1.

INTRODUCTION

&

OBJECTIVES

1. Introduction

Traditional herbal medicinal system, also known as phytotherapy, has evolved from the indigenous knowledge, skills and practice of the holistic health care strategy. It is worldwide recognized and accepted for its role in proper maintenance of health and in the treatment of diseases. Herbal therapies are based on indigenous theories, beliefs and experiences that are handed down from generation to generation (WHO, 2000). Traditional medicine has developed in accordance with the life style and cultural practices of the society throughout the centuries. Hands on practical experiences of the therapeutic efficiencies of different herbal remedies have enriched traditional knowledge around the globe. Indian, Chinese and Arabian traditional medicinal systems are highly developed and have gained importance in other countries too. Herbal medicine has emerged as a mode of complementary and alternative medicine to treat diverse diseases and pathological conditions. Dr. Margaret Chan (2008), WHO Director-General, described traditional medicine as, "For many millions of people, often living in rural areas of developing countries, herbal medicines, traditional treatments, and traditional practitioners are the main – sometimes the only – source of health care …….. this form of care unquestionably soothes, treats many ailments, reduces suffering, and relieves pain." In spite of modern synthetic drugs and antibiotics, herbal drugs still have their place in day-to-day therapy. Their effectiveness, ease in access, low-cost and comparative freedom from serious side-effects makes these medicines not only popular but also an acceptable mode of treating diseases even in modern times. There have been increasing academic and industrial involvement in traditional medicinal research during the last several decades (Figure 1).

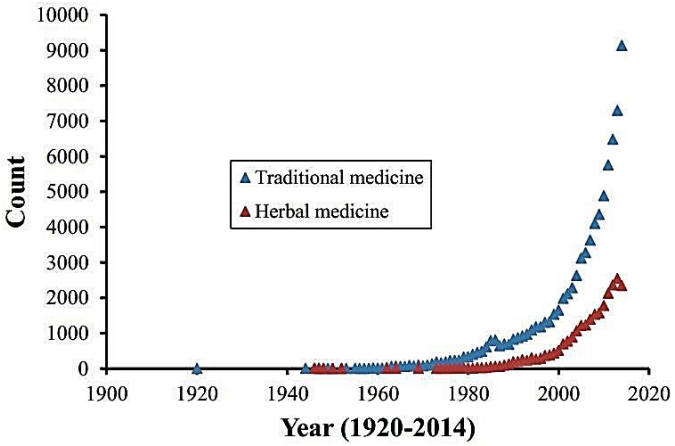

Figure 1: The global trend of increased interest in traditional and herbal medicine. The graphical representation is based on data collected from 'results by year' option of the PubMed database with the keywords 'traditional medicine' and 'herbal medicine'.

The synthetic drugs and antibiotics are sometimes associated with adverse side-effects which include hepatotoxicity, hypersensitivity, immunosuppression and allergic reactions. With the emerging cases of antibiotic resistance in bacteria, there is a constant need for new and

effective therapeutic agents from plants. Thus, the plant derived bioactive chemicals have drawn the main attention as a source of alternative medicines.

In the line of screening of herbal medicines for their therapeutic purpose, one of the first and highly preferred approach is the evaluation of antioxidant and free radical scavenging activities of medicinal herbs. Oxidative stress has emerged as the corner-stone of the pathogenesis of several harmful diseases, disorders and syndromes (Figure 2). The harmful nature oxygen was first proposed by Gerschman and his group in 1954 which marked the

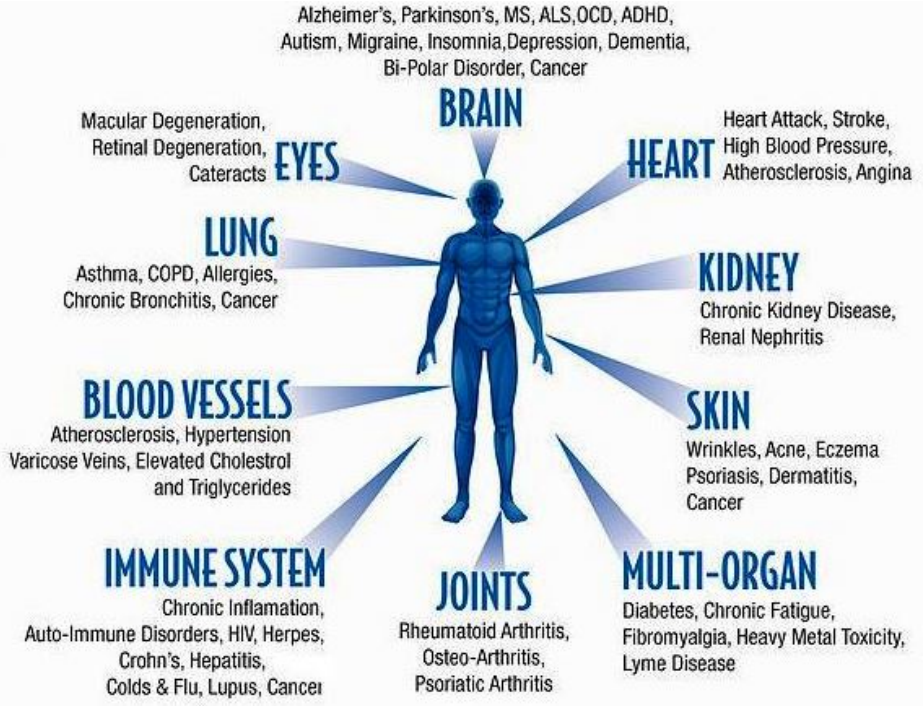

Figure 2: Association of various diseases with free radical mediated oxidative stress (Positive health, 2011).

