). Around 2000 cases of acute liver failure occurs in US every year and out of these cases 50% are associated with DIHT (Dewantara, 2008). Carbon tetrachloride (CCl_4) is considered as a haloalkene model which is extensively used to study the pathophysiological implications of xenobiotic metabolism and hepatoprotective potentialities of natural compounds (Weber *et al*., 2003). Liver

damage by CCl_4 is primarily generated by free radicals mediated tissue injury and inflammatory damages. On the other hand, a number of reactive oxygen or nitrogen species can kick off intracellular signaling cascade that intensify pro-inflammatory gene expression (Anderson *et al*., 1994; Flohe *et al*., 1997). Thus, oxidative stress is directly related with inflammation. CCl_4 is linked to its metabolic activation to short lived reactive intermediates. Terminal oxidase of the oxidase system cytochrome P450 catalyzed CCl_4 to form trichloromethyl radicals (Weisburger, 1977). The radicals formed by the metabolism of CCl_4 are highly energetic and cause lipid per oxidation and membrane damage. Secondary inflammatory response produced by kupffer cells lead to the secretions of several chemokines and cytokines like TNF-α. Repeated cycle of injury, inflammation and repair leads to the fibrosis and eventually hepatocellular carcinoma (HCC), the most common primary malignancy of the liver. The mechanism of HCC is chronic inflammation associated with oxidative stress occurred in cirrhotic liver (Figure 4).

Herbal immunomodulators are plant derived substances, possess the potentiality to stimulate or suppress any component or function of the immune system (both innate and humoral) or work as adjuvant with other compounds (Agarwal and Singh, 1999). Immunostimulants may provide protection against microbial infections as well as enhance body's resistance to allergy, autoimmunity and cancers. Immunosuppressants may provide control of pathological immune response during autoimmune diseases, graft and hypersensitive reactions. Besides, immunoadjuvants may be used to intensify the efficiency of vaccines. Emerging vaccines coupled with botanical immunodrugs are coming up as an exciting field of therapeutics where conventional vaccines fail to deliver required immune response (Bendelac and Medzhitov, 2002). Bioactive polysaccharide containing Japanese traditional medicine Hochu-ekki cause human dendritic cell (DC) maturation by means of up-regulating CD80, CD83 and CD86 expression without the presentation of antigen (Nabeshima, *et al*., 2004). Saponins from *Tripterygium wilfordii* may provide an alternative class of pharmaceuticals because of its down-regulation of around 75% CD80 in human DC in an IL-10 independent manner, (Wang *et al*., 2001). Targeting B-lymphocytes for effective antibody based immunity is also a routine method of immunomodulation. A polyphenol-rich extract of mango (*Mangifera indica* Linn) containing 2.6% mangiferin was demonstrated to elevate the anti-sRBC hemagglutination titre (HA) around 20 fold in murine model (Makare, *et al*., 2001). Furthermore, intraperitoneal immunization with dietary supplement

components (carvone, limonene, and perillic acid) were shown to enhance the anti-sRBC HA titre 10 fold, which was proposed to occur as a result of B-lymphocyte proliferation, resulting in upraised anti-sRBC plaque forming cell (PFC) response (Raphael and Kuttan, 2003). Similar approach was taken by Roy and his group (2013) who demonstrated the modulation of HA titre and PFC values by *Diplazium esculentum* (Koenig ex Retz.) Sw. in experimental mice. Targeting T_H1/T_H2 cell effector function has evolved as a promising strategy where modulation of T_H1 response into T_H2 response appears provocative (Patwardhan & Gautam, 2005). Saikosaponin-D, isolated from Chinese Thoroughwax (*Bupleurum falcatum* L.) enhanced Con A stimulated murine splenic lymphocytes which was associated with elevated IL-2 production (Kato *et al*., 1994). Similar effect was observed in case of a bioactive fraction of *Dioscorea alata* L. underground tuber, which demonstrated the enhanced proliferation of murine splenic lymphocytes and modulated T_H1/T_H2 cytokine balance *in vitro* (Dey and Chaudhuri, 2014).