beginning of oxidative stress and related toxicity (Gerschman, *et al.*, 1954). In the following year, Harman (1956) proposed the role of free radicals in aging. Since then, numerous diseases were found associated with free radicals and oxidative stress, causing direct cellular damage or mediate indirect tissue injury by activating various death-signals in the cell. Oxygen, which is quintessential for the survival of the aerobes, paradoxically, turns toxic due to its oxidizing properties at cellular-molecular level. Inside the cell, through different interconnected reactions, various classes of free radicals are generated which ultimately leads to the damage of cellular biomolecular components (Figure 3). This had leaded the attention towards herbal antioxidants especially from the traditional medicine and dietary supplements for proper maintenance of the body and disease prevention.

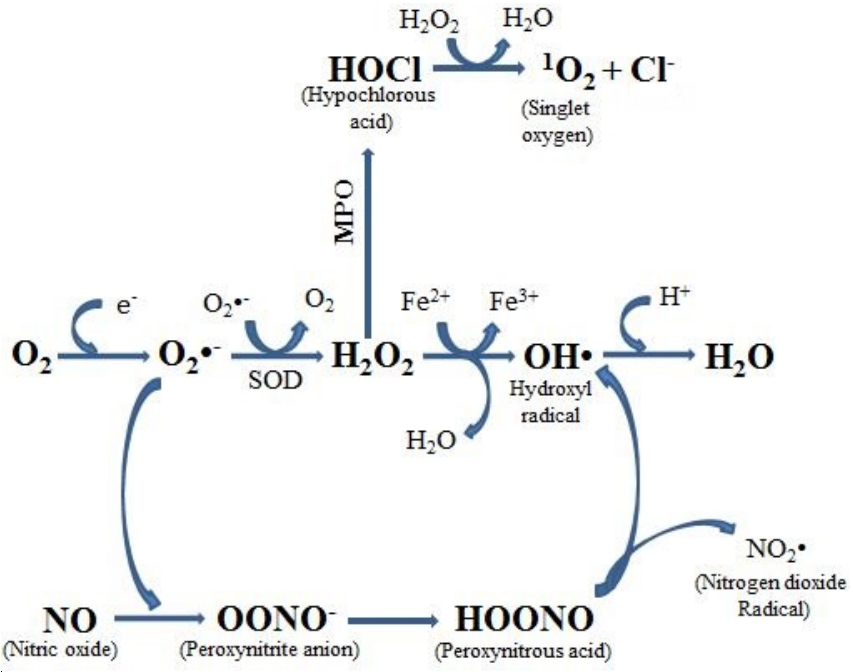

Figure 3: Schematic representation of formation of various free radicals from oxygen (O_2). $O_2•^-$: superoxide radical; SOD: superoxide dismutase; MPO: myeloperoxidase; H_2O_2: hydrogen peroxide.

Antioxidants may be defined as synthetic or natural compounds which inhibits or delays the oxidation process in other molecules (Halliwell, 1997). The basic mode of reactivity of antioxidants is donation of electron to unstable reactive species (Figure 4). In a broad perspective, antioxidants could be categorised into four groups: preventative antioxidants, free-radical scavengers, repair antioxidants and *de novo* antioxidants (Niki, *et al*., 1995; Niki, 2010). Preventative antioxidants averts the formation of reactive chemical species by different methods such as by sequestering metal ions to prevent formation of hydroxyl radical (OH•) through Fenton reaction. Free radical scavengers neutralizes the reactive chemical species

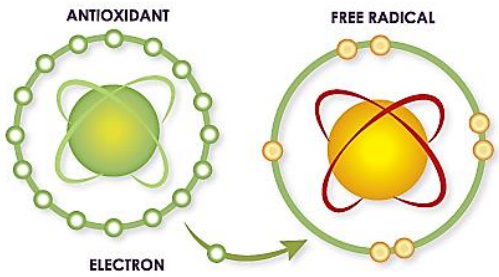

Figure 4: Neutralization of free radicals by antioxidants: Free radicals contain only a single electron in any orbital and are usually unstable toward losing or picking up an extra electron, so that all electrons in the atom or molecule will be paired. Antioxidants donate their electron to the free radical and stabilize the free radicals (Health Savor, 2015).

before they could react with the biomolecules, such as superoxide dismutase (SOD) neutralizes $O_2•^-$ to hydrogen peroxide, which is further broken down to water and oxygen by catalase. Besides, many enzymes functions in parallel to repair free radical mediated damage,

remove tissue and cellular debris, reconstitute loss of cellular functions and participate in the generation of antioxidants and also regulate their functions (Niki, *et al*., 1995; Niki, 2010).