Alternatively, Toll-like receptor (TLR) and associated innate immunity markers are presently being targeted for effective immunoadjuvant actions by botanical immunodrugs (Patwardhan and Gautam, 2005; Chahal *et al*., 2013). Liu *et al*., (2008) demonstrated that a bioactive polysaccharide fraction from *Dioscorea batatas* stimulates murine macrophages trough TLR-4 associated protein kinase signalling in order to induce TNF-α secretion *in vitro*. Very recently Ghochikyan *et al*., (2014) reported Immunomax®, an herbal polysaccharide which possesses TLR-4 agonistic activity may contribute in its anti-cancer efficiency. Cytokines being the central in-house immunoregulators of the system, provides an excellent approach in the treatment and prevention of diseases and infections (Spelman *et al*., 2006). Strategies of cytokine modulation include antagonist, agonist, inhibitory and stimulatory models *in vivo* and *in vitro*. For instance, Hodge *et al*., (2002) proposed the use of garlic extract (*Allium sativum* L.) in the treatment of inflammatory bowel disease through inhibition of TNF-α, IL-1α, IL-6, IL-8 in monocytes and IFN-γ, IL-2, and TNF-α i n T -lymphocytes. A detailed review on selective botanical immunodrugs, including dietary supplements demonstrating cytokine modulatory activity, is given by Sommer (1999). Numerous herbal remedies are presently being targeted towards different inflammatory mediators such as kinins, platelet-activating factors, arachidonic metabolites (prostaglandins), leukotrienes, amines, purines, pro- and anti-inflammatory cytokines, chemokines and celladhesion molecules (CAM) (Levine

and Reichling, 1999). In most anti-inflammatory studies, the strategies involved are: up-regulation of anti-inflammatory cytokines and down-regulation of proinflammatory cytokines, suppression of COX activities leading to inhibition of prostaglandin levels, suppression of NF-κβ activation and suppression of iNOS induction. NF-κB controls the expression of genes encoding different pro-inflammatory cytokines (IL-1, IL-2, IL-6, TNF-α,), chemokines (IL-8,MIP-1α, MCP1), adhesion molecules (ICAM, VCAM, Eselectin), inducible enzymes (COX-2 and iNOS), growth factors, some of the acute phase proteins, and immune-receptors, all of which play a critical role in controlling most of the inflammatory processes (Barnes and Karin, 1997; Ghosh and Karin, 2002).

Cognitive disorders (CDs), one kind of abnormalities that affects brain capability to remember and process information (Trivedi, 2006) at late age, could be balanced by the antioxidative defense system. CDs include dementia, amnesia, Alzheimer disease (AD), Parkinson's disease (PD), trauma, seizures and other neurodegenarative deisorders (NDs) (Chattipakorn *et al*., 2007). Dysfunction of dopaminargic neurons, cholinergic abnormalities, mitochondrial dysfunction and extensive neuronal loss in brain are the main factors of occurring NDs. In addition, ROS generates continuously in brain leading to a progressive accumulation of cellular damage, correlated with the onset of AD and PD (Gandhi and Abramov, 2012). AD is believed to be linked to a deficiency in the brain neurotransmitter, acetylcholin (ACh). Inhibition of acetylcholinesterase enzyme (AChE) is a rational pathway for the systematic treatment of AD (Prince *at el*., 2013). PD, the second most common neurodegenerative disorder after AD, is characterized by resting tremor, bradykinesis, muscular rigidity, and prostural imbalance occurring due to progressive death of substansia nigral cells leading to dysfunction of dopaminergic neurons (Chattipakorn *et al*., 2007). A recent survey reflected that about 35.6 million people lived with dementia worldwide in 2010, likely to be doubled by 2030 (Prince *et al*., 2013). Hence, neurodegenerative disorder has emerged as a great public health concern, thereby demand intervention to ameliorate oxidative stress. Several approved drugs including donepezil, tacrine, rivastigmine, galanthamine etc., to some extent, alleviate the symptoms of cognitive impairments. However, their chronic use is often associated with exerting side effects (Chattipakorn *et al*., 2007). Herbal formulations on the other hand have been documented effective against several cognitive disorders so far (Mathew and Subramanian, 2014).

Potent therapeutic properties of the phyto-medicine are due to the presence of diverse