Inflammation is generally manifested by oxidative stress and the process of induction of pro-inflammatory signals such as cell adhesion molecules and interleukins are primarily induced by reactive chemical species. For example, the sub-clinical pro-inflammatory condition in atherosclerosis, cancer and aging are associated with the mitochondrial over-production of free radicals (Wolhuter & Till, 2010). The defensive role of the immune system against invading pathogens is by itself a source of reactive oxygen species (ROS), since activated neutrophils generate free radicals at a significant extent (Fialkow, *et al*., 2007). In addition, phagocytes, in response to bacterial cell wall component, generates $O_2\bullet^-$ catalysed by NADPH oxidase (Behe & Segal, 2007). The protease/anti-protease equilibrium residing in the intestinal interstitium is hindered by free radicals generated from the neutrophils, resulting in direct tissue injury. Metal ions within the immune system may catalyse the formation of free radicals which in turn induce autoimmune reactions (Brambilla, *et al*., 2008). In rheumatic joints, ROS are generated due to increased fibroblast and leucocyte activities, and antioxidants have proved their efficiency in the treatment of rheumatic arthritis by working as immunological adjuvant (van Vugt, *et al*., 2008; Helmy, *et al*., 2001). Various neurodegenerative diseases like Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis and multiple sclerosis are partially triggered by free radical medicated oxidative and nitrosative stress (Brambilla, *et al*., 2008).

Oxidative stress in diabetic conditions causes different forms of tissue damage. Diabetes is usually accompanied by elevated reactive oxygen species (ROS) and or impaired antioxidative defences (Baynes, 1991; Baynes & Thorpe, 1999; Chang, *et al*., 1993; Halliwell & Gutteridge, 1990; Saxena, *et al*., 1993; McLennan, *et al*., 1991). In diabetes, ROS are chiefly generated through glycation reaction, which play a vital role in the development of diabetic complications (Kaneto, *et al*., 1999). Glucose is converted to reactive ketoaldehydes and to superoxide anion radicals ($O_2\bullet^-$) through an enediol radical anion intermediate. $O_2\bullet^-$ undergoes dismutation to generate H_2O_2. H_2O_2 if not degraded by catalase, then generates highly reactive OH•. Alternatively, $O_2\bullet^-$ can also couple with NO to generate toxic peroxynitrite radical. Pancreatic β-cells are sensitive to ROS because the amount of antioxidative enzymes such as SOD, catalase and glutathione peroxidase is low in β-cells compared to other type of cells (Tiedge, *et al*., 1997). Peroxyl radicals remove proton from

the membrane lipids which cause lipid peroxidation and generating hydroperoxides which further propagate free radical mediated injury in diabetes (Halliwell & Gutteridge, 1990).

Free radicals also actively participate in causing alcoholic and non-alcoholic liver diseases (Videla, 2009; Wu & Cederbaum, 2003). Generally fatty acids function as primary oxidative fuel in the liver. In steatohepatitis, signs of hepatic oxidative stress remain associated with low catalase activity, increment of 3-nitrotyrosine immunoreactivity and production of $O_2^{\bullet -}$ and malondialdehyde by Kupffer cells, elevated NO expression, elevated immunohistochemical reactivity to 8-hydroxydeoxyguanosine and 4-hydroxy-2-nonenal (markers of oxidative DNA damage) and up-regulation of cytochrome P450-2E1 (Videla, 2009). Under oxidative stress, liver damage may include biomolecular degradation resulting in loss of functions and impairment of cell viability and constant activation of redox-sensitive transcription factors (NF-kB, AP-1) leading to consistent expression of pro-inflammatory mediators for the Kupffer cells (Videla, *et al*., 2009). Besides, the cytochrome P450 activated drug derived reactive metabolites lead to protein dysfunction, DNA damage and extensive lipid peroxidation (Lynch & Price, 2007).

Herbal immunomodulators are plant derived substances which possess the potentiality to stimulate or supress any component or function of the immune system (both innate and humoral) or work as adjuvant with other compounds (Agarwal & Singh, 1999). Immunostimulants may provide protection against microbial infections as well as enhance body's resistance to allergy, autoimmunity and cancers. Immunosupresants 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 & 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 antigen presentation (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, 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 & Kuttan, 2003). Similar approach was taken by Roy and his group (2013) who demonstrated 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 a bioactive fraction of *Dioscorea alata* L. underground tuber, which demonstrated enhanced proliferation of murine splenic lymphocytes and modulated T_H1/T_H2 cytokine balance *in vitro* (Dey & Chaudhuri, 2014).

Alternatively, Toll-like receptor (TLR) and associated innate immunity markers are presently being targeted for effective immunoadjuvants actions by the botanical immunodrugs (Patwardhan & 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®, a herbal polysaccharide which possesses TLR-4 agonistic activity, which may contribute in its anti-cancer efficiency. Cytokines being the central in-house immunoregulators of the system, their modulation 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 in T-lymphocytes. A detail review on selective botanical immunodrugs including dietary supplements demonstrating cytokine modulatory activity is given by Sommer (1999).

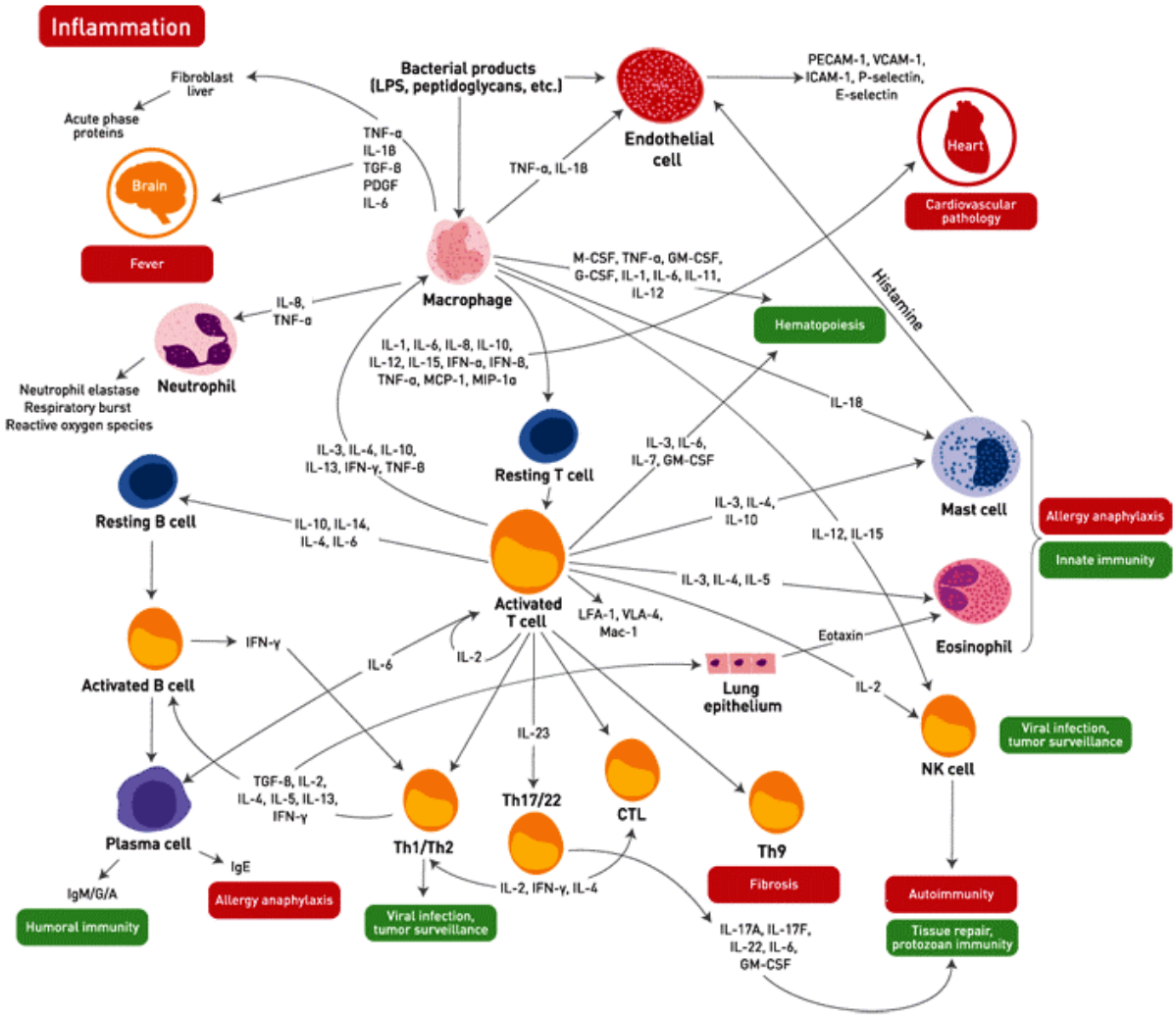

Figure 5: Schematic representation of cytokine interactions during inflammation (Life technologies, 2015).

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 cell-adhesion molecules (CAM) (Levine & Reichling, 1999). In most anti-inflammatory studies, the strategies involved are: up-regulation of anti-inflammatory and down-regulation of pro-inflammatory 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, E-selectin), 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 & Karin, 1997; Ghosh & Karin, 2002).