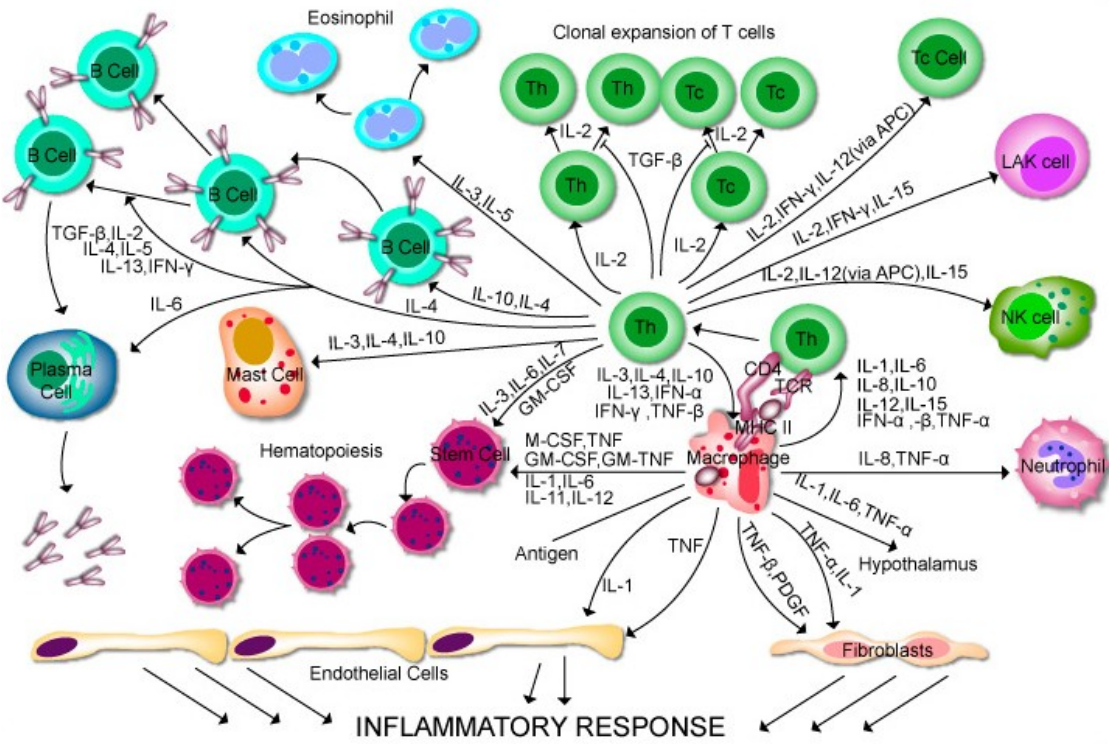

Figure 5: Schematic representation of cytokine interactions during inflammation (GeneCopoeia, 2001).

class of bioactive phytochemicals, most of which are secondary metabolites (Figure 5). Around 30% of FDA approved medicines have a botanical origin (De Smet, 2002; Licciardi and Underwood, 2011) and designer drugs are continuously being synthesized solely based on the structural and functional template of plant derived compounds. Till date, numerous plant derived compounds have been used in clinical practice. Few such major compounds are antineoplastic Paclitaxel from Taxus brevifolia, anti-cancer Vincristine/Vinblastine from Rosy periwinkle, anti-malarial Quinine from Cinchona ledgeriana, cadiotonic Digoxin from Digitalis lanata, analgesic morphine from Papaver somniferum, anti-hypertensive Reserpine from Rauwolfia serpentine, anti-cholinergic Atropine from Atropa belladonna, anti-asthmatic Ephedrine from Ephedra sinica, bronchodilator Theophylline from Camellia sinensis etc. Phytocompounds of diverse chemical natures such as isoflavonoids, indoles, phytosterols, polysaccharides, sesquiterpenes, alkaloids, polyphenols, glucans and tannins are currently being investigated for possible immunomodulatory activities. Few such immunoactive leads from herbal source are Ginsan (polysaccharide from Panax ginseng), Triptolide (terpenoid from Tripterygium wilfordii); Mistletoe lectin (lectin from Viscum album); Piperine (alkaloid from Piper longum), Matrine (alkaloid from Sophora alopecuoides), Sinomenine (alkaloid from Sinomenium

acutum), Artemisinin (lactone from Artemisia annua), Apocynin (glycoside from Picrorhiza kurroa) and Shatavarin (saponin from Asparagus racemosus). Plant derived phenolic compounds possess aromatic rings with one or more hydroxyl group attached to it. These are secondary metabolites comprising a large variety of compounds including simple phenols, phenolic acids, coumarins, flavonoids, stilbens, hydrolysable and condensed tannins, ligans and lignins (Naczk & Shahidi, 2004). Numerous phenolic compounds have been identified to posses various medicinal properties such as antioxidant, anti-carcinogenic/anti-mutagenic, anti-inflammatory, immunomodulatory, anti-diabetic, hepatoprotective, anti-microbial etc. (Huang *et al*., 2010). The physiological and pharmacological properties of the phenolic compounds are mainly attributed to their antioxidant and free radical scavenging capacities (Surh, 2003), which varies in different phenolic compounds depending on the number and position of the hydroxyl groups as well as chemical substitutions (Cai *et al*., 2006; Heim *et al*., 2002). The basic mechanism of their antioxidant capacity includes: (i) ROS scavenging, (ii) inhibiting generation of ROS either by enzymatic suppression or metal chelation and (iii) up-regulating antioxidative defence enzymes.