Liver is one of the essential organs of the body, associated with different physiological process such as growth, immunity, reproduction, energy metabolism and nutrition. Synthesis and excretion of bile, albumin, and prothrombin is carried out by liver. It regulates one of the crucial arms of the humoral immune system by actively participating in production of complements. Liver performs central role in the transformation and metabolism of xenobiotic compounds such as drugs and pharmaceuticals, which in turn cause liver injury, resulting in ionic imbalance, formation of reactive metabolic species (RMS) causing oxidative stress, hindrance in signal transduction pathways, translational inhibition at multiple levels, Ca^{2+} shift, impairment of mitochondrial respiratory chain and β-oxidation. In spite of the fact that liver possess the greatest regenerative capacity, but sub-clinical liver injury is very often caused by the harmful chemicals, especially by their metabolites (Figure 6). Therefore, safeguard of the hepatic system is of utmost importance from the physiological point of view.

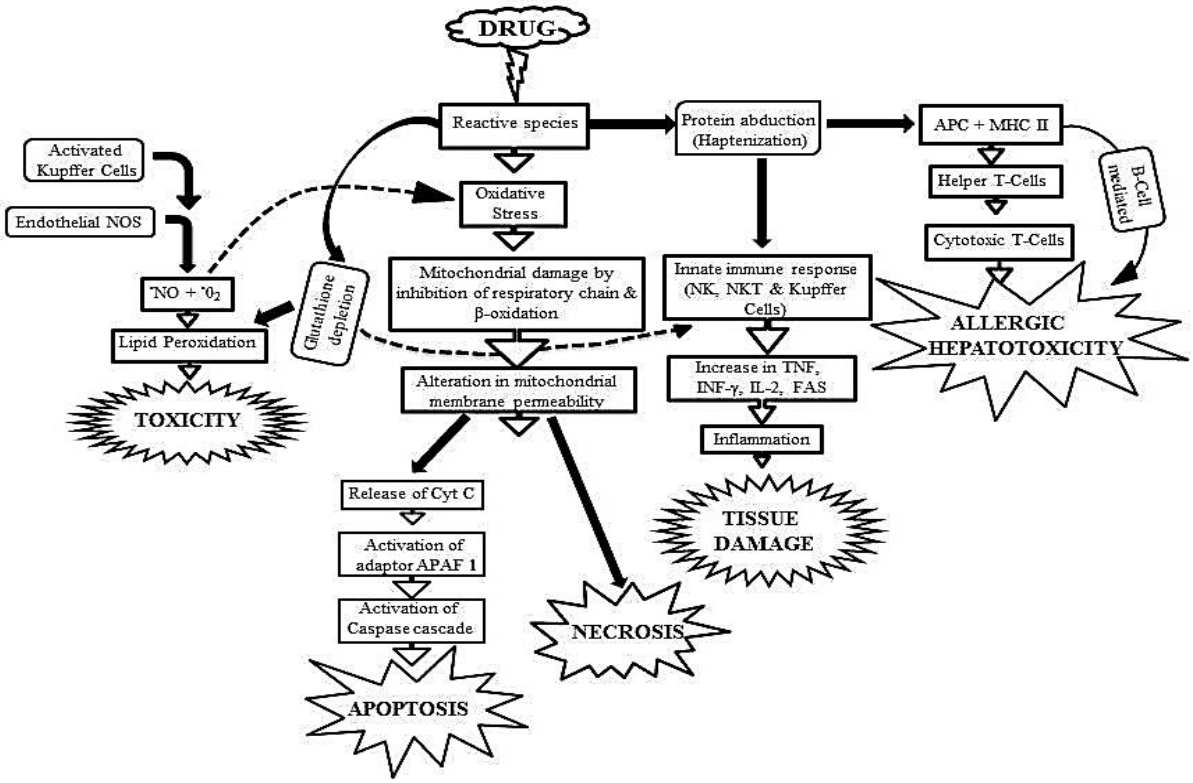

Figure 6: Summarizes the various events which occur during the Drug Induced Hepatotoxicity (Dey, *et al*., 2013).

Drug Induced Hepatotoxicity (DIHT) has emerged as a tremendous concern in medicine in recent years, where around 75% of idiosyncratic drug reactions results in either liver transplantation or proves to be lethal (Mehta, *et al*., 2014). Every year around 2000 cases of acute liver failure occurs in US, out of which around 50% cases are associated with DIHT (Dewantara, 2008). At present there are around 900 compounds in the market in the form of

different drugs and supplements which holds the potential to cause liver injury (Pandit, *et al*., 2012). Hepatotoxicity is one of the major reasons of drugs being withdrawn from the market. Numerous nonsteroidal anti-inflammatory drugs (NSAID) such as amphenac, benoxaprofen, bromofenac, cinchophen, fenclozic acid, fluproquazone, glafanine, isoxepac, pirprofenac, phenylbutazone, sudoxicam etc. were withdrawn from the market due to hepatotoxic nature (Zimmerman, 1990). In practice, hepatotoxicity is primarily classified as (i) elevation of GPT or ALP level in hepatocellular injury; (ii) elevation of ALP and serum bilirubin in cholestatic injury and (iii) GPT and ALP increase in mixed injury (Navarro & Senior, 2006).