Different medicinal plants such as Barringtonia racemosa, Cornus officinalis, Cassia auriculata, Polygonum aviculare, Punica granatum, Rheum officinale, Rhus chinensis, Sanguisorba officinalis, and Terminalia chebula and different dietary supplements like tea, clove and thyme are reported to contain gallic acid which posses a vast array of bioactivities (Huang *et al*., 2010). Bioactive p-coumaric acid, ferulic acid and caffeic acid are the major bioactive constituents of various medicinal herbs, vegetables and fruits (Cai *et al*., 2004). Medicinal plants of the Apocynaceae and Asclepiadaceae family are reported to contain chlorogenic acid, which is ester of caffeic acid (Huang *et al*., 2007). Potent antioxidant phenolic compound rosmarinic acid is abundant in mint, sweet basil, oregano, rosemary, sage, and thyme (Shan *et al*., 2005).

Flavonoids are the most common and widely distributed naturally occurring phenolic compounds in photosynthetic plants (Kumar and Pandey, 2013). Antioxidant activity of flavonoids depends on the arrangement of functional groups around the nuclear structure. The B-ring hydroxyl group configuration is the primary determinant of the antioxidant and free radical scavenging capacity of flavonoids, because it donates proton and an electron to different free radicals and thereby, generating a stable flavonoid radical (Cao *et al*., 1997). Flavonoids possess lower redox potentials and therefore, are thermodynamically capable to reduce some highly oxidizing free

radicals such as OH•, peroxyl, O2•- and alkoxyl radicals (Kumar and Pandey, 2013). Catechin, apigenin, quercetin, naringenin, rutin and different other flavonoids are reported to possess hepatoprotective capacities (Tapas *et al*., 2008). The flavonoid silymarin, composed of silibinin, silydianine, and silychristine, is a well-known hepatoprotective agent and routinely used as a standard in other hepatoprotective studies (Wellington and Jarvis, 2001; Saller *et al*., 2001; Ball and Kowdley, 2005). The pharmacological properties of silymarin includes maintenance of cell membrane permeability and integrity, inhibition of leukotriene, ROS scavenging, suppression of NF-κβ activity, depression of protein kinases, collagen production (He *et al*., 2004) and has clinical application in liver cirrhosis, ischemic injury, and toxic hepatitis (Saller *et al*., 2001). Different flavonoids such as hesperidin, apigenin, luteolin, and quercetin are reported to have anti-inflammatory and analgesic activities. The tyrosine and serine-threonine protein kinases involved in the inflammatory process are affected by Different flavonoids (Nishizuka, 1988). Moreover, flavonoids are also reported to inhibit and/ or down-regulate some of the major inflammatory mediators such as inducible NO synthase, cyclooxygenase and lipooxygenase (Tunon *et al*., 2009). However, this is noteworthy that in traditional medicinal systems, the phytocompounds are not isolated from their natural sources and are administered as whole in the form of crude extracts. Those possibly lead to either prominent bioactivities of one of the lead compound or synergistic activities of the phytochemical cocktail.

1.1. Objectives

C. bonplandianus is known for its therapeutic efficiencies for ages. Numerous surveys have enlisted the ethnopharmacological uses of *C. bonplandianus* by indigenous people for the treatment of diverse ailments. Evidence based research have already demonstrated different pharmacological properties of *C. bonplandianus*. However, majority of those studies were not systematic enough. Therefore, the present study was designed to evaluate certain immunopharmacological properties of *C. bonplandianus*, based on its ethnopharmacological claims. The investigation was primarily divided into six parts which evaluated the immunomodulatory, anti-inflammatory, antioxidant, neuromodulation and hepatoprotective properties of *C. bonplandianus*. Moreover, detailed phytochemical investigations were also aimed to reveal the chemical composition of *C. bonplandianus*. Thus, the present work was based on the following objectives:

1. To qualitatively and quantitatively estimate the amount of different

phytochemicals present in *C. bonplandianus* leaf extract.

2. To study the immunomodulatory effects of the leaf extract of *C. bonplandianus*.
3. To study the anti-inflamatory potential of the leaf extract of *C. bonplandianus*.
4. To estimate the antioxidant profile and reactive oxygen species scavenging activity of leaf extracts of *C. bonplandianus*.
5. To study the hepatoprotective activity of *C. bonplandianus* leaf extract.
6. To investigate the neuromodulatory activity of *C. bonplandianus* leaf extract by studying its effect on the cholinergic nervous system of mouse.