Liver is closely associated with the gastrointestinal system and therefore, received large amount of blood containing toxic chemicals, xenobiotics and drugs. Biotransformation of these chemicals through cytochrome P450 generates reactive metabolites of the chemicals (Lynch & Price, 2007). These metabolites reacts with cellular biomolecules causing protein dysfunction, lipid peroxidation, DNA damage, further leading to oxidative stress. This results in ionic imbalance and disruption of intracellular Ca^{2+} storage causing mitochondrial dysfunction. Generation of the free radicals activates pro-inflammatory signals in the hepatocytes which trigger various immunoregulatory cells such as natural killer T (NKT) cells, Kupffer cells and natural killer (NK) cells. These cells further secret inflammatory cytokines such as TNF-α, interferons and interleukins which promotes tissue damage (Blazka, *et al*., 1995).

Use of herbal hepatoprotective remedies is popular in Ayurveda, Siddha, Unani, Amchi and Chinese systems of traditional medicine. Around 77 herbs are mentioned in Ayurveda to possess hepatoprotective potentials (Kshirsagar, *et al*., 2011). At present there are around 89 Ayurvedic formulations which are used by 37 Indian pharmaceutical companies to prepare hepatoprotective drugs (Kshirsagar, *et al*., 2011). Different herbal formulations of Ayurvedic, Siddha and Unani medicinal systems have already been approved by The Indian Medicinal Practitioner's Co-operative Pharmacy and Stores (Thyagarajan, 1996). The bioactive constituent silymarin (consists of isomeric flavolignans-silybin, silydianin and silychristen) isolated from milk thistle seeds (*Silybum marianum*) has been proved to possess tremendous hepatoprotective capacity and accounts for 180 million US dollars business in Germany alone (Pradhan & Girish, 2006). Another polyherbal formulation Liv 52, which is a phytococtail of around 18 plants, is extensively used as hepatoprotective medicine (Singh, *et al*., 1983) and several studies have already demonstrated the hepatoprotective properties of Liv 52 (Chauhan & Kulkarni, 1991; Huseini, *et al*., 2005; Gopumadhavan, *et al*., 1993). Among the Indian

medicinal plants, Kalmegh (*Andrographis paniculata*), Bhuia amla (*Phyllanthus niruri*), Indian bearberry (*Berberis aristata*), Turmeric (*Curcuma longa*), Kutki (*Picrorhiza kurroa*), Mulethi (*Glycyrrhiza glabra*), Punarnava (*Boerhavia diffsa*), Tulsi (*Ocimum sanctum*), Chicory (*Cichorium intybus*), Bhringa raja (*Eclipta alba*), Kanak champa (*Pterospermum acerifolium*), Guduchi (*Tinispora cordifolia*), Chirayata (*Swertia chirata*) etc. are routinely used to ameliorate liver related complications.

Traditional herbal remedies are also extensively used worldwide for the treatment of diabetic complications which is one of the major challenge to the global healthcare system. Diabetes prevalence, according to International Diabetes Federation (2013), is expected to rise to 552 million by the year 2030 from the present count of 371 million in 2012. It sounds horrible that every 6 seconds, a person dies from diabetes and it has been estimated that by the end of 2013, globally 5.1 million deaths might have occurred due to diabetes costing $546 billion USD which accounted for 11% of expense in global healthcare (International Diabetes Federation, 2013).

Diabetes mellitus is a combined phenotype of several metabolic complaints which affects different organs of the body (Figure 7). Increasing evidence suggests that oxidative stress (Figure 8) plays a key role in the pathogenesis of diabetes mellitus and its complications (Brownlee, 2001). Glucose metabolism gets severely hindered due to improper insulin production from the pancreatic β-cells (Jarald, *et al*., 2008). Glycogen catabolism in liver increases due to low insulin level resulting in low hepatic glycogen content in diabetes. Hepatic damage induced in such condition may demonstrate elevation of the liver marker enzymes such as transaminases and phosphatases (Amarapurkar & Das, 2002). Diabetic nephropathy results in further increase of urea, uric acid and creatinine level in serum. Besides, hyperglycaemia induces oxidative stress exacerbates the pathogenesis of diabetic complications (Johansen, *et al*., 2005).

The earliest known use of herbal medicine for the treatment of diabetic complications is found in the Ebers Papyrus of about 1550 BC (Pushparaj, 2004). Today, a staggering 80% people suffering from diabetes belong to low- and middle-income countries (International Diabetes Federation, 2013) and due to easy availability and low cost, traditional medicine is the primary therapeutic approach in those countries. Around 21,000 plants were listed by WHO to possess medicinal properties and 800 of them were reported to possess anti-diabetic properties (Rizvi & Mishra, 2013). Numerous Indian medicinal plants have been identified which holds

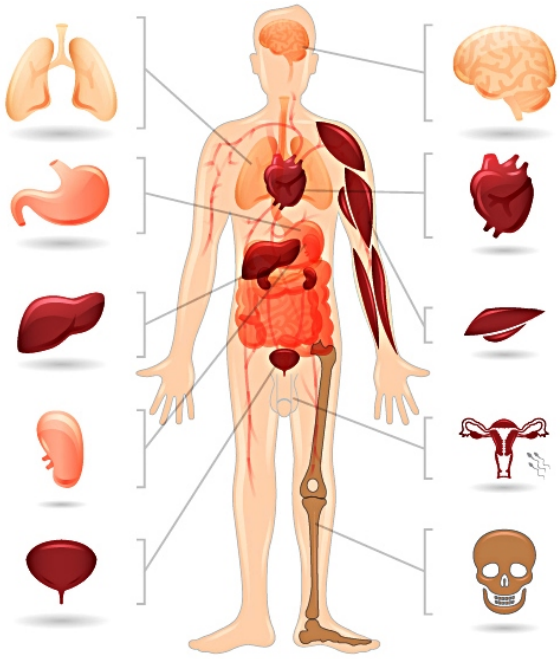

Figure 7: The effect of diabetes on different systems of the body including (anti-clockwise) respiratory, digestive, endocrine, immune, urinary, skeletal, reproductive, muscular, circulatory and nervous system (Diabetes and the body, 2015)

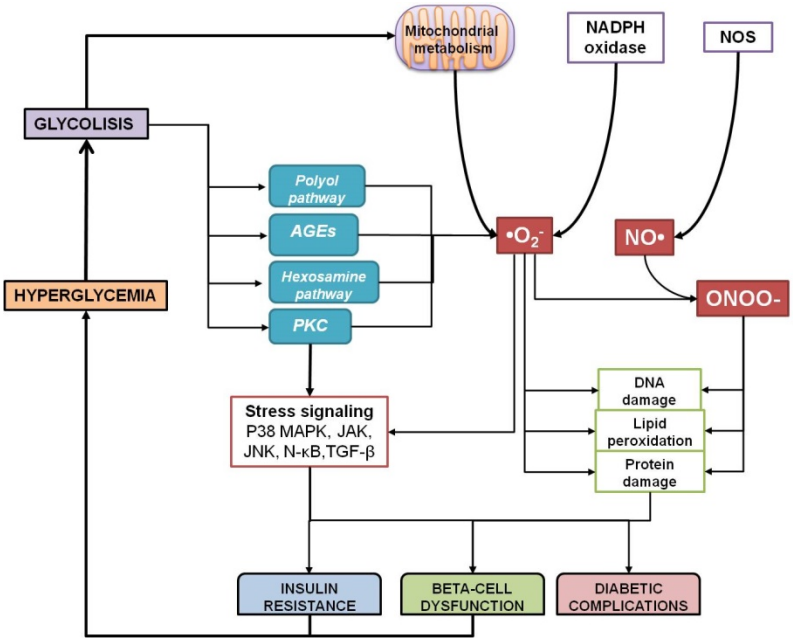

Figure 8: Oxidative stress pathways in diabetes mellitus. Several studies of diabetic micro and macrovascular complications suggests mitochondrial superoxide overproduction as the main cause of metabolic abnormalities of diabetes (Morales-Gonzalez, 2013).

the potentialities to lower blood glucose levels and diabetes associated metabolic complications such as Babul (*Acacia arabica*), Bael (*Aegle marmelose*), Onion (*Allium cepa*), garlic (*Allium sativum*), Ghrita kumara (*Aloe vera*), Neem (*Azadirachta indica*), Beetroot (*Beta vulgaris*), Fever nut (*Caesalpinia bonducella*), Ivy gourd (*Coccinia indica*), Eucalyptus (*Eucalyptus globules*), Banyan tree (*Ficus benghalenesis*), Gurmar (*Gymnema sylvestre*), Sweet potato (*Ipomoea batatas*), Karela (*Momordica charantia*), Mulberry (*Morus alba*), Basil (*Ocimum sanctum*), Pomegranate (*Punica granatum*), Jamun (*Syzygium cumini*), Methi

(*Trigonella foenum-graecum*), etc. Various groups of researchers have also identified some active ingredients with anti-diabetic potentialities in different medicinal plants. For instance, castanospermine (alkaloid) isolated from seeds of *Castanospermum australe*; epicatechin (flavonoid) isolated from the heartwood of *Pterocarpus marsupium*, and neomyrtillin (glycoside) isolated from *Vaccinium myrtillus* were claimed to possess anti-hyperglycaemic activities (Day, 1990). A systemic review of anti-diabetic herbs and dietary supplements by Yeh and her group (2003) revealed that among more than 100 trials of anti-diabetic herbal supplements, significant therapeutic efficiencies were reported in 88% cases of those examining single herbs, 60% cases of those examining combination herbs, and 67% cases of those examining vitamin or mineral supplements. Silymarin from milk thistle have demonstrated to lower glucose level in blood and urine in addition to HbA1c, C-peptide levels and insulin requirement in diabetic conditions (Velussi, *et al*., 1997). Different varieties of Ginseng (genus Panax) have demonstrated excellent anti-diabetic properties through modulation of blood glucose, insulin, HbA1c, homeostasis model assessment of insulin resistance (HOMA-IR) levels in several controlled clinical trials (Shishtar, *et al*., 2014). Moreover, different traditional medicinal systems around the globe utilizes various herbal remedies for the treatment of diabetogenic complications and their efficiencies are continuously being established through *in vivo* and *in vitro* bioassays by different groups of researchers.

Potent therapeutic properties of the phytomedicin are due to the presence of diverse class of bioactive phytochemicals, most of which are secondary metabolites (Figure 9). Around 30% of FDA approved medicines have a botanical origin (De Smet, 2002; Licciardi & 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 anti-neoplastic 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*).

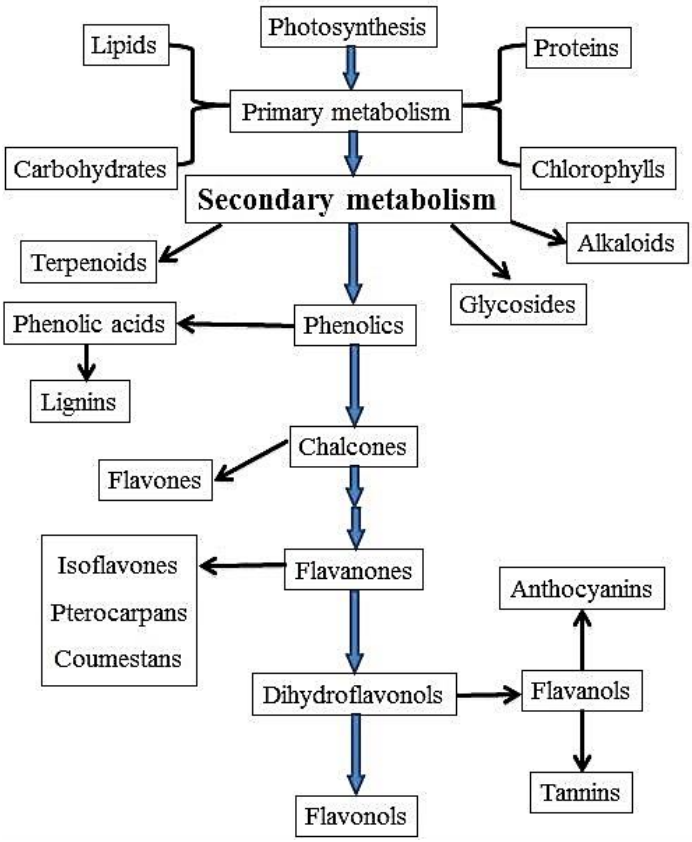

Figure 9: Simplified representation of the metabolic pathway of formation of various classes of plant secondary metabolites (Morales-Gonzalez, 2013)

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 possess 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 were reported to contain gallic acid which possesses 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 & 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, $O_2^{\bullet -}$ and alkoxyl radicals (Kumar & 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 & Jarvis, 2001; Saller *et al*., 2001; Ball & Kowdley, 2005). The pharmacological properties of silymarin includes maintenance of cell membrane permeability and integrity, inhibition of leukotriene, ROS scavenging, suppression of NF-κB 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 were reported to have anti-inflammatory and analgesic activities. Different flavonoids affects the enzymatic cascade of the inflammatory

process i.e. the tyrosine and serine-threonine protein kinases (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.

Objectives

N. indicum is known for its therapeutic efficiencies for ages. Numerous surveys have enlisted the ethnopharmacological uses of *N. indicum* by indigenous people for the treatment of diverse ailments. Evidence based research have already demonstrated different pharmacological properties of *N. indicum*. However, majority of those studies were either confined mainly to evaluate the anti-cancer properties or the studies were not systematic enough. Besides, most of the former studies evaluated the therapeutic efficiencies of the oleander leaf only and therefore, the potentiality of stem and root remained unexplored. Therefore, the present study was designed to evaluate certain immunopharmacological properties of *N. indicum*, based on its ethnopharmacological claims. The investigation was primarily divided into six parts which evaluated the immunomodulatory, anti-inflammatory, antioxidant, anti-diabetic and hepatoprotective properties of *N. indicum*. Moreover, detailed phytochemical investigations were also aimed to reveal the chemical composition of *N. indicum*. Thus, the present work took up the following objectives:

i. Study of the immunomodulatory effects of the leaf, stem and root of *N. indicum*.

ii. Study of the anti-inflammatory potentials of *N. indicum*.

iii. Investigation of the antioxidant profile and reactive oxygen species scavenging activities of leaf, stem and root extracts of *N. indicum*.

iv. Evaluation of the hepatoprotective activities of *N. indicum*.

v. Study of the anti-diabetic activities of *N. indicum*.

vi. Quantitative and qualitative estimation of different phytochemicals in leaf, stem and root of *N. indicum*.